

***PUBLIC REVIEW DRAFT***

**TECHNICAL SUPPORT DOCUMENT: INTERIM SPECIFIC GROUND  
WATER CRITERION FOR PERFLUOROOCCTANOIC ACID (PFOA, C8)  
(CAS #: 335-67-1; Chemical Structure:  $\text{CF}_3(\text{CF}_2)_6\text{COOH}$ )\***

***Primary Authors:***

**Gloria B. Post, Ph.D., DABT;**

**NJDEP Division of Science, Research & Environmental Health**

**Jessie A. Gleason, M.S.P.H.**

**NJDOH Environmental & Occupational Health Surveillance Program**

**Division of Science, Research & Environmental Health  
New Jersey Department of Environmental Protection**

**January 2019**

**\*This document is based on an evaluation of PFOA by the Health Effects Subcommittee of the New Jersey Drinking Water Quality Institute. Members of the Subcommittee are Jessie A. Gleason, Keith R. Cooper, Judith B. Klotz, Gloria B. Post, and George Van Orden.**

## **TABLE OF CONTENTS**

ABSTRACT.....	1
EXECUTIVE SUMMARY .....	2
INTRODUCTION .....	18
BACKGROUND INFORMATION .....	19
GUIDANCE AND STANDARDS DEVELOPED BY NEW JERSEY, OTHER STATES, AND USEPA .....	22
ENVIRONMENTAL FATE, TRANSPORT, AND OCCURRENCE.....	25
HUMAN BIOMONITORING.....	34
SOURCES OF HUMAN EXPOSURE.....	39
TOXICOKINETICS .....	42
HEALTH EFFECTS - ANIMAL TOXICOLOGY .....	102
MODE OF ACTION.....	177
DEVELOPMENT OF ISGWQC .....	203
DISCUSSION OF UNCERTAINTIES .....	220
CITATIONS .....	222

## **APPENDICES**

Appendix 1 - Literature Search Criteria and Documentation – PFOA.....	2
Appendix 2 - Comparison of USEPA Office of Water Health Advisory and NJDEP ISGWQC for PFOA.....	4
Appendix 3 - Risk Assessment Considerations for Butenhoff et al. (2002) Subchronic Cynomolgus Monkey Study.....	17
Appendix 4 - Individual Tables for Epidemiology Studies.....	20
Appendix 5 - Individual Tables for Toxicology Studies.....	77
Appendix 6 - Benchmark Dose Modeling for Mammary Gland Development .....	113
Appendix 7 - Benchmark dose analysis of relative liver weight in response to PFOA (linear/branched) using BMR of 10% increase relative to controls (Loveless et al., 2006).....	114
Appendix 8 - Benchmark dose analysis of testicular tumor data in response to PFOA using BMR of 5% tumor incidence (Butenhoff et al., 2012) .....	128

**TABLE OF TABLES**

Table 1. PFOA concentrations in raw or finished water from PWS included in NJDEP database ..... 32

Table 2. New Jersey versus national UCMR3 PFC occurrence data as of January 2016..... 33

Table 3. Serum PFOA concentration from NHANES (ng/ml) ..... 35

Table 4: Serum/plasma elimination half-lives of PFOA..... 44

Table 5. Increase in serum PFOA concentrations predicted from various concentrations of PFOA in drinking water ..... 58

Table 6A. Summary of findings from epidemiologic studies of PFOA and serum lipids ..... 90

Table 6B. Summary of findings from epidemiologic studies of PFOA and liver enzymes/bilirubin..... 94

Table 6C. Summary of findings from epidemiologic studies of PFOA and thyroid hormones and diseases ..... 97

Table 6D. Summary of findings from epidemiologic studies of PFOA and uric acid..... 100

Table 6E. Summary of findings from epidemiologic studies of PFOA and antibody concentrations (following vaccination)..... 101

Table 7. Relative Liver Weight (compared to controls) in 90 Day Rhesus Monkey Study (Goldenthal, 1978)..... 104

Table 8. Liver enzymes (relative to control) in serum of mice exposed to PFOA in drinking water for 21 days (Son et al., 2008)..... 114

Table 9. Developmental events in human and rodent mammary tissue (Fenton, 2006)..... 127

Table 10. Summary of Increased Relative Liver Weight Data from Rodents Studies Using Doses < 1 mg/kg/day, and 90 Day Non-Human Primate Study ..... 150

Table 11. Summary of toxicological studies of effects of oral exposure to PFOA on the immune system ..... 152

Table 12: Summary of studies of effects of gestational/lactational exposure to PFOA in mice (most sensitive effect(s) in each study are shown in *red italics*)\* ..... 157

Table 13. Identification of most sensitive endpoints in mouse developmental studies of PFOA\*..... 167

Table 14A. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Includes studies with exposure during pregnancy, gestation, and/or lactation (6 publications/10 studies). ..... 171

Table 14B. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Studies with peripubertal exposure (3 publications) ..... 175

Table 15. Mammary gland development parameters selected for dose-response modeling from PND 21 female offspring after exposure on GD 10-17 .....	207
Table 16. Benchmark Dose modeling of serum PFOA data (PND1) for mammary gland developmental effects (PND 21) in CD-1 mouse pups .....	208
Table 17: Serum PFOA and relative liver weight in Male CD-1 mice dosed with branched/linear PFOA for 14 days.....	212
Table 18. Benchmark Dose analysis for a 10% increase in relative liver weight from linear/branched PFOA in male mice (Loveless et al., 2006) <sup>a</sup> .....	213
Table 19. BMD modeling (0.05 BMR; 5% response) of rat testicular tumor data (Butenhoff et al., 2012) <sup>a</sup> .....	219

**TABLE OF FIGURES**

Figure E-1. Increases in serum PFOA concentrations predicted from mean and upper percentile consumption of drinking water with various concentrations of PFOA, as compared to U.S median and 95 <sup>th</sup> percentile serum PFOA levels (NHANES, 2011-12). .....	6
Figure E-2. From Verner et al. (2016a). Modeling simulation of the ratio of PFOA in blood plasma in breast fed infants/children to plasma concentration in mother. ....	7
Figure 1. Increases in serum PFOA concentrations predicted from consumption of drinking water with various concentrations of PFOA .....	25
Figure 2. Major transport pathways of PFCs to the Arctic (and other remote locations), by Annika Jahnke (Butt et al., 2010) .....	27
Figure 3. APFO (PFOA) transport near discharge source (Davis et al., 2007).....	29
Figure 4. Structure of diPAPs 8:2 .....	29
Figure 5. PFOA concentration in cord blood and blood collected in infants around six and nineteen months after birth.....	52
Figure 6. Serum PFOA concentrations over time in 12 infants from Mogensen et al. (2015). .....	53
Figure 7. Monte Carlo simulations (n = 10,000) of child/mother ratios of plasma PFOA levels (ng/ml; right side of figure) and doses (ng/kg/day; left side of figure) for a breastfeeding period of 30 months ...	53
Figure 8. Increases in serum PFOA concentrations predicted from mean and upper percentile consumption of drinking water with various concentrations of PFOA, as compared to U.S median and 95 <sup>th</sup> percentile serum PFOA levels (NHANES, 2011-12) .....	58
Figure 9. Adjusted-predicted total cholesterol change with increasing group median deciles. (A). Adults, Steenland et al., 2009 (B). Adolescents, .....	89

Figure 10. Timeline of critical periods of mammary gland development and potential effects of endocrine disrupting compounds on mammary gland development (Fenton, 2006)..... 127

Figure 11. White et al. (2011b) study design and experimental timeline. .... 133

Figure 12. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male cynomolgus monkeys dosed with PFOA for 6 months (Butenhoff et al., 2002). 180

Figure 13. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male mice dosed with linear/branched, linear, or branched PFOA for 14 days (Loveless et al., 2006). .... 181

Figure 14. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male rats dosed with linear/branched, linear, or branched PFOA for 14 days (Loveless et al., 2006). .... 182

Figure 15. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male rats and mice dosed with PFOA for 4, 7, or 13 weeks (Perkins et al., 2004). .... 183

Figure 16. Changes in PFOA levels in breast-fed infants from birth to later timepoints (Fromme et al., 2010; Mogensen et al., 2015)..... 216

Figure 17. Monte Carlo simulations of child/mother ratios of plasma PFOA levels (ng/ml) a breastfeeding period of 30 months. Black line - 50th percentile; blue line - 5th percentile; red line - 95th percentile; dotted lines - minimum and maximum values ..... 217

## **ABBREVIATIONS**

ALP — alkaline phosphatase  
ALT /SGPT — alanine aminotransferase  
APFO — ammonium perfluorooctanoate, the ammonium salt of PFOA  
AST /SGOT — aspartate aminotransferase  
BMD — Benchmark Dose  
BMDL — lower 95% confidence limit on the Benchmark Dose  
BMR — Benchmark Response  
CAR — constitutive androstane receptor  
C8 — a synonym for PFOA  
DWQI — New Jersey Drinking Water Quality Institute  
FTOH — fluorotelomer alcohol  
GD — gestational day  
GFR — glomerular filtration rate  
GGT — gamma-glutamyl transferase  
GM — geometric mean  
H & E — hematoxylin and eosin  
HDL — high-density lipid cholesterol  
IARC — International Agency for Cancer Research  
IRIS — USEPA Integrated Risk Information System  
ISGWQC – Interim Specific Ground Water Quality Criterion  
LDL — low-density lipid cholesterol  
LOAEL — Lowest Observed Adverse Effect Level  
MCL — Maximum Contaminant Level  
NHANES — National Health and Nutrition Examination Survey  
NJDEP — New Jersey Department of Environmental Protection  
NJDOH — New Jersey Department of Health  
NOAEL — No Observed Adverse Effect Level  
NTP — National Toxicology Program  
OR — odds ratio  
PAPs — polyfluoroalkyl phosphoric acid diesters  
PCO — palmitoyl CoA oxidase  
PFC — perfluorinated compound  
PFOA — perfluorooctanoic acid  
PFOS — perfluorooctane sulfonate  
PFNA — perfluorononanoic acid  
PND — postnatal day  
POD — Point of Departure  
PPAR — peroxisome proliferator activated receptor  
PTFE – polytetrafluoroethylene  
PWS – public water supplies  
PXR — pregnane X receptor  
RfD — Reference Dose  
RL — Reporting Level  
RSC — Relative Source Contribution  
SAB — Science Advisory Board

SDWA — Safe Drinking Water Act  
SMR — standardized mortality ratio  
TSH — thyroid stimulating hormone  
TT3 — total triiodothyronine  
TT4 - total thyroxine  
UCMR3 — Unregulated Contaminant Monitoring Rule 3  
UF — uncertainty factor  
VLDL — very low-density lipid cholesterol  
USEPA — United States Environmental Protection Agency  
WY — Wyeth 14,643 (4-Chloro-6-[2,3-xylidino]-2-pyrimidinylthio)acetic acid), a model  
PPAR-alpha activating compound

## **ABSTRACT**

An Interim Specific Ground Water Quality Criterion (ISGWQC) for perfluorooctanoic acid (PFOA, C8) was developed to protect for chronic (lifetime) drinking water exposure. Although human data are not used as the quantitative basis for the risk assessment, a public health-protective approach in developing an ISGWQC based on animal toxicology data is supported by associations of PFOA with numerous health effects in the general population and communities with drinking water exposure, as well as its biological persistence and bioaccumulation from drinking water in humans. PFOA was described as “likely to be carcinogenic to humans” by the USEPA Science Advisory Board and “possibly carcinogenic to humans” by the International Agency for Research on Cancer (IARC), and both non-carcinogenic and carcinogenic effects were evaluated for ISGWQC development. Two sensitive non-carcinogenic endpoints, delayed mammary gland development after developmental exposures and increased liver weight, were evaluated. For each endpoint, benchmark dose modeling of serum PFOA levels from mouse studies was performed and appropriate uncertainty factors were applied to develop a Target Human Serum Level (analogous to a Reference Dose but on a serum level basis). A clearance factor ( $1.4 \times 10^{-4}$  L/kg/day) which relates serum PFOA concentrations to human PFOA doses was applied to the Target Human Serum Levels to develop Reference Doses. For delayed mammary gland development, the Target Human Serum Level is 0.8 ng/ml, which is below the median serum PFOA level in the U.S. general population. The Reference Dose for this endpoint is 0.11 ng/kg/day. Because the use of delayed mammary gland development as the basis for quantitative risk assessment is a currently developing topic, an ISGWQC using this endpoint as its primary basis was not recommended. However, the occurrence of this and other effects at similarly low doses clearly requires application of an uncertainty factor to protect for these more sensitive effects. An ISGWQC protective for increased relative liver weight was derived based on a study in which male mice were exposed to PFOA for 14 days. For increased relative liver weight, the Target Human Serum Level is 14.5 ng/ml and the Reference Dose is 2 ng/kg/day. This Target Human Serum Level and Reference Dose incorporate uncertainty factors to protect sensitive human subpopulations, toxicodynamic differences between human and experimental animals, and more sensitive endpoints that occur from developmental exposures (delayed mammary gland development, hepatic toxicity, and others). Using default values for drinking water exposure assumptions and Relative Source Contribution factor, a health-based water concentration of 14 ng/L was developed based on increased relative liver weight. A cancer slope factor,  $0.021$  (mg/kg/day)<sup>-1</sup>, was developed based on increased incidence of testicular tumors in a chronic rat study. This slope factor was used to develop a health-based water concentration protective for cancer effects at the  $1 \times 10^{-6}$  (one in one million) lifetime cancer risk level of 14 ng/L, identical to the health-based water concentration based on non-cancer endpoints. Since ISGWQC are rounded to one significant figure, the ISGWQC is therefore 10 ng/L (0.01 µg/L).

## **EXECUTIVE SUMMARY**

Perfluorooctanoic acid (PFOA, C8) is a member of the group of substances called perfluorinated compounds (PFCs), chemicals that contain a totally fluorinated carbon chain which varies in length and a functional group such as carboxylic or sulfonic acid. PFCs are part of a larger group of chemicals called poly- and perfluoroalkyl substances (PFAS).

Interim Specific Ground Water Quality Criteria are based on chronic (lifetime) exposure, a one in one million ( $10^{-6}$ ) risk for carcinogenic effects, and no adverse physiological effects for non-carcinogenic effects.

The development of the ISGWC is based primarily on an evaluation of PFOA by the Health Effects Subcommittee of the New Jersey Drinking Water Quality Institute (DWQI, 2017). This report closely reflects the report produced by the DWQI with text revised by the New Jersey Department of Environmental Protection to describe the development of the ISGWC.

### **Manufacturing and Use**

Because carbon-fluorine bonds are among the strongest found in organic chemistry, PFOA and other PFCs are extremely stable and resistant to chemical reactions. PFOA has been produced for use in commercial products and industrial processes for over 60 years. Its unique surfactant properties and resistance to chemical and thermal degradation make it useful in many applications including water-, soil-, and stain-resistant coatings, fire-fighting foams, and industrial uses. Large amounts of PFOA were used industrially as a processing aid (emulsifier) in the production of fluoropolymers and fluoroelastomers for use as non-stick coatings.

Because of concerns about its ubiquitous presence in environmental media (including wildlife) and human blood serum worldwide, its persistent and bioaccumulative nature, and its potential health effects, the eight major U.S. producers of PFOA entered into a voluntary agreement with USEPA in 2006 to reduce emissions and product content of PFOA and its precursors by 95% by 2010 and to work towards eliminating them by 2015. However, other manufacturers and users of PFOA that are not participants in the voluntary agreement with USEPA continue to emit large amounts of PFOA to the environment, particularly overseas. Although the production and use of PFOA and its precursors has been phased out by major U.S. manufacturers, environmental contamination and resulting human exposure to PFOA are anticipated to continue for the foreseeable future due to its persistence, formation from precursor compounds, and continued production by other manufacturers.

### **Environmental Fate and Transport**

Because of the extreme stability of their carbon-fluorine bonds, PFOA and other PFCs are extremely resistant to degradation in the environment and thus persist indefinitely. PFOA and other PFCs are found in many environmental media and in wildlife worldwide including in remote polar regions. PFOA is much less bioaccumulative in fish than PFOS or perfluorinated

carboxylates with more than eight carbons, and PFOA concentrations in wildlife are generally lower than for these other PFCs. PFOA and other PFCs can be taken up into plants from contaminated soil or irrigation water. In general, PFOA and other longer chain PFCs are preferentially taken up into the root and shoot parts of the plant.

PFOA and some other PFCs are distinctive from other persistent and bioaccumulative organic compounds because of their importance as drinking water contaminants. PFOA does not bind well to soil, migrates readily from soil to ground water, and is highly water-soluble. These properties of PFOA differ from those of other well-known persistent and bioaccumulative organic pollutants such as polychlorinated dioxins and polychlorinated biphenyls (PCBs) that have a high affinity for soil and sediments but low water solubility.

PFOA that is released into the environment can contaminate surface water and groundwater used as drinking water sources. Environmental sources include industrial discharge to soil, air, and water; release of aqueous firefighting foams; disposal in landfills; wastewater treatment plant discharge; street and storm water runoff; and land application of biosolids, industrial solid waste, and wastewater. PFOA also enters the environment through the breakdown of precursor compounds such as the fluorotelomer alcohol 8:2 FTOH and larger molecules that can release 8:2 FTOH. These precursor compounds are used industrially and in consumer products. They are converted to PFOA by microbes in soil, sludge, and wastewater and through atmospheric chemical reactions.

As is the case for other ground water contaminants, PFOA can reach drinking water wells via migration of a ground water plume. Unlike many other environmental contaminants, PFOA emitted to air from industrial facilities can also contaminate distant groundwater wells through air transport, followed by deposition from air onto soil, and migration through the soil to groundwater.

### **Occurrence in Drinking Water**

PFOA and other PFCs are not effectively removed from drinking water by standard treatment processes but can be removed from drinking water by granular activated carbon (GAC) or reverse osmosis. Therefore, unless specific treatment for removal of PFCs is in place, concentrations of PFOA detected in raw drinking water can be considered to be representative of concentrations in finished drinking water.

The occurrence of PFOA and other PFCs in public water supplies (PWS) has been evaluated more extensively in New Jersey than in most or all other states. More than 1,000 samples from 80 NJ PWS were analyzed with relatively low Reporting Levels (RLs; generally  $\leq 5$  ng/L) in 2006-2016. PFOA was the most frequently detected PFC and was found in samples from approximately 60% of the 80 NJ PWS tested. In the 2013-2015 USEPA Unregulated Contaminant Monitoring Rule 3 (UCMR3) survey of all large (>10,000 users) and a subset of smaller PWS in the U.S., PFOA was detected more than five times more frequently in New

Jersey PWS (10.5%) than nationally (1.9%). The RL in UCMR3 was 20 ng/L, much higher than the RLs for most other NJ PWS monitoring. PFOA has also been detected in NJ private wells near sources of industrial discharge.

### **Human Biomonitoring**

PFOA and other PFCs are found ubiquitously in the blood serum of the general population in the U.S. and worldwide. The 2011-2012 National Health and Nutrition Examination Survey (NHANES), a representative sample survey of the U.S. general population conducted by the U.S. Centers for Disease Control and Prevention (CDC), determined the geometric mean and 95<sup>th</sup> percentile serum PFOA concentrations as 2.08 and 5.68 ng/ml, respectively. Serum PFOA levels in the U.S. general population have declined since the first NHANES monitoring in 1999-2000 when the geometric mean and 95<sup>th</sup> percentile values were 5.21 and 11.9 ng/ml. In communities exposed through contaminated drinking water, serum PFOA levels are elevated compared to the general population. Exposures to industrially-exposed workers or others with occupational exposure are much higher than in the general population. Serum PFOA concentrations of greater than 100,000 ng/ml (100 ppm) have been reported in industrially exposed workers, although levels in most workers were lower.

### **Sources of Exposure**

Sources of exposure to PFOA and/or its precursors include drinking water, food and food packaging, treated fabrics, protective sprays and waxes, cosmetics and personal care products, house dust, and inhalation of indoor and outdoor air. Most studies predict that food and food packaging are the predominant exposure sources, and several studies suggest that PFOA and its precursors in indoor air and/or house dust can be a major exposure source. It should be noted that migration of PFOA from polytetrafluoroethylene (PTFE)-coated non-stick cookware into food is not considered to be a significant source of exposure. The contribution of ingested drinking water to total exposure from all sources (e.g. diet, consumer products, etc.) is dependent on the concentration of PFOA in the drinking water, and relatively low concentrations in water substantially increase human body burden. Inhalation from showering, bathing, laundry, and dishwashing, and dermal absorption during showering, bathing, or swimming, are not expected to be significant sources of exposure from contaminated drinking water.

Exposures to PFOA may be higher in young children than in older individuals because of age-specific behaviors such as greater drinking water and food consumption on a body weight basis, hand-to-mouth behavior resulting in greater ingestion of house dust, and more time spent on floors where treated carpets are found.

## **Toxicokinetics**

PFOA is well absorbed orally, and it was also absorbed dermally and by inhalation in toxicological studies. It is water soluble and distributes primarily to the liver and serum, and, to a lesser degree, to the kidney. Unlike most other bioaccumulative organic compounds, it does not distribute to fat. In the serum, PFOA is almost totally bound to albumin and other proteins. Since it is chemically non-reactive, it is not metabolized. The rate of excretion is largely dependent on the extent of secretion and reabsorption by organic anion transporters in the kidney. The excretion rate varies widely among species, and in some cases between males and females of the same species.

PFOA's half-life in humans is several years and is similar in males and females. Because of its long half-life, it remains in the human body for many years after exposures cease. PFOA is persistent in both male and female mice and in male rats, with half-lives of days to weeks. However, PFOA is rapidly excreted in female rats (half-life of 2-4 hours); thus, this species is not an ideal model for studying potential human developmental effects. Because of the large variation in half-lives, the internal dose resulting from a given administered dose varies widely among species and, in some cases, genders of the same species. For this reason, interspecies (e.g. animal-to-human) comparisons are made on the basis of internal dose, as indicated by serum level, rather than administered dose.

### **Relationship between drinking water exposure and human serum levels**

Data from communities with contaminated drinking water indicate that ongoing human exposure to PFOA in drinking water increases serum levels, on average, by at least 100 times the drinking water concentration. A human clearance factor for PFOA of  $1.4 \times 10^{-4}$  L/kg/day was developed by USEPA researchers (Lorber and Egeghy, 2011) to relate serum PFOA concentration to administered dose. Assuming an average U.S. daily water consumption rate, the clearance factor predicts a serum:drinking water ratio of 114:1, consistent with the ratios that have been observed in exposed communities.

Continued exposure to even low drinking water concentrations results in substantially increased serum PFOA levels. Based on the clearance factor, each 10 ng/L in drinking water is predicted to increase serum PFOA by 1.1 ng/ml with an average water consumption rate, and 2.0 ng/ml with an upper percentile water consumption rate. These increases in serum PFOA from drinking water can be compared to the NHANES (2011-2012) geometric mean, 2.08 ng/ml, and 95<sup>th</sup> percentile, 5.68 ng/ml, serum PFOA concentrations. Increases in serum PFOA levels predicted from average and upper percentile drinking water consumption at various drinking water PFOA concentrations are shown in Figure E-1.

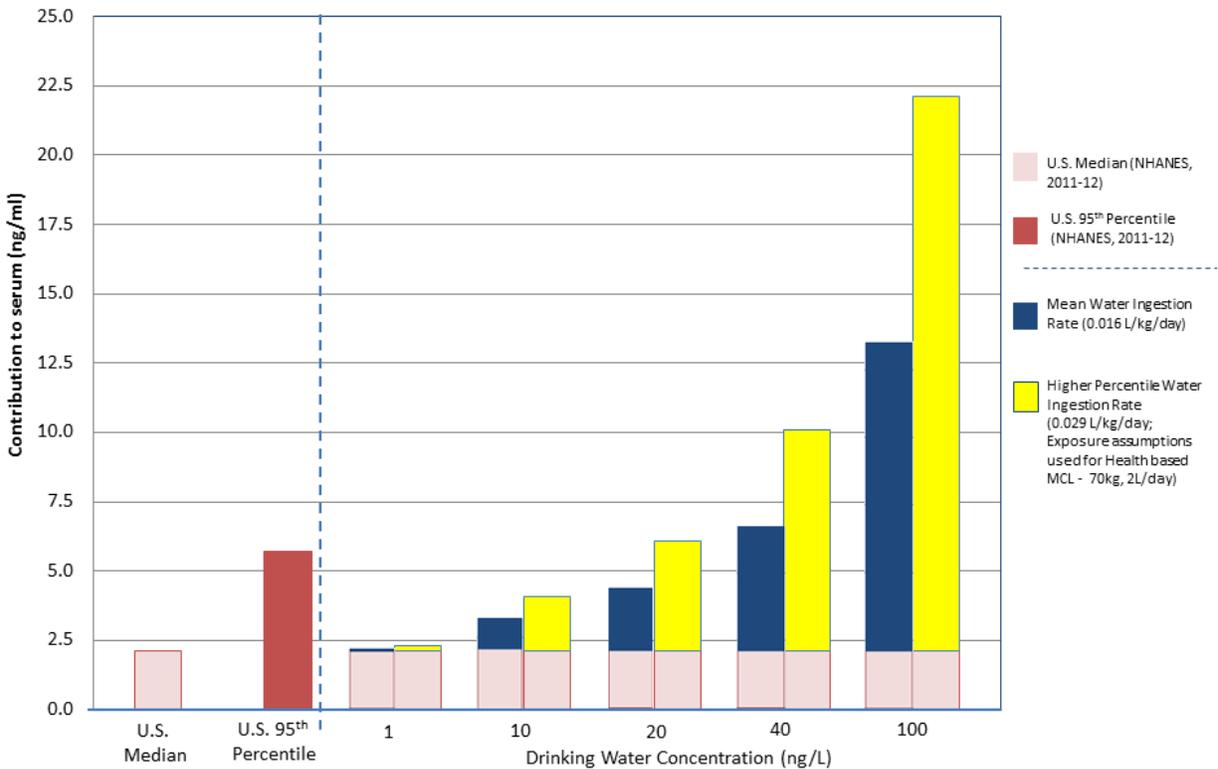


Figure E-1. Increases in serum PFOA concentrations predicted from mean and upper percentile consumption of drinking water with various concentrations of PFOA, as compared to U.S median and 95<sup>th</sup> percentile serum PFOA levels (NHANES, 2011-12).

### Exposures to infants

In humans, PFOA has been measured in amniotic fluid, maternal serum, umbilical cord blood, and breast milk. Serum PFOA concentrations in infants at birth are similar to those in maternal serum. Both breast-fed infants whose mothers ingest contaminated drinking water and infants fed with formula prepared with contaminated drinking water receive much greater exposures to PFOA than older individuals who consume drinking water with the same PFOA concentration. PFOA exposure in breast-fed infants is greatest during the first few months of life because both PFOA concentrations in breast milk and the rate of fluid consumption are highest then. As a result, serum PFOA concentrations in breast-fed infants increase several fold from levels at birth within the first few months of life (Figure E-2). Exposures to infants who consume formula prepared with contaminated water are also highest during this time period. While serum PFOA levels peak during the first year of life, they remain elevated for several years. These elevated exposures during infancy and early childhood are of particular concern because early life effects are sensitive endpoints for the toxicity of PFOA.

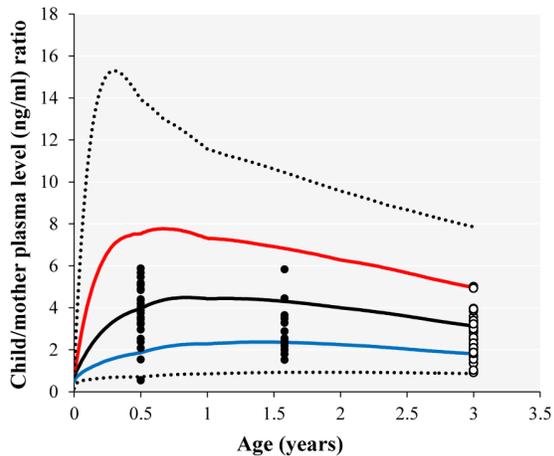


Figure E-2. From Verner et al. (2016a). Modeling simulation of the ratio of PFOA in blood plasma in breast fed infants/children to plasma concentration in mother. Black line - 50th percentile. Blue line - 5th percentile. Red line - 95th percentile. Dotted lines - minimum and maximum values.

### **Health Effects**

Because the scientific database related to health effects of PFOA is very large, evaluation was focused on specific endpoints from human and animal studies. Relevant studies were identified through literature searches of the PubMed database, from earlier evaluations of PFOA by the Health Effects Subcommittee, and through backwards searching.

### **Epidemiology**

The choice of endpoints selected for comprehensive review from epidemiology studies was largely based on knowledge gained from previous evaluations by the DWQI Health Effects Subcommittee. Health endpoints evaluated comprehensively were serum cholesterol/lipids, liver enzymes/bilirubin and liver disease, uric acid, thyroid function and thyroid disease, and antibody concentrations following vaccination. In total, 54 epidemiological studies were evaluated in depth, including studies from the general population, communities with drinking water exposures including most notably the C8 Health Study - a large study of about 70,000 Ohio and West Virginia residents exposed to a wide range of PFOA concentrations (>50 ng/L to over 3000 ng/L) in drinking water, and occupationally exposed workers. Recent comprehensive reviews by other authoritative scientific groups were evaluated for two additional critical endpoints, fetal growth following developmental exposure and cancer.

Of the endpoints that were evaluated comprehensively, the evidence for associations with PFOA was strongest for increases in serum levels of cholesterol, the liver enzyme ALT, and uric acid. PFOA was associated with clinically defined hypercholesterolemia in a community exposed through drinking water. The epidemiological evidence supports multiple criteria for a causal relationship between PFOA and both serum cholesterol and ALT. Notably, the steepest dose-response for associations with these endpoints was within the range of serum PFOA

concentrations found in the general population and communities with drinking water exposures, with a much flatter curve at higher serum concentrations.

For some other endpoints that were comprehensively reviewed, limited evidence of an association with PFOA was found. Although there is consistent evidence of decreased antibody concentrations following vaccination, most of the vaccine types were evaluated in only one or two studies and there is limited evidence of exposure-response. Other endpoints with limited evidence of an association include LDL, the liver enzymes GGT and AST, bilirubin, liver disease, and thyroid disease. There was limited or no evidence of association of PFOA with TSH and thyroid hormones, and no evidence for association with HDL or the liver enzyme ALP.

A systematic review using the Navigation Guide methodology concluded that there is “sufficient” human evidence, the strongest descriptor for strength of evidence, that developmental exposure to PFOA reduces fetal growth (e.g. birth weight) in humans (Johnson et al., 2015). It was concluded that the basis for this conclusion is reasonable and supportable. Maternal glomerular filtration rate (GFR) was evaluated as a potential confounding factor for this effect, and it was concluded that decreased GFR does not account for the major portion of the decrease in fetal growth associated with PFOA.

PFOA was associated with increased incidence of testicular and kidney cancer in communities with drinking water exposure. These studies accounted for smoking history and other relevant factors. The USEPA SAB (2006) described PFOA as “likely to be carcinogenic to humans.” based on the criteria provided in USEPA (2005b) cancer risk assessment guidance. More recently, IARC (2016) concluded that PFOA is possibly carcinogenic to humans, and the USEPA Office of Water (2016a) described it as having suggestive evidence of carcinogenic potential.

Although the magnitude of change for some of the parameters associated with PFOA was relatively small, they are of public health concern because population-level changes of this magnitude will result in a shift in the overall distribution of values such that the number of individuals with clinically abnormal values is increased. Additionally, small changes in a clinical biomarker may be an indicator of other effects that were not evaluated. For example, relatively small decreases in birth weight may be an indication of changes in other more subtle developmental parameters which were not assessed.

In summary, associations of PFOA with numerous health endpoints have been found in human populations with evidence supporting criteria for causality for some endpoints. These health endpoints include non-carcinogenic effects in the general population, and both non-carcinogenic effects and cancer in communities with drinking water exposure. The epidemiologic data for PFOA are notable because of the consistency between results among human epidemiologic studies in different populations, the concordance with toxicological findings from experimental animals, the use of serum concentrations as a measure of internal exposure, the potential clinical

importance of the endpoints for which associations are observed, and the observation of associations within the exposure range of the general population. These features of the epidemiologic data distinguish PFOA from most other organic drinking water contaminants and justify concerns about exposures to PFOA through drinking water. Although there is evidence to support causality for some epidemiological endpoints, the human data have limitations and therefore are not used as the quantitative basis for the ISGWQC. Instead, the potential ISGWQCs developed below are based on sensitive and well-established animal toxicology endpoints that are considered relevant to humans based on mode of action data.

### Toxicology

The toxicological database for PFOA includes evaluation of numerous effects in non-human primates and rodents. The review for development of the ISGWQC focused on endpoints that were identified as sensitive and potentially appropriate for use in risk assessment. The effects selected for detailed review were hepatic toxicity, developmental effects, immune system toxicity, and carcinogenicity. As discussed above, effects relevant to these endpoints have been associated with PFOA in human epidemiological studies. Additionally, information is presented on general toxicity in non-human primates, as well as thyroid, neurobehavioral, and male reproductive effects.

The non-human primate studies have limitations that preclude their consideration as the basis for risk assessment. These include very small numbers of animals, severe toxicity at the lowest dose, loss of animals during the study due to toxicity and/or mortality, and lack of dose-response for key endpoints (e.g. increased liver weight).

Increased liver weight is a sensitive toxicological endpoint for PFOA which has been observed in many studies in both non-human primates and rodents. Increased liver weight can co-occur with and/or progress to more severe hepatic effects including hepatocellular necrosis, fatty liver, increased serum liver enzymes, and hyperplastic nodules. Recent studies show that developmental exposure to low doses of PFOA in mice causes cellular changes indicative of liver toxicity that persist until adulthood.

Reproductive or developmental effects of PFOA have not been studied in non-human primates. The mouse is an appropriate species for evaluating effects on reproduction and development since the female mouse excretes PFOA slowly, as do humans. In contrast, rats and rabbits are not ideal models for studying these effects because they excrete PFOA very quickly, with a half-life of a few hours. Effects from developmental exposures in mice include full litter resorptions, decreased postnatal survival and growth, delayed development, accelerated sexual maturation in males, persistent liver toxicity (noted above), and delayed mammary gland development. PFOA also causes reproductive toxicity in male mice.

Delayed mammary gland development and persistent liver toxicity after perinatal (prenatal and/or neonatal) exposure are sensitive endpoints which occur in mice at lower doses of PFOA than other developmental effects. Delayed mammary gland development has been reported in nine separate studies presented in five publications, while only one study which has several general problematic issues did not find this effect. Gestational and/or lactational exposures to PFOA caused delayed mammary gland development in pregnant dams and/or female offspring in two strains of mice. Histological changes in the mammary gland of exposed offspring occurred in a dose-related fashion, persisted until adulthood, and were considered permanent. However, available toxicological information is not sufficient to make conclusions about the effects of PFOA on lactational function. Maternal PFOA exposure has been associated with shorter duration of breastfeeding in humans, and there is no information indicating that the histological changes observed in mice are not relevant to humans.

Additional studies evaluated effects of peripubertal (around the time of puberty) exposure on mammary gland development in mice. These studies cannot be directly compared to studies of perinatal exposure because effects on mammary gland development differ depending on the lifestage when exposure occurs. Additionally, interpretation of the peripubertal studies is problematic because each PFOA dose level was used in only one study in each of the strains of mice evaluated, such that dose-response interpretations can only be made by combining data from different studies.

PFOA suppressed the immune system in studies of rhesus monkeys and mice. Decreased bone marrow cellularity and lymphoid atrophy occurred in monkeys, while effects in mice included decreased spleen and thymus weights, decreased thymocyte and splenocyte counts, decreased immunoglobulin response, and changes in total numbers and/or specific populations of lymphocytes. Immune system effects were not observed in two rat studies which included doses higher than those which generally caused these effects in mice.

Review of the toxicological data indicates that increased liver weight is an endpoint that is as sensitive or more sensitive than immune system toxicity or reproductive/developmental effects, with the exception of delayed mammary gland development.

PFOA caused tumors of the liver, pancreatic acinar cells, and testicular Leydig cells in male rats. Since PFOA is rapidly excreted by female rats, chronic studies in another species in which PFOA is persistent in both sexes, such as the mouse, would provide important information specific to females. A recent study suggests that prenatal exposure to PFOA in mice caused an increased incidence of liver tumors. However, this study was not designed as a carcinogenicity bioassay and does not provide definitive information on this issue. Additional research on carcinogenicity later in life after developmental exposures to PFOA is needed.

## **Mode of Action**

The mode(s) of action of PFOA have not been fully characterized. Based on the information reviewed in this report, the toxicological effects of PFOA are generally considered relevant to humans for the purposes of risk assessment.

PFOA is not chemically reactive. Thus, it is not metabolized to reactive intermediates and does not covalently bind to nucleic acids and proteins. Consistent with these properties, available data indicate that it is not genotoxic.

### **Activation of nuclear receptors and role of PPAR-alpha**

Effects of PFOA occur through multiple modes of action including activation of receptors that control the expression of genes involved in many biological pathways. Much attention has been focused on the potential human relevance of effects that occur through activation of the nuclear receptor, peroxisome proliferator-activated receptor-alpha (PPAR-alpha). This question arises because many PPAR-alpha activating compounds cause rodent liver tumors; the human relevance of these tumors is subject to debate due to lower levels and/or differences in intrinsic activity of PPAR-alpha in human liver. However, the uncertainty about human relevance does not necessarily apply to PPAR-alpha mediated effects other than liver tumors. Both human and mouse PPAR-alpha are activated by PFOA *in vitro*, and the results do not clearly indicate that human PPAR-alpha is less sensitive than rodent PPAR-alpha in these *in vitro* systems.

### **Hepatic effects**

Studies of non-human primates, standard strains of rats and mice, PPAR-alpha null mice, and humanized PPAR-alpha mice support the conclusion that hepatic effects of PFOA are relevant to humans for the purposes of risk assessment. As noted above, PFOA is associated with increased liver enzymes in human epidemiological studies.

In a subchronic study of cynomolgus monkeys, a species in which human relevance of hepatic effects is not in question, PFOA caused increased liver weight and peroxisomal proliferating activity similar in magnitude to that seen in rats, demonstrating that hepatic PPAR-alpha activity in response to PFOA is not limited to rodents. In this study, several animals exhibited notably increased liver weight, highly elevated serum liver enzymes, and/or severe hepatic toxicity.

Observations in standard strains of laboratory rodents indicate that PFOA causes PPAR-alpha independent hepatic effects in rodents with normal PPAR-alpha function. In these strains, increased relative liver weight caused by PFOA did not directly correspond with hepatic peroxisome proliferating activity. Additionally, PFOA caused fatty liver in these standard strains, although PPAR-alpha activation decreases hepatic lipids. Finally, developmental exposure to PFOA caused abnormal mitochondria in livers of a standard mouse strain, with no evidence of peroxisome proliferation.

PFOA caused decreased serum lipids, typically associated with PPAR-alpha activation, in rodents, while increased serum lipids are associated with PFOA exposure in humans. Recent studies suggest that these differences may be related to the low fat diet generally used in laboratory rodent studies versus the higher fat content of a typical Westernized human diet, rather than solely to interspecies differences.

Studies comparing wild type (with normal PPAR-alpha) and PPAR-alpha null (lacking PPAR-alpha) mice provide further evidence that hepatic effects occur through both PPAR-alpha dependent and independent pathways. PFOA caused similar increases in liver weight in wild type and PPAR-alpha null strains. Increased liver enzymes and histopathological changes, particularly damage to the bile duct, also occurred in PFOA-treated PPAR-alpha null mice. Additionally, developmental exposures to PPAR-alpha null mice caused persistent histopathological changes in the liver.

Studies of strains of mice which express human PPAR-alpha in the liver (humanized PPAR-alpha mice) indicate that PFOA causes hepatic effects through activation of human PPAR-alpha. In humanized PPAR-alpha mice, PFOA caused increased liver weight similar to that in wild type mice, activation of hepatic genes associated with PPAR-alpha, and histopathological changes in the liver. Fetal liver weight was increased similarly in wild type and humanized PPAR-alpha mice after *in utero* exposure, and expression of genes associated with PPAR-alpha in fetal liver was increased to a greater degree in humanized PPAR-alpha mice than in wild type mice.

#### Immune system effects

PFOA suppresses the immune system in both non-human primates and mice. As noted above, decreased response to vaccinations has been associated with PFOA in human epidemiological studies. Data from mouse studies indicate that these effects on the immune system occur through both PPAR-alpha dependent and independent modes of action. Both PPAR-alpha dependent and independent effects on the immune system are considered relevant to humans for the purposes of risk assessment.

#### Developmental and reproductive effects

As noted above, decreased fetal growth is associated with PFOA in human epidemiological studies. Developmental effects of PFOA in rodents appear to occur primarily through PPAR-alpha dependent mechanisms, while some reproductive effects such as full litter resorptions appear to be PPAR-alpha independent. PPAR-alpha and other PPARs are present in human fetal tissues and are expected to have important roles in reproduction and development. Therefore, PPAR-alpha mediated effects of PFOA on development are considered relevant to humans for the purposes of risk assessment. Toxicity to the placenta may play a role in PFOA's developmental effects such as fetal growth retardation; more research is needed on this question.

Delayed mammary gland development after developmental exposure is a sensitive endpoint for PFOA toxicity in mice. The rodent is considered a good model for human mammary gland development, and there is no mode of action evidence suggesting that the effects of PFOA on this endpoint are not relevant to humans.

PFOA also causes male reproductive toxicity in mice, and there is no mode of action information to suggest that these effects are not relevant to humans.

#### Carcinogenicity

As noted above, PFOA has been associated with increased incidence of kidney and testicular cancer in communities exposed through drinking water after adjustment for smoking and other relevant factors. The USEPA Science Advisory Board (2006) concluded that the liver tumors caused by PFOA in rats are potentially relevant to humans, based on similarities in hepatic effects of PFOA in monkeys and rodents and the limited evidence available at the time on hepatic effects of PFOA in PPAR-alpha null mice. Subsequent studies in PPAR-alpha null mice have provided substantial additional relevant data. Importantly, hepatic cell proliferation, a causal event for tumor formation, is increased similarly by PFOA in wild type and PPAR-alpha null mice. Although a carcinogenicity bioassay of PFOA has not been conducted in PPAR-alpha null mice, a recent study suggests that developmental exposures to PFOA may cause hepatic tumors in adulthood in this strain. Finally, studies in rainbow trout, a species used as a model for human liver cancer because it lacks PPAR-alpha, suggest that PFOA causes liver tumors through an estrogenic mode of action.

The mode of action for the testicular and pancreatic tumors caused by PFOA in rats has not been established. Therefore, they are considered relevant to humans for the purposes of risk assessment.

#### Additional modes of action

A number of other modes of action for PFOA have been suggested including effects on intercellular gap junction communication, effects on mitochondria, changes in expression of microRNAs (miRNAs), and effects related to transporter proteins such as organic anion transporters (OATs) and multidrug resistance-associated proteins (MRPs).

#### **Development of ISGWQC**

ISGWQC are intended to be protective for chronic (lifetime) exposure through drinking water. They are based on a one in one million lifetime cancer risk level for carcinogens and no adverse effects from lifetime ingestion for non-carcinogens. PFOA was described as “likely to be carcinogenic to humans” by the USEPA Science Advisory Board and “possibly carcinogenic to humans” by the International Agency for Research on Cancer (IARC), and as having “suggestive evidence of carcinogenic potential” by the USEPA Office of Water. As such both non-

carcinogenic and carcinogenic effects were evaluated using approaches consistent with USEPA risk assessments guidance and previous risk assessments developed by NJDEP.

Both the human epidemiology data and the animal toxicology data were considered as part of the overall weight of evidence for the potential human health effects of PFOA. As discussed above, PFOA is associated with non-carcinogenic effects in the general population, and with both non-carcinogenic effects and cancer in communities with drinking water exposure. Although the data for some endpoints support multiple criteria for causality, the human epidemiology data have limitations and are therefore not used as the quantitative basis for the ISGWQC. As such, the ISGWQC is based on sensitive and well established animal toxicology endpoints that are considered relevant to humans. Notwithstanding, the human data suggest that continued human exposure to even relatively low concentrations of PFOA in drinking water results in elevated body burdens that increase the risk of health effects, indicating a need for caution about exposures from drinking water. Therefore, the human epidemiological data support the use of a public health-protective approach in developing a ISGWQC based on animal toxicology data.

#### ISGWQC based on non-carcinogenic effects

Delayed mammary gland development and increased relative liver weight were identified as the most sensitive non-carcinogenic endpoints with data appropriate for dose-response modeling, and it was concluded that these endpoints are relevant to humans for the purposes of risk assessment. Benchmark dose (BMD) modeling of serum PFOA data from toxicological studies was performed to determine the BMDLs (lower 95% confidence limit on the doses corresponding to a minimal response) for the serum concentrations that are used as the points of departure (PODs) for these endpoints. Only studies that provide serum PFOA data were considered for dose-response modeling for these effects, since measured serum levels are associated with less uncertainty than serum level estimates from pharmacokinetic modeling or interspecies extrapolations based on half-life differences.

#### Effects on mammary gland development

Delayed mammary gland development is the most sensitive systemic endpoint with data appropriate for dose-response modeling, and a Reference Dose (RfD) was developed for this endpoint. It is believed that this endpoint has not previously been used as the primary basis for health-based drinking water concentrations or other human health criteria. Because the use of delayed mammary gland development as the basis for quantitative risk assessment is a currently developing topic, an ISGWQC with this RfD as its primary basis was not recommended. However, it was concluded that an additional uncertainty factor (UF) should be incorporated into the RfD based on increased liver weight (the endpoint used as the basis for the ISGWQC - see below) to protect for mammary gland effects, persistent liver toxicity, and other effects from developmental exposures at doses far below those that cause increased relative liver weight.

A study of exposure to pregnant mice on days 10-17 of gestation (Macon et al., 2011) is the only developmental exposure study of mammary gland development that provides serum PFOA data appropriate for dose-response modeling. Of the multiple time points assessed in this study, delays in mammary gland development were most evident on postnatal day (PND 21). Of the several endpoints related to mammary gland development that were evaluated, decreases in mammary gland developmental score and number of terminal end buds were selected for dose-response modeling because they showed a statistically significant dose-related decrease at PND 21. BMD modeling was based on serum levels at PND 1, since they were higher at this time than at later time points. The serum concentration BMDLs for a 10% change in decreased developmental score and decreased number of terminal end buds were 24.9 and 22.9 ng/ml, respectively.

A total UF of 30, including UFs of 10 for intra-human variability and 3 for animal-to-human toxicodynamic differences, was applied to the serum level BMDL for decreased number of terminal end buds, 22.9 ng/ml, to derive a Target Human Serum Level of 0.8 ng/ml. The typical UF of 3 for toxicokinetic variability between species is not included because the risk assessment is based on comparison of internal dose (serum levels) rather than administered dose. The Target Human Serum Level is analogous to a RfD in terms of internal, rather than administered, dose. This Target Human Serum Level for delayed mammary gland development of 0.8 ng/ml is below the median serum PFOA level in the U.S. general population (2.1 ng/ml). The clearance factor mentioned above,  $1.4 \times 10^{-4}$  L/kg/day, was applied to the Target Human Serum Level, 0.8 ng/ml, to calculate an RfD of 0.11 ng/kg/day.

#### Hepatic effects

Increased relative liver weight is a well-established effect of PFOA which is more sensitive than most other toxicological effects such as immune system toxicity and most reproductive/developmental effects (Table 12 of Animal Toxicology section). The ISGWQC for non-carcinogenic effects is based on this endpoint.

A study of male mice exposed to branched/linear PFOA for 14 days (Loveless et al., 2006) that showed a dose-related increase in relative liver weight was selected for dose-response modeling. This isomeric mixture is relevant to environmental contamination and human exposure, and it was used in almost all toxicological studies of PFOA. Because review of studies of increased relative liver weight indicated that the magnitude of this effect does not increase with exposure durations longer than 14 days, this study was considered to be of sufficient duration for use as the basis for an ISGWQC. BMD modeling of the serum PFOA data from the study determined a serum level BMDL for a 10% increase in relative liver weight of 4350 ng/ml.

A total UF of 300 was applied to the serum level BMDL of 4350 ng/ml to derive a Target Human Serum Level of 14.5 ng/ml. This UF includes UFs of 10 for intra-human variability, 3

for animal-to-human toxicodynamic differences, and 10 to protect more sensitive toxicological effects. These more sensitive effects, including delayed mammary gland development and hepatic toxicity after developmental exposures, occurred at doses 100-fold lower than the Lowest Observed Adverse Effect Level (LOAEL) for increased liver weight. Although the study duration was only 14 days and the ISGWQC is intended to protect for chronic exposure, a UF for less-than-chronic duration of exposure was not applied because increased liver weight does not appear to increase in magnitude when exposures continue beyond two weeks. The clearance factor mentioned above,  $1.4 \times 10^{-4}$  L/kg/day, was applied to the Target Human Serum Level, 14.5 ng/ml, to calculate an RfD of 2 ng/kg/day.

#### Relative Source Contribution factor

A Relative Source Contribution (RSC) factor that accounts for non-drinking water sources including food, soil, air, water, and consumer products is used by USEPA, NJDEP, and the DWQI in the development of health-based drinking water concentrations based on non-carcinogenic effects. The default value for the RSC is 20%, meaning that 20% of total exposure is assumed to come from drinking water and 80% from non-drinking water sources. If supported by available data, a higher chemical-specific value (up to 80%) can be used (i.e. the percent exposure from non-drinking water sources is lower than the default assumption of 80%). It was concluded that there are insufficient data to develop a chemical-specific RSC for PFOA. USEPA UCMR3 monitoring shows that PFOA occurs (at concentrations greater than 20 ng/L) more frequently in PWS located throughout New Jersey (10.5%) than nationwide (1.9%).

There are no New Jersey-specific biomonitoring data for PFOA, and the more frequent occurrence in NJ PWS suggests that New Jersey residents may also have higher exposures from non-drinking sources, such as contaminated soils, house dust, or other environmental media, than the U.S. general population. Additionally, the default RSC of 20%, while not explicitly intended for this purpose, also partially accounts for the greater exposures to infants who are breast-fed or consume formula prepared with contaminated drinking water, as compared to older individuals. These higher exposures during infancy must be considered because short term exposures to infants are relevant to the effects of concern (delayed mammary gland development and increased relative liver weight). Therefore, the default RSC of 20% was used to develop the ISGWQC.

#### ISGWQC based on non-carcinogenic effects

The health-based water concentration protective for increased liver weight, based on the RfD of 2 ng/kg/day, standard drinking water exposure assumptions (2 L/day water consumption; 70 kg body weight), and a 20% RSC is 14 ng/L.

#### ISGWQC based on carcinogenic effects

Testicular tumor data from the chronic dietary exposure rat study (Butenhoff et al., 2012) are the only tumor data appropriate for dose-response modeling and were used to develop a cancer potency factor. The BMDL for 5% tumor incidence is 2.36 mg/kg/day, and the corresponding cancer potency factor is  $0.021 \text{ (mg/kg/day)}^{-1}$ . The dose in rats corresponding to a  $1 \times 10^{-6}$  risk level,  $4.8 \times 10^{-5}$  mg/kg/day, was converted to the human equivalent dose of  $4 \times 10^{-7}$  mg/kg/day (0.4 ng/kg/day) using a pharmacokinetic adjustment based on the ratio of half-lives in the two species. Using default drinking water assumptions (2 L/day water consumption; 70 kg body weight), the health-based water concentration at the  $1 \times 10^{-6}$  lifetime cancer risk level is 14 ng/L. This value is identical to the health-based water concentration based on non-cancer endpoints developed above.

Since ISGWQC are rounded to one significant figure, the ISGWQC is 10 ng/L.

#### Potential for additive toxicity with other PFCs

Available information indicates that the target organs and modes of action are generally similar for PFOA and some other PFCs, such as PFNA. Therefore, the toxicity of PFOA and other PFCs may be additive. Although PFOA and other PFCs, including PFNA, are known to co-occur in NJ ground water, the potential for additive toxicity of PFOA and other PFCs was not considered in development of the ISGWQC.

**The ISGWQC is 10 ng/L (0.01 µg/L).**

## **INTRODUCTION**

### **Development of ISGWQC by NJDEP**

Development of an ISGWQC for perfluorooctanoic acid (PFOA, C8) was requested of the New Jersey Department of Environmental Protection (NJDEP) Division of Science, Research and Environmental Health by the NJDEP Site Remediation Program under N.J.A.C 7:9C.

Interim specific ground water quality criteria are intended to be protective from lifetime cancer risk at the one in one million ( $10^{-6}$ ) risk level and from any adverse non-cancer effects resulting from chronic (lifetime) exposure.

### **Document development process**

The ISGWC is based primarily on an evaluation of PFOA by the Health Effects Subcommittee of the DWQI. The information in this document is very similar to that in the DWQI Health-Based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA) (DWQI, 2017). Text has been revised by the New Jersey Department of Environmental Protection to describe the development of the ISGWC.

The evaluation of PFOA began by formulating an approach for the review of information and document development. Because the scientific database related to health effects of PFOA is very large, it was decided to focus on specific endpoints from human and animal studies for hazard identification and/or dose-response. Criteria for selection of the human and animal endpoints that were reviewed in depth are discussed in the Epidemiology and Toxicology sections.

A literature search of PubMed databases through April 2015 was conducted using relevant search terms which are provided in Appendix 1. The U.S. National Library of Medicine's Toxline database was searched using similar keywords as the PubMed search strings. The Toxline search yielded a significant number of non-peer reviewed literature including articles, policy papers, and grant proposals, and ultimately Toxline did not identify additional peer-reviewed literature for inclusion in the review. Studies evaluated also included relevant citations from the earlier DWQI Health Effects Subcommittee evaluations of PFOA, as well as backward searching. PubMed is also searched on a monthly basis, and an ongoing title review of these searches was conducted to identify any additional studies for inclusion.

The original PubMed search identified 2,016 references. All of these references were screened by title, abstract and/or full text. Title and abstract review was used to sort studies into inclusion categories for consideration for detailed evaluation related to hazard identification and/or dose-response evaluation using EndNote (Appendix 1). Studies were excluded if they were "Unrelated" which includes those studies which either did not assess PFOA, were proposals or reviews, or if they were "Non-Health" which includes studies of analytical methods, environmental occurrence, sources of human and wildlife exposure, and other topics unrelated to

health effects. Some studies categorized as “Non-Health” are cited in relevant sections of the document, as appropriate. Remaining studies were identified as either “in vitro”, “Experimental Animal”, or “Human”. Further study inclusion categories are described in more detail in the Epidemiology and Toxicology sections. The numbers of records retrieved, and numbers of studies sorted into inclusion/exclusion categories are also provided in Appendix 1. Following study inclusion identification, data were extracted from included studies into individual study tables and/or summary tables, as described in the Epidemiology and Toxicology sections. Individual study tables for the Epidemiology section are provided in Appendix 4 and for the Toxicology section in Appendix 5.

Some sections of the document that provide background information but do not impact development of the ISGWQC (e.g. Environmental Sources, Fate, and Occurrence) are based on updates of the Subcommittee’s previous evaluation of PFOA in 2009-10 and a comprehensive review of PFOA as an emerging drinking water that was published in 2012 (Post et al., 2012).

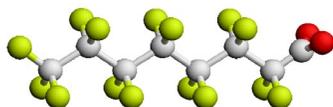
In 2014, NJDEP posted a request for public input regarding data or technical information about the toxicology, epidemiology, toxicokinetics, or other health effects topics related to PFOA that should be considered in evaluation of PFOA. One submission on PFOA was received, and relevant comments from this submission were considered.

## **BACKGROUND INFORMATION**

PFOA is a member of a class of anthropogenic chemicals called perfluorinated chemicals (PFCs) with structures consisting of a totally fluorinated carbon chain of varying length and a charged functional group, such as carboxylate or sulfonate (Lindstrom et al., 2011a). PFCs are members of a larger class of compounds, poly- and perfluoroalkyl substances (PFAS) which also includes fluorinated compounds with structures that differ from PFCs (Buck et al., 2011). The eight-carbon PFCs, PFOA and PFOS, were the most intensively investigated compounds in earlier studies, while current research focuses on a wider range of PFAS.

### **Physical and Chemical Properties (PubChem, 2016)**

Chemical Name:	Perfluorooctanoic acid
Synonyms:	PFOA, C8
CAS #:	335-67-1
Chemical Formula:	C <sub>8</sub> HF <sub>15</sub> O <sub>2</sub>
Chemical Structure:	CF <sub>3</sub> (CF <sub>2</sub> ) <sub>6</sub> COOH



Molecular Weight:	414.07
-------------------	--------

Physical State:	Solid
Melting Point:	54.3 °C
Boiling Point:	189 – 192.4 °C
Vapor Pressure:	0.017 mm Hg at 20 °C
Density:	1.8 g/cm <sup>3</sup> at 20 °C
Water Solubility:	9.5 g/L at 25 °C
Log octanol/water partition coefficient:	Not applicable (see below).
Taste Threshold (water):	No data
Odor Threshold (water):	No data
Odor Threshold (air):	No data

PFOA is a fully fluorinated carboxylic acid. Because carbon-fluorine bonds are among the strongest found in organic chemistry due to fluorine's electronegativity, PFOA and other PFCs are extremely stable and resistant to chemical reactions. PFOA is resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis, and is not known to degrade in the environment (Vaalgamaa et al., 2011).

PFOA contains a long perfluorocarbon tail that is both hydrophobic and oleophobic (repels both water and oil) and a charged end (the carboxylate group) that is hydrophilic. Because it forms a separate layer when mixed with hydrocarbons and water, measurement of the octanol:water partition coefficient is not practical (Prevedouros et al., 2006).

PFOA has been manufactured as salts such as ammonium perfluorooctanoate (APFO) or sodium perfluorooctanoate (NaPFOA) which dissociate in water. The  $PK_a$  of PFOA is 2.8. At the pH range found in drinking water (6.5-8.5) and within the body, PFOA is present almost totally in the non-volatile anionic form, the perfluorooctanoate anion (Goss, 2008; Rayne and Forest, 2010).

### **Production and Use**

PFOA and other PFCs have been produced for use in commercial products and in industrial processes for over 60 years. Because of their unique surfactant properties and their resistance to chemical and thermal degradation, they have been used in many applications including water-, soil-, and stain-resistant coating for fabrics used in clothing, upholstery, and carpets, oil-resistant coatings for food contact paper, aviation hydraulic fluids, fire-fighting foams, paints, adhesives, waxes, and polishes, and other products. They are used industrially as surfactants, emulsifiers, wetting agents, additives, and coatings. PFOA is used as a processing aid (emulsifier) in the production of fluoropolymers such as polytetrafluoroethylene (PTFE) and fluoroelastomers used as non-stick coatings on cookware, membranes for waterproof/breathable clothing, electrical

wire casing, fire and chemical resistant tubing, and plumbing thread seal tape (Lau et al., 2007; Buck et al., 2011; Lindstrom et al., 2011a; Post et al., 2012).

PFOA has been produced by two different manufacturing methods, electrochemical fluorination (ECF) and telomerization. The ECF process was primarily used from 1947 to 2002. In this process, 1-heptanecarbonyl fluoride is dissolved in anhydrous hydrogen fluoride, and an electrical current is passed through the solution causing all hydrogen atoms on the carbon backbone to be replaced with fluorine atoms. This process produces a mixture of isomeric forms including branched, linear, and cyclic isomers of various chain lengths (Prevedouros et al., 2006; Buck et al, 2011; Lindstrom et al., 2011a).

The second process, telomerization, has been primarily used since 2002. This process involves reacting pentafluoroiodoethane with tetrafluoroethane in the molar ratio that gives the desired chain length. The product of this reaction is then oxidized to form the carboxylic acid. This process produces straight chain (linear) PFOA (Prevedouros et al., 2006; Buck et al, 2011; Lindstrom et al., 2011a).

Historically, PFOA and PFOS were the two PFCs produced in the greatest amounts. PFOS was principally manufactured by the 3M Company, which completed its phase-out of production of this chemical in 2002. In 2006, the eight major U.S. producers of PFOA voluntarily agreed to reduce emissions and product content of PFOA and related substances, including precursors of PFOA, on a global basis by 95% by 2010 and to work towards elimination of these substances by 2015 (USEPA, 2016b). According to USEPA, reports submitted by the participating companies in 2013 and 2014 indicated that they were on track to achieve the goal of phasing out these chemicals by the end of 2015. However, other manufacturers and users of PFOA that are not participants in the voluntary agreement with USEPA continue to emit large amounts of PFOA to the environment, particularly in nations overseas including China, India, Russia, and Poland (USEPA, 2009; Lindstrom et al., 2011; OECD, 2015).

In 2009, the USEPA Office of Pollution Prevention and Toxics (OPPT) developed action plans for several groups of chemicals of concern including PFCs (USEPA, 2009a). According to the USEPA Action Plan, concerns about PFOA and other PFCs include their worldwide presence in the environment, wildlife, and humans; their persistence in the environment and bioaccumulative potential in humans and wildlife; and the significant adverse effects observed in wildlife and laboratory animals. USEPA stated that “given the long half-life of these chemicals in humans (years), it can reasonably be anticipated that continued exposure could increase body burdens to levels that would result in adverse outcomes.”

USEPA (2009a) stated that PFOA and other long-chain PFCs are of concern for children’s health, based on studies in laboratory animals that have demonstrated developmental toxicity,

including neonatal mortality. They stated that: “Children’s exposures are greater than adults due to increased intakes of food, water, and air per pound of body weight, as well as child-specific exposure pathways such as breast milk consumption, mouthing and ingestion of non-food items, and increased contact with the floor. Biomonitoring studies have found PFCs in cord blood and breast milk, and have reported that children have higher levels of some PFCs compared to adults. Thus, given the pervasive exposure to PFCs, the persistence of PFCs in the environment, and studies finding deleterious health effects, USEPA will examine the potential risks to fetuses and children.”

USEPA (2009a) stated that it intended to propose actions to address the potential risks from long-chain PFCs in 2012 under the Toxic Substances Control Act (TSCA). USEPA stated that potential actions could include banning or restricting their manufacture (including import), processing, and use, depending on the findings of more detailed analysis of information on these compounds.

In 2013, the European Chemical Agency (ECHA) Member State Committee unanimously agreed that PFOA should be classified as a Substance of Very High Concern (SVHC) because it has potential to cause reproductive toxicity and is persistent, bioaccumulative, and toxic (ECHA, 2013). ECHA (2015) is currently considering restrictions on the manufacture, marketing and use of PFOA, its salts and PFOA-related substances, as well as of articles and mixtures containing these substances.

## **GUIDANCE AND STANDARDS DEVELOPED BY NEW JERSEY, OTHER STATES, AND USEPA**

### **New Jersey Health-based Drinking Water Guidance**

New Jersey DEP developed chronic (lifetime) drinking water guidance for PFOA in drinking water of 40 ng/L in 2007 (NJDEP, 2007). The basis for the NJDEP guidance was subsequently published in a peer-reviewed journal (Post et al., 2009a).

The New Jersey guidance is based on the NOAELs (No Observed Adverse Effects Levels) and LOAELs from toxicology studies identified in the draft USEPA (2005a) PFOA risk assessment and considered the conclusions of the USEPA Science Advisory Board (2006) review of this draft risk assessment. The draft USEPA (2005a) risk assessment compared PFOA exposures prevalent within the U.S. general population with NOAELs and LOAELs for various life stages identified in toxicology studies. As such, the USEPA (2005a) draft risk assessment did not develop a Reference Dose or a cancer slope factor for PFOA, and it did not address the relationship between drinking water concentration and human body burden, as measured by serum level.

Because the half-life of PFOA is much longer in humans (several years) than in the animal species used in the toxicological studies (several hours to 30 days), a given external dose (mg/kg/day) results in a much greater internal dose (as indicated by serum level) in humans than in animals. Therefore, comparisons between effect levels in animal studies and human exposures were made on the basis of serum levels rather than external dose. This approach was recommended by USEPA (2005a) and the USEPA Science Advisory Board (2006).

Target Human Serum Levels (analogous to RfDs, but on a serum level basis) were derived by applying UFs to the measured or modeled serum levels at the NOAELs or LOAELs identified by USEPA (2005a). The default RSC of 20% was applied to the Target Human Serum Levels to account for contributions to serum PFOA from non-drinking water exposures. The default RSC value is used when the relative contributions of drinking water versus non-drinking water sources are not fully characterized, as is the case for PFOA.

USEPA (2005a) classified PFOA as having “suggestive evidence of carcinogenic potential”, whereas the USEPA Science Advisory Board (2006) disagreed and recommended a classification of “likely to be carcinogenic to humans”. For the cancer end point, the serum level resulting in a one in one million ( $10^{-6}$ ) risk level was estimated by linear extrapolation from the modeled serum level in animals at a dose resulting in an approximate 10% tumor incidence.

The mean ratio of approximately 100:1 between serum PFOA levels and drinking PFOA water concentrations in exposed communities was used to determine the drinking water concentrations that are expected to result in a given increase in serum PFOA level (Post et al., 2009a). Data supporting a ratio of 100:1 or greater is discussed in the Toxicokinetics section below. Because this approach is based on the observed relationship between serum and drinking water concentrations, assumptions for body weight, volume of water ingested daily, or half-life of PFOA in humans or experimental animals were not explicitly used in the calculation of the health-based drinking water concentrations.

The range of health-based drinking water concentrations for the seven endpoints assessed was 0.04-0.26  $\mu\text{g/L}$ , and several of the concentrations fell within a similar range (0.04, 0.05, 0.06, 0.07, and 0.08  $\mu\text{g/L}$ ). The most sensitive endpoints, resulting in a drinking water concentration of 40 ng/L, were decreased body weight and hematological effects in the adult female rat in a chronic dietary study (Sibinski, 1987). This value was determined to be protective for carcinogenic effects, as the drinking water concentration at the  $10^{-6}$  cancer risk level was estimated as 60 ng/L.

It should be noted that a large body of health effects information, including toxicology studies reporting sensitive developmental effects in mice and epidemiology studies reporting associations of PFOA with numerous health effects, has become available subsequent to the

USEPA (2005a) risk assessment that served as the basis for the New Jersey guidance. These data were therefore not considered in the development of the NJDEP (2007) guidance, and they are considered in the development of the ISGWQC presented in this document.

### **USEPA Drinking Water Health Advisory**

In May 2016, the USEPA Office of Water finalized a drinking water Health Advisory for PFOA of 70 ng/L (USEPA, 2016a). This Health Advisory is intended to apply to both lifetime exposure and short-term exposure. It replaces the earlier the USEPA Office of Water (2009b) Provisional Health Advisory for PFOA of 400 ng/L, developed in 2009, which was stated to be intended to protect for “short-term exposure” (defined by the USEPA Integrated Risk Information System (IRIS) as up to 30 days; USEPA, 2011a).

USEPA (2016c) also finalized a Health Advisory for PFOS of 70 ng/L, and USEPA (2016d) states that the total concentration of PFOA and PFOS in drinking water should not exceed 70 ng/L.

A detailed discussion of the basis for the USEPA (2016a) Health Advisory for PFOA and a comparison with the NJDEP ISGWQC are provided in Appendix 2. In summary, the USEPA Health Advisory is based on a Reference Dose (RfD) of 20 ng/kg/day. The RfD is based on delayed ossification and accelerated puberty in male offspring in a mouse developmental toxicology study (Lau et al., 2006). The default Relative Source Contribution factor of 20% was used to account for non-drinking water exposures. The USEPA Health Advisory uses a drinking water consumption rate of 0.054 L/kg/day, based on the 90<sup>th</sup> percentile for lactating women, which is higher than the default consumption rate of based on adult exposure factors.

Figure 1 shows the predicted increases in serum PFOA levels from ongoing exposure in drinking water at the USEPA Health Advisory (70 ng/L), the NJDEP (2007) guidance (40 ng/L), and the health-based water concentration (14 ng/L) developed in this document. Predictions based on both average (0.016 L/kg/day) and upper percentile (0.029 L/kg/day) drinking water ingestion rates are shown. A clearance factor developed by USEPA scientists (Lorber and Egeghy, 2011) to relate human PFOA exposures to human serum PFOA levels was used to predict the increases in serum PFOA from exposures to these level in drinking water. With average water consumption, ongoing exposure to 70 ng/L (the USEPA Health Advisory) is predicted to increase serum PFOA by 8.0 ng/ml, a 4.8-fold increase from the U.S. general population (NHANES) median of 2.1 ng/ml (CDC, 2015). With upper percentile water consumption, the increase in serum PFOA level from 70 ng/L is predicted as 14 ng/ml, a 7.7-fold increase from the general population (NHANES) median.

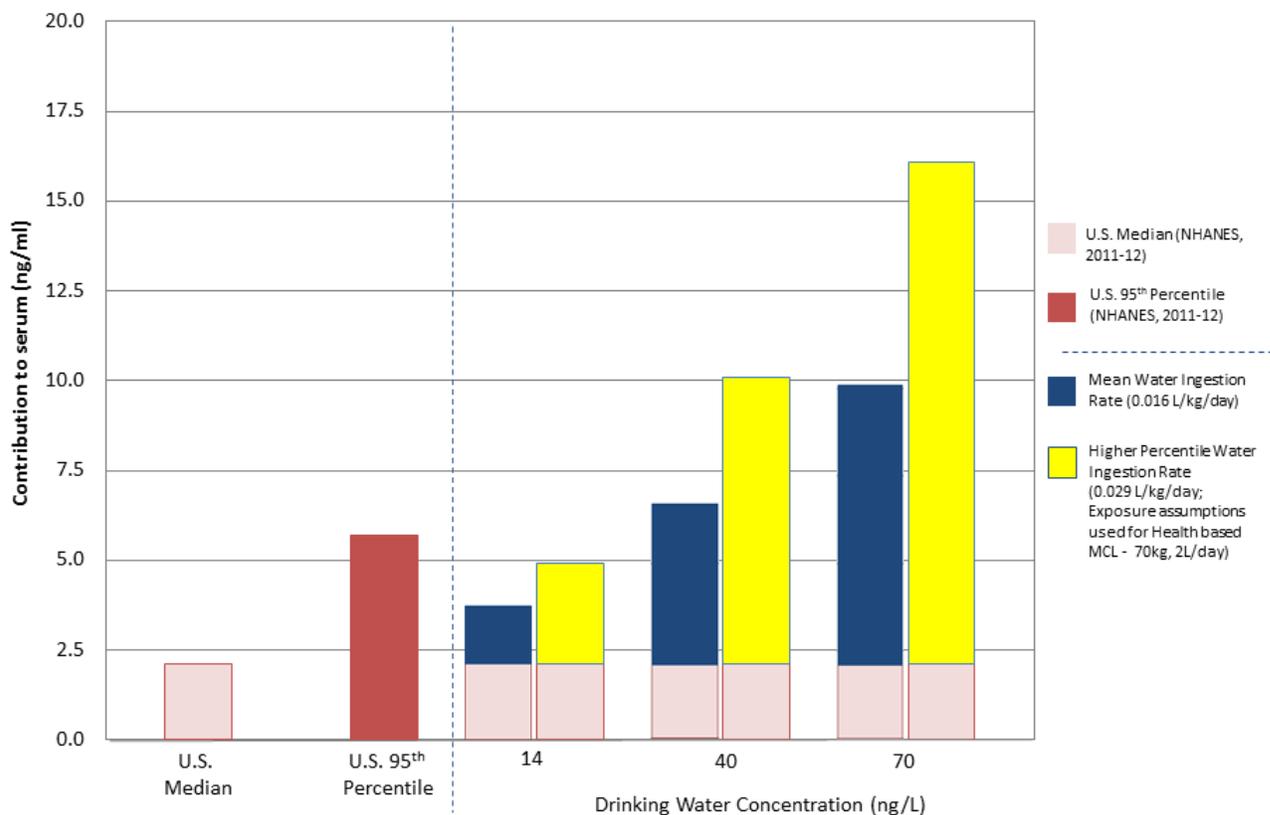


Figure 1. Increases in serum PFOA concentrations predicted from consumption of drinking water with various concentrations of PFOA (14 ng/L –NJDEP health-based drinking water concentration; 40 ng/L – NJDEP guidance (2007); 70 ng/L – USEPA Lifetime Health Advisory).

### **Agency for Toxic Substances and Disease Registry (ATSDR) Draft Minimum Risk Level (MRL)**

Agency for Toxic Substances and Disease Registry (ATSDR) Draft Minimum Risk Level (MRL) ATSDR (2018) has recently released a draft Toxicological Profile for Perfluoroalkyls that includes Intermediate MRLs for several PFCs including PFOA. ATSDR (2018) states that “an MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure.” MRLs are therefore similar in concept to RfDs developed by USEPA, NJDEP, and DWQI, except that RfDs are intended to protect for chronic (lifetime) exposure, while MRLs are developed for several different exposure durations (Acute – up to 14 days; Intermediate – 15 to 364 days; Chronic – 365 days or longer). For PFOA, the the ATSDR Intermediate MRL of 3 ng/kg/day, close to but slightly higher than the New Jersey RfD of 2 ng/kg/day presented herein. Both values are based on sensitive toxicological endpoints from mouse studies. As discussed herein, the New Jersey RfD is based on increased liver weight (Loveless et al., 2012) with an additional uncertainty factor to protect for sensitive developmental effects that may occur at much lower doses, and the ATSDR Intermediate MRL is based on behavioral effects (Onishchenko et al., 2011) and permanent effects on bone structure (Koskela et al., 2016) from

developmental exposures. Since Intermediate MRLs are intended to protect for a shorter exposure duration (15 to 364 days) than chronic (lifetime) Reference Doses, it is logical and consistent that the Intermediate MRL would higher than the New Jersey Reference Dose.

### **Guidance and standards of other states**

The Interstate Technology and Regulatory Council (ITRC, 2018) has developed tables that provide the PFOA drinking water standards and guidance developed by USEPA, states, and other nations (Table 4 of ITRC, 2018), and the basis for the USEPA and state PFOA values (Table 5-2 of ITRC, 2018).

California (California State Water Resources Control Board, 2018; California EPA, 2018) has recently adopted the NJ DWQI Health-based MCL of 14 ng/L, which has the same technical basis as the ISGWQC proposed herein, as an interim Notification Level for detections of PFOA in California public water systems.

Vermont has adopted drinking water and ground water standards (Vermont DEC, 2017) for PFOA, PFOS, and the total of the two compounds of 20 ng/L. These Vermont values are based on the Reference Dose (RfD) of  $2 \times 10^{-5}$  mg/kg/day from the draft USEPA (2016a) PFOA Health Advisory, drinking water exposure assumptions for a child less than 1 year of age (instead of default adult exposure assumptions), and the default Relative Source Contribution (RSC) factor of 20%. Vermont (2018) drinking water guidance applies the total of 20 ng/L to PFOA, PFOS, and three additional PFCs (perfluoroheptanoic acid [PFHpA], perfluorononanoic acid [PFNA], and perfluorohexane sulfonate [PFHxS]).

Minnesota Department of Health (2017) has updated its earlier Health Risk Limit (HRL) for PFOA in drinking water to 35 ng/L. This value is based on the USEPA Reference Dose of 20 ng/kg/day and exposure modeling for breast-fed and formula-fed infants. The Reference Dose was derived by incorporation of an additional database uncertainty factor of 3, for potentially more sensitive immunotoxic effects, into the USEPA PFOS Reference Dose which is based on decreased offspring weight as described above.

Several other states use the USEPA (2016) Health Advisory of 70 ng/L for PFOA, PFOS, or the total of both compounds as drinking water guidance or have adopted it as an enforceable standard. Connecticut (2016) and Massachusetts (2018) use 70 ng/L as guidance for the total of PFOA, PFOS, PFHpA, PFNA, and PFHxS.

## **ENVIRONMENTAL FATE, TRANSPORT, AND OCCURRENCE**

### **Environmental Fate and Transport**

Because of the extreme stability of their carbon–fluorine bonds, PFOA and other PFCs are extremely resistant to degradation in the environment and thus persist indefinitely (Buck et al., 2011; Lindstrom et al., 2011a). As discussed above, the production and use of PFOA and its precursors has been phased out by major U.S. manufacturers. However, environmental contamination and resulting human exposure to PFOA are anticipated to continue for the

foreseeable future due to its environmental persistence, formation from precursor compounds, and continued production by other manufacturers.

PFOA and other PFCs are found in many environmental media (e.g. drinking water, surface water, groundwater, air, sludge, soils, sediments, outdoor and indoor dust, and ice caps) in locations around the world including remote polar regions (Lau et al., 2007). PFCs are also found in wildlife (fish, birds, mammals) including in remote polar regions. However, the bioconcentration factor for PFOA is lower than for PFOS or longer chain perfluorocarboxylates such as PFNA (Martin et al., 2003; Conder et al., 2008), and concentrations of PFOA in wildlife in remote locations are generally lower than for these other compounds (Butt et al., 2010).

Two major pathways have been proposed for long-range transport of PFOA and other PFCs to remote locations worldwide, including the Arctic (Figure 2; Lau et al., 2007, 2013; Butt et al., 2010). The relative contributions of each of these pathways are not known. The first pathway involves the atmospheric transport of volatile precursors such as 8:2 fluorotelomer alcohol (8:2 FTOH), followed by oxidation of the precursors to PFOA and other PFCs which are then deposited onto the land or the water. The second pathway involves long-range aqueous transport of emitted perfluorinated carboxylates such as PFOA in their anionic forms to remote locations by currents on the ocean's surface.

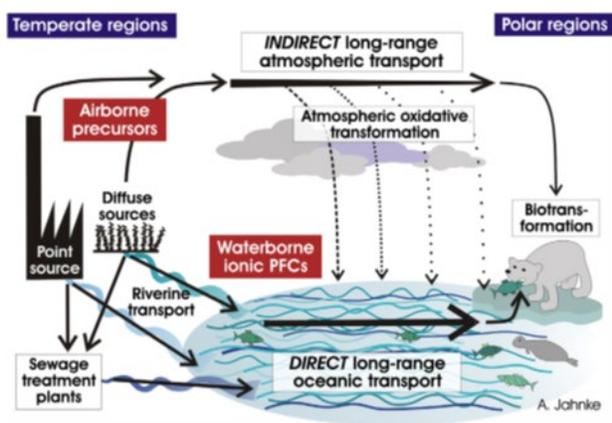


Figure 2. Major transport pathways of PFCs to the Arctic (and other remote locations), by Annika Jahnke (Butt et al., 2010)

### Fate and Transport Relevant to Drinking Water Contamination

PFOA and some other PFCs are distinct from other persistent and bioaccumulative organic compounds because of their importance as drinking water contaminants. PFOA exists predominantly as an anion under environmental conditions, does not bind well to soil, migrates readily from soil to groundwater, and is highly water-soluble (Davis et al., 2007). These properties of PFOA differ from those of other persistent and bioaccumulative organic pollutants such as polychlorinated dioxins and furans, PCBs, and pesticides like chlordane and DDT. These other compounds are generally not significant as drinking water contaminants because they have

high octanol/water partition coefficients. Thus, they have a high affinity for soil and sediments but low water solubility (Post et al., 2011).

PFOA that is released to the environment can contaminate surface water and groundwater used as sources of drinking water. Sources of PFOA in the environment include discharge to air and water from industrial facilities where it is made or used (Davis et al., 2007); release of aqueous firefighting foams, particularly at military sites, airports, and fire fighter training facilities (Moody et al., 2003; Backe et al., 2013); disposal in landfills (Eggen et al., 2010); discharge from wastewater treatment plants treating domestic and/or industrial waste (Sinclair and Kannan, 2006); street runoff (Murakami et al., 2009); storm water runoff (Kim and Kannan, 2007); land application of biosolids (sludge) from wastewater treatment plants treating industrial waste (Clarke and Smith, 2011; Lindstrom et al., 2011b; Sepulvado et al., 2011); land application of wastewater from industrial sources (Konwick et al., 2008); and use of contaminated industrial waste as a soil amendment (Skutlarek et al., 2006; Hölzer et al., 2008).

Environmental transport pathways that can result in surface water and groundwater contamination by PFOA after release from an industrial source are shown in Figure 3 (Davis et al., 2007) and were reviewed by Lau et al. (2007) and Butt et al. (2010).

As is the case for other groundwater contaminants, PFOA can reach drinking water wells via the well-established pathway of migration of a groundwater plume that has been contaminated either directly from surface spills or by contaminated surface water mixing with groundwater drawn in by pumping wells. Unlike many other environmental contaminants, PFOA can also reach groundwater from air emissions from nearby industrial facilities, followed by deposition from air onto soil, and migration through the soil to groundwater (Davis et al., 2007).

In West Virginia and Ohio, drinking water wells as far as 20 miles away were contaminated with PFOA by releases from an industrial facility where it was used as a processing aid in fluoropolymer production. Groundwater contamination occurred via soil deposition of PFOA that had been emitted into the air followed by migration to groundwater, and, to some extent, recharge of the groundwater aquifer with contaminated surface water from the Ohio River (Steenland et al., 2009a; Shin et al., 2011). PFOA was detected in public water supply wells in this vicinity at levels up to > 4000 ng/L (DuPont and URS Diamond Corporate Remediation Group, 2008) and in private wells at up to >13,000 ng/L (Hoffman et al., 2011). In New Jersey, PFOA was detected at up to 190 ng/L in shallow unconfined wells of a public water supply located near an industrial source (Post et al., 2009a), and at > 40 ng/L, with a maximum above 400 ng/L, in 59 of 104 private wells within a radius of slightly more than 2 miles of this facility (DuPont, 2009); contamination of the distant wells was likely due to air deposition (Post et al., 2012).

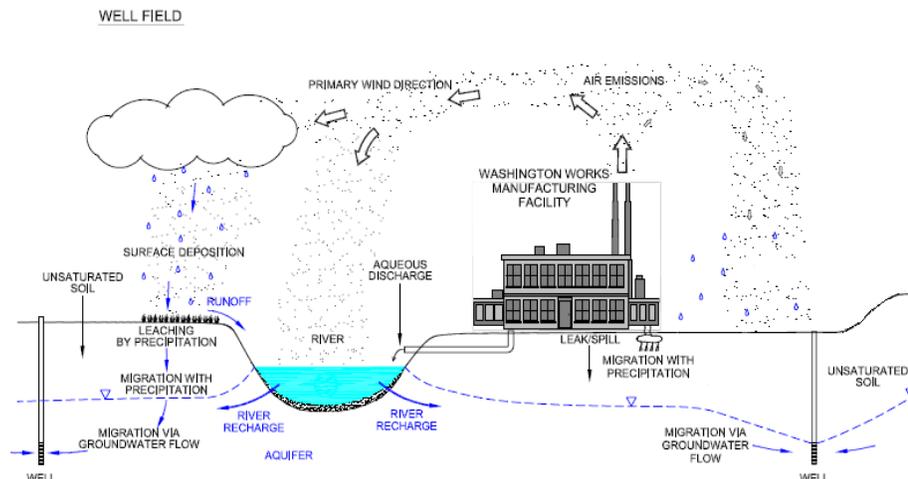


Figure 3. APFO (PFOA) transport near discharge source (Davis et al., 2007)

### Formation from precursor compounds

An additional source of PFOA in the environment is the breakdown of precursor compounds such as the fluorotelomer alcohol, 8:2 FTOH [ $F_3(CF_2)_7CH_2CH_2OH$ ], used industrially and in consumer products (Butt et al., 2010; Buck et al., 2010; Butt et al., 2014).



Larger molecules such as polyfluoroalkyl phosphoric acid diesters (diPAPs) (e.g. diPAPs 8:2; Figure 4) are found in greaseproof food contact papers, wastewater treatment plant sludge, and paper fibers from paper mills (D'eon et al., 2009). These larger molecules release 8:2 FTOH that can degrade to PFOA.

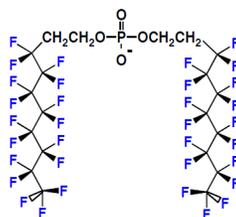


Figure 4. Structure of diPAPs 8:2

PFOA is formed from these precursor compounds through biodegradation in soil, sludge, and wastewater (Sinclair and Kannan, 2006; Lee et al., 2010) as well as through chemical reactions in the atmosphere (Figure 2). PFOA and other PFCs have been found at higher concentrations in effluent than influent at wastewater treatment plants. This increase is believed to result from the biodegradation of telomer alcohols and other precursors from domestic and industrial sources within the wastewater treatment plant (Sinclair and Kannan, 2006; Lee and Mabury, 2011). Fluoroacrylate polymers, used in commercial products, may also degrade in soil to release FTOH

which can degrade to PFCs such as PFOA (Russell et al., 2008; Washington et al., 2009). Since PFOA, once formed, does not degrade appreciably, environmental PFOA levels are increased by conversion of even a small fraction of the precursors to the terminal breakdown product, PFOA.

### **Occurrence in drinking water**

PFOA and other PFCs occur in raw and finished drinking water from both groundwater and surface water sources in New Jersey, other parts of the United States, and nations around the world (reviewed by Mak et al., 2009; Post et al., 2012; Post et al., 2013). PFOA and other PFCs are found in drinking water impacted by discharges from industrial facilities, release of aqueous firefighting foam, and other known sources of contamination, as well as where the source is unknown (Post et al., 2012).

PFOA has been detected at high frequency in some river basins that are important sources of drinking water. For example, it was detected (>1 ng/L) in 82.3% of samples from 80 locations throughout the Cape Fear River (North Carolina) drainage basin, population 1.7 million, at a median of 12.6 ng/L and a maximum of 287 ng/L (Nakayama et al., 2007). In the Upper Mississippi River drainage basin in the Midwestern U.S., population 30 million, it was detected (>1 ng/L) in 73% of 88 locations with a median of 2.07 ng/L and a maximum of 125 ng/L. Elevated levels at certain sites were attributed to point sources in this study (Nakayama et al., 2010). In the Tennessee River in Alabama, PFOA levels were 395±128 ng/L in samples from the 35 river miles downstream of the site of discharge from a fluorochemical manufacturing facility, with the highest levels (521-598 ng/L) in the 6 river miles furthest downstream (Hansen et al., 2002). In Germany, PFOA and other PFCs in organic material applied to agricultural land contaminated the Moehne and Ruhr Rivers, important sources of drinking water. PFOA was detected at up to 33,900 ng/L in a creek near the site of contamination upstream of these rivers, and at up to 519 ng/L in drinking water from the Moehne River (Skutlarek et al., 2006).

PFOA and other PFCs are not effectively removed from drinking water by standard treatment processes such as coagulation/flocculation, sand filtration, sedimentation, medium-pressure ozonation, chloramination, and chlorination. However, PFOA can be removed from drinking water by granular activated carbon (GAC) or reverse osmosis (Rumsby et al., 2009, Bartell et al., 2010a, Tagaki et al., 2011; Eschauzier et al., 2012; Appleman et al., 2014; DWQI, 2015b). Therefore, unless specific treatment for removal of PFCs is in place, concentrations of PFOA and other PFCs detected in raw drinking water can be considered to be representative of concentrations in finished drinking water (Post et al., 2013).

### **Occurrence in New Jersey drinking water**

Considerable information is available on the occurrence of PFOA and other PFCs in New Jersey public water systems (PWS). This includes data from 53 PWS from two NJDEP occurrence studies of PFCs, substantial additional data submitted to NJDEP by PWS and other parties, and

data from the nationwide USEPA Unregulated Contaminant Monitoring Rule 3 (UCMR3) survey. For the two NJDEP occurrence studies and most of the additional data submitted to NJDEP, analysis of samples was performed by certified laboratories with Reporting Levels (RLs) that were generally 4-5 ng/L or lower. It is believed that statewide drinking water studies of PFOA with sensitive RLs such as these have not been conducted in states other than New Jersey. In contrast, the RL for PFOA in USEPA UCMR3 is much higher (20 ng/L) than the RLs in the other NJ PWS monitoring data.

*NJDEP studies of occurrence in New Jersey public water systems*

Following detection of PFOA in a New Jersey PWS at up to 190 ng/L in a groundwater source and up to 64 ng/L in tap water, two statewide studies of the occurrence of PFOA and other PFCs in drinking water were conducted by NJDEP. The 2006 study tested 23 PWS for PFOA and PFOS, and the 2009-10 study tested 33 additional PWS for PFOA, PFOS, and eight other PFCs (NJDEP, 2007b; NJDEP, 2014; Post et al., 2009a; Post et al., 2013).

The 2006 NJDEP study included 29 samples of raw and/or finished water from 23 NJ PWS including 14 with groundwater sources, 8 with surface water sources, and one using both groundwater and surface water. In the 4 PWS where both raw and finished water were analyzed, PFOA concentrations were similar in both samples. Of the PWS in this study, PFOA was detected in 15 of 23 systems (65%) at or above the RL (4 ng/L), and in 3 of 23 systems below the RL. PFOA was detected above the RL (9 of 13) at up to 33 ng/L, or below the RL (1 of 13), in 10 of 13 groundwater samples (77%) from unconfined or semiconfined aquifers, but was not detected in the two groundwater samples from confined aquifers. Additionally, PFOA was detected above the RL (7 of 9; 78%) at up to 39 ng/L, or below the RL (2 of 9; 22%), in samples from all 9 PWS using surface water sources. In this study, PFOS was detected (>4 ng/L) in 30% of the PWS, less frequently than PFOA (NJDEP, 2007; Post et al., 2009a).

The 2009-2010 NJDEP study tested raw water from 30 PWS for PFOA, PFOS, and 8 other PFCs. The sites for this study were chosen for geographic diversity, representing 19 of NJ's 21 counties. The study included 18 PWS with groundwater sources (17 unconfined, one confined) and 12 PWS with surface water sources. One or more PFC was detected (>5 ng/L) at 21 sites (70%), with the number of individual compounds detected varying from one (in 8 samples) to a maximum of 8 in one sample. PFOA was the most commonly detected PFC (17 of 30 samples; 57%), including 6 of 18 of groundwater samples (33%) and 11 of 12 of surface water samples (92%). When PFOA was detected, other PFCs were often but not always found in the same sample. PFOA was found at the highest maximum concentration of any of the PFCs analyzed in the study, 100 ng/L. This highest detection was in a PWS intake from a river, and the likely source was subsequently identified as discharge from an upstream facility that made and used products containing PFOA and other PFCs (Post et al., 2013; NJDEP, 2014).

NJDEP database of PFCs in New Jersey Public Water Systems

The NJDEP Division of Science, Research and Environmental Health maintains an internal database of PFC results from NJ PWS including the two NJDEP occurrence studies, additional raw and finished water data submitted to NJDEP by PWS and other parties, and detections from UCMR3 data. As of January 2016, the database included 1035 samples (423 raw water, 549 finished water, and 63 distribution system) from 282 sampling locations in 80 PWS (including 72 PWS with data from NJDEP studies and/or submitted to NJDEP, and 8 additional PWS with PFC detections in UCMR3). Of these samples, 374 were analyzed for only PFOA and PFOS, and 661 were analyzed for a broader suite of PFCs.

PFOA was the most frequently detected PFC in NJ PWS. It was detected at some level in 65% of 72 PWS included in the NJDEP database (excluding UCMR3 data; Table 1). The highest detection in finished water was 100 ng/L, and concentrations exceeding 40 ng/L were reported in at least one finished water sample from 12 of 72 PWS (17%). It was also detected at >20 ng/L in UCMR3 monitoring in finished water from six additional PWS that are not otherwise included in the database, including two PWS that had levels above 40 ng/L.

PFOA Concentration (ng/L)	Number of PWS	% of PWS
ND**	25	35%
RL - <10**	15	21%
10 - <20**	10	14%
20 - <40	10	14%
>40	12	17%

\*Data shown are highest concentration found in raw or finished water from the PWS. Levels in finished water from some water supplies included may be lower because several raw water sources are blended in the treatment plant.

\*\*Reporting levels (RLs) vary among samples and range from 2.5 - 20 ng/L. Therefore, the percentage of PWS with RL-<10, and 10 - <20, may actually be higher than shown.

Comparison of NJ occurrence to nationwide UCMR3 data and studies from other nations

Data on PFOA in PWS in New Jersey and nationwide is available through the USEPA UCMR3. Under UCMR3, nationwide monitoring of finished water for 30 unregulated contaminants, including PFOA and 5 other PFCs, was conducted in 2013–2015 by all U.S. large PWS (serving more than 10,000 people) and 800 representative smaller PWS (serving less than 10,000 people) (USEPA, 2012b). UCMR3 data therefore provide useful information on occurrence of PFCs in NJ in comparison to the rest of the United States. However, comparison of the UCMR3 PFC data with other New Jersey PFC occurrence data is complicated by the fact that the UCMR3 RLs for PFOA (20 ng/L) and other PFCs are much higher than the RLs for other PFC data in the NJDEP database (generally  $\leq 5$  ng/L).

UCMR3 monitoring in New Jersey includes all 165 large community PWS, 13 of about 435 small community PWS, and 8 of about 700 non-transient non-community water systems. A comparison of national versus New Jersey PFC data from UCMR3 reported through January 2016 is shown in Table 2 (data obtained from USEPA, 2016f). PFOA was detected ( $\geq 20$  ng/L) in PWS at locations throughout the state, and was detected more than five times more frequently in New Jersey PWS (10.53%) than nationally (1.93%). PFNA was also detected much more frequently in NJ (2.34%) than nationally (0.22%). However, PFNA was detected only in the vicinity of a likely industrial source located in Gloucester County (DWQI, 2015c) but not in other parts of New Jersey. The occurrence of the other PFCs included in UCMR3 (PFHpA, PFOS, PFHxS, PFBS) was similar or slightly higher in New Jersey compared to nationally.

Compound*	Reporting Level (RL) (ng/L)	New Jersey			United States (other than NJ)		
		Number of PWS	Number above RL	Percent above RL	Number of PWS	Number above RL	Percent above RL
PFOA	20	171	18	10.53 %	4617	89	1.93 %
PFNA	20	171	4	2.34 %	4617	10	0.22 %
PFHpA	10	171	5	2.92 %	4617	77	1.67 %
PFOS	40	171	5	2.92 %	4617	88	1.91 %
PFHxS	30	171	2	1.17 %	4617	52	1.13 %
PFBS	90	171	0	0 %	4617	6	0.13 %

\*PFHpA – perfluoroheptanoic acid (C7); PFBS – perfluorobutane sulfonate; PFHxS – perfluorohexane sulfonate.

The occurrence of PFCs in NJ PWS in the 2009-10 NJDEP study was also compared to similar occurrence studies in other nations by Post et al. (2013). PFOA was detected more frequently and at a higher maximum concentration in the 2009-10 New Jersey PWS study than in comparable drinking water studies in France, Spain, and China which had RLs similar to the RL in the NJ study.

#### Occurrence in NJ private wells

A statewide study of PFOA or other PFCs in New Jersey private wells has not been conducted. PFOA was detected at  $>40$  ng/L, with a maximum above 400 ng/L, in 59 of 104 private wells within a radius of slightly more than 2 miles of a New Jersey industrial source (DuPont, 2009); contamination of the distant wells was likely due to air deposition. More recently, PFOA has been detected in private wells near another facility which used and discharged a mixture of PFCs that consisted primarily of PFNA and also contained PFOA (DWQI, 2015c).

## **HUMAN BIOMONITORING**

Human biomonitoring studies show that exposure to PFOA and/or its precursors is ubiquitous in the U.S. and throughout the world. PFOA has a human half-life of several years and remains in the body for a long period of time after exposure occurs. Data on blood serum concentrations from the general population, communities with contaminated drinking water, and workers with occupational exposure are summarized below. Consumption of contaminated drinking water results in increased blood serum concentrations, while the highest blood serum concentrations have been found in occupationally exposed workers. PFOA is detected in human breast milk, amniotic fluid, and umbilical cord blood, demonstrating that exposure occurs during prenatal and postnatal development, and it has also been detected in human seminal fluid.

### **Blood Serum**

#### **General population**

PFOA and other PFCs are present in the serum of the general population in the United States and in countries worldwide. This topic was recently comprehensively reviewed by Kato et al. (2015).

Data from archived serum samples from the United States and Norway indicate that human exposure to PFOA has been ongoing for decades, and that exposure increased greatly in the 1980s in these two locations. Analysis of serum samples collected up to 56 years ago found that the median level in serum from pregnant California women sampled in 1960-63 (n=40) was 0.27 ng/ml, approximately 10-fold lower than the median in serum from California women sampled in 1981-86 (n=30) and 2009 (n=35), which were 2.71 and 2.08 ng/ml, respectively (Wang et al., 2011a). In pooled serum samples from Norwegian men (age 40-50) collected over a 29 year period (1977-2006), PFOA levels gradually increased from 0.58 ng/ml in 1976 to 4.9 ng/ml in 2001, an 8-fold increase, followed by a yearly decline to 2.7 ng/ml in 2006. A similar temporal pattern was seen in serum samples collected from Norwegian children and male and female adults of other age groups between 1976 and 2007 (Haug et al., 2009).

The largest studies of the U.S. general population are from the National Health and Nutrition Examination Survey (NHANES) conducted by the U.S. Centers for Disease Control and Prevention (CDC) (Kato et al., 2011; CDC, 2015) and American Red Cross blood donors (Olsen et al., 2012). PFOA is one of four PFCs (PFOA, PFOS, PFNA, and perfluorohexane sulfonate [PFHxS]) that have been detected in the serum of greater than 99% of a representative sample of the U.S. population, age 12 or older, in NHANES (Kato et al., 2011). PFOA and these other PFCs are biologically persistent, with human half-lives of several years, as discussed in the Toxicokinetics section below.

Data from six cycles of NHANES monitoring between 1999-2000 and 2011-12 show that serum PFOA levels have decreased in the U.S. general population during this time period (Table 3). In

the first NHANES (1999-2000), the geometric mean serum concentration was 5.21 ng/ml and the 95<sup>th</sup> percentile was 11.9 ng/ml, while the most recent NHANES (2011-12) found a geometric mean and 95<sup>th</sup> percentile of 2.08 and 5.68 ng/ml, respectively. In the NHANES surveys, PFOA concentrations were lower in those 12-19 years of age than in older individuals and were somewhat higher in males than females. In data from the three ethnic groups that were analyzed over time, levels were consistently lowest in Mexican Americans, intermediate in non-Hispanic blacks, and highest in non-Hispanic whites (CDC, 2015).

Year	Geometric Mean	Percentile				n
		50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>	
2011-12	2.08	2.08	3.03	4.35	5.68	1904
2009-10	3.07	3.20	4.60	6.00	7.50	2233
2007-08	4.12	4.30	5.90	7.90	9.60	2100
2005-06	3.92	4.20	6.20	9.00	11.3	2120
2003-04	3.95	4.10	5.80	7.80	9.80	2094
1999-2000	5.21	5.20	6.90	9.40	11.9	1562

CDC, 2015

A similar pattern of decreasing serum PFOA concentrations over time was seen in three studies of American Red Cross blood donors in 2000-2001, 2006, and 2010 (Olsen et al., 2012). Each study included samples from 600-645 subjects from six locations throughout the U.S., with an approximately equal number in each of five 10-year age categories (20-29 through 60-69 years of age) from each location. Geometric means and 95<sup>th</sup> percentile concentrations, respectively, were 4.7 and 12.0 ng/ml in 200-01, 3.44 and 7.9 ng/ml in 2006, and 2.44 and 6.6 ng/ml in 2010. As in the NHANES studies, serum concentrations were generally higher in males than females.

Serum PFOA levels are generally comparable to those found in the U.S in developed countries throughout the world, including Europe, Asia, and Australia (Post et al., 2012; Kato et al., 2015). In contrast to industrialized nations where serum PFOA is almost universally detected, PFOA was detected at > 0.5 ng/ml in only 12 of 55 serum samples from Afghan children and adults, with a maximum of 1.5 ng/ml; relatively low serum levels have also been reported in other developing countries where exposure to PFOA and other PFCs may be lower than in industrialized nations (Hemat et al., 2010).

PFOA concentrations in pooled serum samples from children (age 3-11) in 2001-2002 NHANES ranged from about 6-8 ng/ml, significantly higher than in pooled serum samples from adults in this study (Kato et al., 2009). Median and maximum serum PFOA levels in 300 Texas children, age <1 to 12 years, were 2.85 ng/ml and 13.50 ng/ml; adults were not included in this study. In the Texas study, the median level did not differ between genders, and was lower in those less

than 3 years of age than in the older age groups (Schechter et al., 2011). Exposures to infants and young children are discussed in detail in the section on developmental exposures below.

#### Communities with drinking water exposures

Continued exposure to even relatively low concentrations of PFOA in drinking water concentrations results in substantial increases in serum levels. The quantitative relationship between drinking water exposure and human serum PFOA levels is discussed below.

A recent study (Hurley et al., 2016) found substantially increased serum PFOA levels in individuals served by PWSs reporting detection of PFOA in UCMR3 monitoring. PFOA detections were relatively low, ranging from 20 ng/L (the UCMR3 RL) to 53 ng/L, with a mean of 28 ng/L. The study group consisted of middle aged and older California women (n=1,333; 70% between 60 and 79 years of age). Of this group, 4.5% resided in a zipcode where a PWS reporting detection of PFOA in UCMR3 monitoring is located. The distribution of serum concentrations differed significantly ( $p < 0.0001$ ) in those served by a PWS where PFOA was detected (“exposed”) as compared to those served by a PWS without a detection (“unexposed”). The median serum PFOA concentrations in the “exposed” group was 38% higher (3.46 ng/ml) than in the “unexposed” group (2.51 ng/ml). The authors note that the contribution of drinking water to serum PFOA is likely actually greater than observed in the study since some of those classified as “exposed” may have received their drinking water from another point of entry (e.g. treatment plant) within the PWS that detect PFOA. Additionally, the serum PFOA levels of some participants classified as “not exposed” may have been increased by PFOA in drinking water at concentrations below the UCMR3 RL of 20 ng/L.

Public water supply wells and private wells in several Ohio and West Virginia communities were contaminated by PFOA emissions from an industrial facility. In Little Hocking, Ohio, the concentration in drinking water was 3550 ng/L in 2002-2005, and the median serum PFOA concentration in 282 individuals tested in 2004-2006, with occupationally exposed individuals excluded, was 371 µg/L (Emmett et al., 2006a). The C8 Health Study (described in more detail below) is a much larger study of Little Hocking and several other communities in this vicinity with drinking water PFOA concentrations ranging from >50 ng/L to over 3000 ng/L (Post et al., 2009a). In approximately 69,000 C8 Health Study participants, including some with occupational exposures, the median serum PFOA concentration in 2005-2006 was 28.2 ng/ml, as compared to the median of 4 ng/ml in the 2003-2004 NHANES study (Steenland et al., 2009a). The upper 25% of C8 Health Study participants had serum PFOA levels greater than 71 ng/ml, only slightly below the highest concentration found in 2003-2004 NHANES, 77.1 ng/ml. As in the NHANES and Red Cross blood donor studies of the general population discussed above, the median serum concentrations in males (33.7 ng/ml) were higher than females (23.7 ng/ml) in this large study of an exposed population.

Emmett et al. (2006a) observed higher serum levels in children ages 2-5 than in older children and adults in their study of Little Hocking, Ohio residents with exposure to PFOA in drinking water. Adults over 60 years of age also had higher levels than other age groups. In the larger C8 Health Study population, serum concentrations were also higher in children and older adults, with the lowest levels in those age 20-29 (Steenland et al., 2009a). More recently, Shin et al. (2011) estimated a median one-year old child:maternal serum ratio of 1.27:1 from data on 40 child-mother pairs in the C8 Health Study. In a much larger study of almost 5000 mother-child pairs from the C8 Health Study, serum levels in children up to age 12 were higher than in their mothers in this population; in the youngest age category ( $\leq 5$  years), mean levels were 44% higher than maternal levels (Mondal et al., 2012).

Serum PFOA levels were also higher than in the general population in several other communities with exposure from drinking water. These include communities in Germany whose surface water source of drinking water was contaminated by runoff from industrial waste used as a soil amendment (Hölzer et al., 2008); in Alabama where a river used as a public water supply source was contaminated by industrial discharge, and PFOA from contaminated biosolids applied to agricultural land reached drinking water wells (ATSDR, 2013); in Minnesota communities where private wells and public water supply wells were contaminated by disposal of industrial waste (Landsteiner et al., 2014); in Pease, NH where public water supply wells were contaminated by military use of aqueous firefighting foam (NHDHHS, 2015); and in Hoosick Falls, NY, where drinking water was contaminated by releases from an industrial facility (NYS DOH, 2016)

#### Occupationally exposed workers

Serum PFOA levels in workers at facilities where PFOA is made or used in fluoropolymer production are much higher than in the general population. Biomonitoring data from workers at such facilities were reviewed by Olsen (2015). Mean or median serum concentrations of several 1000 ng/ml (several ppm) were reported for some job categories at some facilities, with maximum serum concentrations of over 100,000 ng/ml (100 ppm), although levels in most workers were lower. In the C8 Health Study participants, the median serum level among those currently working at the Washington Works plant where PFOA was used in fluoropolymer production (n=1,171) was 148 ng/ml, as compared to 24 ng/ml in those who did not work there at or prior to the time of sampling (Steenland et al., 2009a).

Serum concentrations of PFOA and other PFCs are also elevated in professional ski waxing technicians due to exposures to fluorinated ski waxes that contain both the compounds themselves and their precursors (reviewed by Olsen, 2015).

#### **Other human biological matrices**

##### Seminal plasma

PFOA and other PFCs were found in human seminal plasma in a study of Sri Lankans. The mean and median concentrations were 6.38 and 4.02 ng/ml, respectively, and PFOA concentrations were significantly correlated with serum PFOA concentrations (Guruge et al., 2005).

#### Amniotic fluid

PFOA was detected in amniotic fluid in a study in the United States (Stein et al., 2012). The median blood serum:amniotic fluid concentration ratio was about 13:1.

#### Umbilical cord blood serum

PFOA and other PFCs were detected in numerous studies of umbilical cord blood from the general population worldwide in studies reviewed by Post et al. (2012). Locations of these studies included Baltimore, Maryland (Abelberg et al., 2007), Ontario, Canada (Monroy et al., 2008), Denmark (Fei et al., 2007), Germany (Midasch et al., 2007), Norway (Gützkow et al., 2011), the Faroe Islands (Needham et al., 2011), Australia (Toms et al., 2009), South Africa (Hannsen et al., 2010), Korea (Kim et al., 2011), and Taiwan (Lien et al., 2011). Mean serum (or plasma) levels in these studies ranged from 1.1 ng/ml in Korea (Kim et al., 2011) to 4.4 ng/ml in Taiwan (Lien et al., 2011). No geographic pattern was apparent from this dataset, as the levels reported from Africa, Australia, Europe, and North America fell in between the levels in the two Asian studies. More recent additional data are reviewed by Kato et al. (2015).

#### Breast milk

PFOA was detected in human breast milk in studies from locations worldwide (reviewed by Liu et al., 2010; White et al., 2011a; Post et al., 2012) including Massachusetts (Tao et al., 2008a), Japan (Tao et al., 2008b), China (So et al., 2006; Liu et al., 2010), Korea (Kim et al., 2011), Belgium (Roosens et al., 2010), Spain (Llorca et al., 2010), Norway (Haug et al., 2011; Thomsen et al., 2010), and Sweden (Sunstrom et al., 2011). Concentrations in breast milk were generally similar in studies from different parts of the world. In studies using sensitive analytical methods enabling detection of lower concentrations, median PFOA levels were 36 ng/L (Massachusetts; Tao et al., 2008a), 67 ng/ml (Japan; Tao et al., 2008b), and 46 ng/L (China; Liu et al., 2010), while PFOA was not detected or was infrequently found in breast milk in some other studies with higher detection limits (Fromme et al., 2010; von Ehrenstein et al., 2009). In the studies cited above, PFOA was frequently found in breast milk at concentrations higher than 40 ng/L, with some detections exceeding 100 ng/L (for example, in Belgium; Roosens et al., 2010).

Notably, breast milk concentrations were much higher in both rural and urban samples from Shanghai province (urban mean, 616 ng/L; rural mean, 814 ng/L) than in 12 other Chinese provinces (mean, 46 ng/L). Maternal exposures were likely higher in Shanghai than in the other areas sampled because PFOA levels are higher in Shanghai drinking water and surface water, likely because many fluorochemical manufacturing plants are located there (Liu et al., 2010).

## **SOURCES OF HUMAN EXPOSURE**

The human body burden of PFOA results from exposure to both PFOA itself and to precursor compounds that can be metabolized to PFOA, such as FTOH and diPAPs (D'eon and Mabury, 2011a; Lee and Mabury, 2011). Sources of exposure to PFOA and/or its precursors include drinking water, food, migration from food packaging into food, treated fabrics (carpets, upholstery, and clothing), protective sprays, ski waxes, cosmetics and personal care products, house dust, and inhalation of indoor and outdoor air (Trudel et al., 2008; Guo et al., 2009; Gewurtz et al., 2009; Freberg et al., 2010; Nilsson et al., 2010; Fraser et al., 2012; Knobeloch et al., 2012; Fujii et al., 2013; Fraser et al., 2013; Kotthoff et al., 2015). Migration into food from non-stick (PTFE-coated) cookware is not considered to be a significant exposure source (Trudel et al., 2008).

The relative contributions from direct exposure to PFOA and exposure to its precursors, used in products including food contact paper and stain resistant carpet and textile coatings, is uncertain and varies among individuals (D'eon and Mabury, 2011b; Gebbink et al., 2015a). Other classes of precursor molecules, some of which are known or suspected to be metabolically converted to PFOA in humans, have also been detected in human serum (Lee and Mabury, 2011). Some of these precursor compounds are known to be present in consumer products at much higher levels than PFOA itself (Lee and Mabury, 2011).

Efforts have been made to model the relative contributions of consumer products, indoor and outdoor air, house dust, diet, and/or other sources to exposures of PFOA and other PFCs in the general population. Some of these studies estimated the contributions of precursors (Fromme et al., 2008; Vestergren and Cousins, 2009; Gebbink et al., 2015b) while others did not (Washburn et al., 2005; Tittlemeier et al., 2007; Trudel et al., 2008; Cornelius et al., 2012); a high level of uncertainty is associated with the precursor estimates.

Most of these studies predict that diet is the predominant exposure source. Typical adult total exposures of about 2-3 ng/kg/day in Europe or North America were estimated in several studies (Fromme et al., 2009; Trudel et al., 2008; Vestergren and Cousins, 2009), while some more recent studies give higher dietary estimates (6.1 ng/kg/day in Flanders, Belgium; Cornelis et al., 2012) or lower dietary estimates (0.6 ng/kg/day in Norway, Haug et al., 2010a; 0.2 ng/kg/day in The Netherlands, Noorlander et al., 2011; 0.16 ng/kg/day (location not specified), Gebbink et al., 2015b). Such dietary exposure estimates, in general, are highly uncertain because there are relatively few data on PFOA levels in food, analytical methods for food lack sufficient sensitivity, detection limits vary greatly among food types, and PFOA levels differ greatly in samples of the same foods obtained from different sources and/or locations.

PFOA has been detected in at least some samples of several types of foods including milk, butter, meats, fish, vegetables (including potatoes), bread, and microwave popcorn, but was not

detected in most food samples tested (reviewed by D'Hollander et al., 2010; Domingo et al., 2012).

Commercially available infant formula products do not appear to be a major source of exposure to PFOA or other PFCs in the U.S. Tao et al. (2008a) evaluated PFCs in 21 samples of five brands of infant formula representing >99% of the U.S. market. Products tested included milk-, organic- and soy-based formula, packed in cans, glass, or plastic, in liquid, powdered, and concentrated liquid forms. PFOA was not detected (<0.048 ng/L) in any sample. Other PFCs (for which detection levels varied) were also not detected or were infrequently found (PFOS – one detection at 11.3 ng/L; perfluorohexane sulfonate (PFHxS)-two detections at up to 3.59 ng/L). In this study, PFCs were also analyzed in 12 samples of 11 brands of dairy milk purchased in Albany, NY in 2008, and there was only one detection of PFHxS at 3.83 ng/L. However, it should be noted that exposure to infants occurs when powdered or concentrated formula is prepared with drinking water contaminated with PFOA.

Llorca et al. (2010) analyzed two brands of dry infant cereal and three brands of powdered milk-based infant formula purchased in Spain. PFOA concentrations in the cereals were 166 and 438 ng/kg, and in the formulas, 374, 488, and 723 ng/kg. The concentrations in these products when prepared for consumption were not given.

PFOA and other PFCs can be taken up into plants grown on contaminated soil (e.g. from application of PFOA-contaminated biosolids) or irrigated with contaminated water, including into the parts of some vegetables and grains that are consumed by humans and by grazing livestock (Stahl et al., 2009; Lechner and Knapp, 2011; Yoo et al., 2011). The potential for human exposure to PFOA through this route generally depends on the part of the plant that is consumed. In general, shorter chain PFCs are preferentially taken up into the fruit of the plant, while longer chain PFCs such as PFOA are preferentially taken up into the root and shoot parts of the plant (Blaine et al., 2013a, b, 2014; Felizeter et al., 2012, 2014). In the C8 Health Study population, consumption of locally grown or home grown vegetables was associated with higher serum PFOA levels (Emmett et al., 2006a; Steenland et al., 2009a; Hoffman et al., 2011).

PFOA is much less bioaccumulative in fish than other PFCs that have a longer fluorinated carbon chain, including PFOS (Conder et al., 2008). Thus, consumption of fish from waterways contaminated with PFOA does not result in the high exposures typical of other persistent organic contaminants, including PFOS, which are bioaccumulative in fish (Hölzer et al., 2011). However, PFOA has been detected in edible fish and other seafood, and consumption of aquatic organisms may represent a significant portion of total dietary exposure in some populations (Haug et al., 2010 b; Zhang et al., 2011).

Several studies suggest that PFOA and its precursors in indoor air and/or house dust may be a major exposure source for some individuals (Haug et al., 2011; Shoeib et al., 2011; Schlummer et al., 2013; Gebbink et al., 2015a). Fraser et al. (2013) reported that the concentration of the PFOA precursor 8:2 FTOH in indoor air in offices is a predictor of serum PFOA concentration. Levels of this compound were greatly elevated in offices with new carpets compared to other offices.

Greater exposures to PFOA may occur in young children than in older individuals because of age-specific behaviors such as greater drinking water and food consumption on a body weight basis, hand-to-mouth behavior resulting in greater ingestion of house dust, and more time spent on floors where treated carpets are found (Section 5.1; Trudel et al., 2008; Shoeib et al., 2011).

Occupational exposure to PFOA is believed to occur primarily through inhalation (Vestergren and Cousins, 2009).

### **Exposures from drinking water**

It is well established that serum PFOA concentrations are greatly elevated in communities with highly contaminated drinking water resulting from environmental discharges (discussed in Biomonitoring, above). As discussed in Biomonitoring (above) and Toxicokinetics (below), continued exposure to even relatively lower drinking water concentrations which are more widespread (Section 3 above) can also substantially increase total human exposure, as indicated by serum PFOA levels.

The total exposure studies discussed above provide varying conclusions about the relative importance of drinking water to total exposure; these conclusions are highly dependent on the concentration of PFOA in drinking water assumed in the analyses. For example, Fromme et al. (2008) and Cornelis et al. (2012) concluded that drinking water contributed <1% to total exposure, assuming drinking water levels of 1 ng/L and 2 ng/L, respectively, while Noorlander et al. (2011) estimated that 55% of exposure comes from drinking water, assuming 9 ng/L. Vestergren and Cousins (2009) and Thompson et al. (2011) demonstrated that the contribution of drinking water to total exposure depends on the concentration of PFOA, and Thompson et al. (2011) predicted that a drinking water level of 9.66 ng/L contributed 24% to total exposure.

PFOA exists in drinking water in its non-volatile anionic form, and the formation of inhalable water droplets during showering or bathing is minimal. Therefore, inhalation exposure is not expected to be significant from non-ingestion uses of drinking water such as showering, bathing, laundry, and dishwashing (Trudel et al., 2008; USEPA, 2016e). In contrast, these are important exposure routes for volatile drinking water contaminants. Similarly, dermal absorption of PFOA during showering, bathing, or swimming is not expected to be significant compared to exposure through ingestion, based on analysis by NJDOH (2014) using skin permeability data from Franko et al. (2012).

## **TOXICOKINETICS**

### **Absorption, Distribution, Metabolism, and Excretion**

#### Summary

PFOA is well absorbed orally and can also be absorbed dermally and by inhalation. The ammonium (APFO) or sodium (NaPFO) salts dissociate to PFOA (the anionic form) in the body. PFOA is water soluble and distributes primarily to the liver and serum, and, to a lesser degree, to the kidney. Unlike most other bioaccumulative organic compounds, it does not distribute to fat. In the serum, PFOA is almost totally bound to albumin and other proteins. Since it is chemically non-reactive, it is not metabolized. The rate of excretion of PFOA varies widely among species, and in some cases between males and females of the same species. The excretion rate is largely dependent on the extent of secretion and reabsorption by organic anion transporters in the kidney. In humans, the half-life is several years, and half-lives in male and female mice and male rats are days to weeks, so that PFOA reaches steady-state in these species/genders after continued dosing. However, PFOA is rapidly excreted in female rats (half-life of 2-4 hours) and does not reach steady-state after continued once-daily dosing. For this reason, the rat is not an ideal model for studying developmental effects of PFOA. Because of the large variations in half-lives, the internal dose resulting from a given administered dose varies widely among species and, in some cases, genders of the same species. For this reason, interspecies (e.g. animal-to-human) comparisons are made on the basis of internal dose, as indicated by serum level, rather than administered dose.

#### Absorption

PFOA is well absorbed by the oral route (Lau et al., 2007). More than 95% of a single dose of 0.1 to 25 mg/kg APFO (the ammonium salt of PFOA) was absorbed in male and female rats (Kemper, 2003). It was also well absorbed in mice, rats, hamsters, and rabbits in studies by Hundley et al. (2006). About 98.7% of an oral dose given to pregnant rats on gestation day (GD) 8 or 9 was excreted in the urine within 24 hours (Gibson and Johnson, 1983). Additionally, a recent study in mice (Fujii et al., 2015) estimated the oral absorption of PFOA as 98.7% in males and 99.8% in females. The extent of oral absorption was determined by comparing fecal excretion after intravenous dosing (representing biliary excretion of PFOA into the gastrointestinal tract) and oral dosing (representing both unabsorbed PFOA and biliary excretion). PFOA is well absorbed by humans exposed orally, as demonstrated by elevated serum concentrations in residents of communities with contaminated drinking water (discussed above).

PFOA penetrated rat and human skin in an *in vitro* system (Fasano et al., 2005), and dermal exposure caused liver toxicity in rats (Kennedy, 1985) and immune effects in mice (Fairley et al., 2007). The dermal permeability coefficient of PFOA (14,000 ng/L [14 µg/L] in water, pH 5.01) was estimated as  $8.8 \times 10^{-5}$  cm/hr (Fasano et al., 2005). As above, dermal absorption is not

expected to be a significant source of exposure from contaminated drinking water (NJDOH, 2014).

Inhalation exposure to APFO in rats caused hepatic effects (Kennedy et al., 1986). Elevated serum levels of PFOA in workers in facilities making or using PFOA are likely to result primarily from inhalation exposure (Olsen, 2015). As above, PFOA does not volatilize from water, and inhalation is not expected to be a significant source of exposure from contaminated drinking water (Trudel et al., 2008; USEPA, 2016e).

### Distribution

After oral administration, the highest concentrations of PFOA are found in the liver and serum, followed by the kidney, with lower concentrations in other organs (Vanden Heuvel et al., 1991; Kemper, 2003; Hundley et al., 2006; Fujii et al., 2015). After *in utero* exposure to mice, PFOA persisted in bone until adulthood (Koskela et al., 2016). In the serum, PFOA is almost totally bound to albumin and other proteins (SRI, 2003; Han, 2003). Unlike many other persistent bioaccumulative compounds, PFOA does not distribute to fat, and the concentrations of PFOA in the fat after dosing are very low (Vanden Heuvel et al., 1991; Hundley et al., 2006; Fujii et al., 2015).

The fraction of the dose found in the liver is dose-dependent and varies between male and female animals. For example, in male rats two hours after a single intravenous dose, 52% of a low dose (0.041 mg/kg) and 27% of a higher dose (16.56 mg/kg) were found in liver (Kudo et al., 2007). Serum concentrations were similar in male and female CD-1 mice after a single oral gavage dose of 1 or 10 mg/kg PFOA (Lou et al., 2009). However, the concentration in the liver was higher in males than in females.

In another study in which rats were given a single dose of 25 mg/kg, the absolute concentrations in the livers of males were higher than in females, as expected based on the slower excretion by male rats (see below), and the percentage of PFOA in the cytosolic fraction of the liver was higher in females (49%) than in males (26%) (Han et al., 2005).

The subcellular distribution within the liver of male rats was also found to be dose-dependent (Kudo et al., 2007). Over 40% of a low dose (0.041 mg/kg) distributed to the 8000xg pellet (nuclei, mitochondria, and cellular debris), followed by lysosomes and peroxisomes, microsomes, with the least amount found in cytosol (less than 5%), while over 43% of a higher dose (16.56 mg/kg) distributed to cytosol, followed by the 8000xg pellet, lysosomes and peroxisomes, and microsomes. In another part of this study using a range of doses, there was a dose-dependent increase in the percentage found in cytosol.

### Metabolism

PFOA is chemically unreactive due to its carbon-fluorine bonds, one of the strongest found in organic chemistry (Vaalgamaa et al., 2011). Therefore, it is not metabolized by biological systems.

### Excretion

PFOA is excreted in the urine and the feces, and the proportion in the urine versus the feces varies among species (Hundley et al., 2006; Cui et al., 2010). It is believed that PFOA undergoes enterohepatic circulation (Kudo and Kawashima, 2003) since oral administration of cholestyramine (an anion exchange resin which is not absorbed from the gastrointestinal tract) increased the fecal elimination of PFOA in male rats by 10-fold (Johnson et al., 1984).

Data from several studies indicate that blood loss (e.g. through menstruation, blood donation, or venesection) is an additional excretion route for PFCs (Harada and Koizumi, 2009; MDH, 2013; Taylor et al., 2014; Lorber et al., 2015)

The half-life of PFOA varies among species, and it also differs between males and females in some species, most notably rats and hamsters (Table 4; Hundley et al., 2006, Lau et al., 2007). PFOA is excreted much more quickly in female rats ( $t_{1/2}$  = 2-4 hours) than in male rats ( $t_{1/2}$  = 4-6 days), while the excretion rate in hamsters is much more rapid in males than in females. The half-life in both sexes of mice is similar (17 days in females and 19 days in males), while excretion is rapid in both sexes of rabbits with half-lives of 5.5 hours in males and 7 hours in females.

The differences in excretion rates between species and between male and female rats are thought to be due to variations in renal clearance rates. These rates are controlled by specific organic anion transporters that are responsible for the active transport (secretion or reabsorption) of many organic anions, including endogenous substances and xenobiotics, across membranes in several organs including the kidney (Weaver et al., 2010; Han et al., 2012). The specific transporters believed to be responsible for renal reabsorption of PFOA have been identified in male rats as *Oatp1a1* and in humans as OAT4 and URAT1 (Han et al., 2012).

<i>Species</i>	<i>Females</i>	<i>Males</i>	<i>References</i>
Rat	2–4 hours	4–6 days	Johnson et al. (1979); Kemper and Jepson (2003)
Mouse	17 days	19 days	Lau et al. (2005)
Rabbit	7 hours	5.5 hours	Hundley et al. (2006)

Dog	8–13 days	20–30 days	Hanhijarvi et al. (1988)
Cynomolgus Monkey	30 days	21 days	Butenhoff et al. (2004a)
Human (males and females combined)	3.8 years (retired workers)		Olsen et al. (2007)
	2.3 years (adults after cessation of exposure from contaminated drinking water)		Bartell et al. (2010a)
	3.3 years (average of adults and children after cessation of exposure from contaminated drinking water)		Brede et al. (2010)
	Adults and children after cessation of exposure to contaminated drinking water.  Highly exposed group: 2.9 years (initial 4 years post-exposure); 10.1 years (>4 years post-exposure).  Less exposed group: 8.5 years (initial 9 years post-exposure); no apparent decline (>9 years post-exposure).		Seals et al. (2011)

Adapted from Lau, 2012

Renal excretion of PFOA appears to be under hormonal control (Ylinen et al., 1989; Kudo et al., 2002). Kudo et al. (2002) found that the clearance of PFOA in female rats (15 ml/min/kg) is greater than the glomerular filtration rate (10 ml/min/kg), suggesting that PFOA is actively excreted through renal tubular secretion, while the renal clearance was much lower in male rats, 0.6 ml/min/kg (Kudo et al., 2002). Castration of male rats increased the clearance to a rate similar to that in females, and this increase was reversed by administration of testosterone to the castrated rats (Ylinen et al., 1989; Kudo et al., 2002). Administration of estradiol to male rats increased the renal excretion of PFOA, and administration of testosterone to female rats reduced the clearance to a rate similar to that of male controls (Ylinen et al., 1989; Kudo et al., 2002). Probenecid, an inhibitor of renal tubular secretion of organic anions, greatly reduced the clearance of PFOA in female and castrated male rats, but had little effect on the excretion rate in control male rats (Hanhijarvi et al., 1982; Kudo et al., 2002).

The gender-dependent differences in excretion rate in rats appear to develop between 3 and 5 weeks of age (Hinderliter et al., 2006). At 4 weeks of age, serum concentrations 24 hours after a single oral dose of 10 mg/kg PFOA were similar (within 3-fold) in male and female rats, while at 5 weeks and older, serum levels were at least 30-fold higher in males than in females receiving the same dose. This greater difference in older rats resulted from age-dependent changes in both males and female. At 5 weeks of age or older, serum levels in males at this dose were about 5-fold higher than serum levels at 4 weeks, while serum levels in females 5 weeks or older are 2-3 fold lower than at 4 weeks of age.

### **Human half-life**

The half-life of PFOA in humans is several years and does not appear to differ significantly between males and females. Inter-individual differences in half-life may be due to differences in renal transport by OATs. A mean half-life of 3.8 years was estimated from data from 26 retired workers with occupational exposure, with no difference found between men and women (Olsen et al., 2007). Bartell et al (2010a) estimated a half-life of 2.3 years in a more heterogeneous study population consisting of 200 adults exposed to PFOA in drinking water. This estimate was based on average decreases in serum level of 26% and 24% for a one-year period after treatment to remove PFOA was initiated in two water districts contaminated by PFOA emissions from a West Virginia manufacturing facility. There was no evidence of age- or gender-dependence in elimination rates in this study. During the second year of follow-up of the same individuals, serum PFOA levels decreased more slowly (9% and 15% in the two water districts), suggesting either ongoing exposures from sources other than residential drinking water or that kinetics do not follow first-order elimination (Bartell et al., 2010b).

Seals et al. (2011) studied the rate of decline of serum PFOA levels in former residents of Little Hocking (n = 602) and Lubeck (n = 971), the two water districts with the highest drinking water PFOA levels of the six districts included in the C8 Health Study. Median serum levels in current and former residents of Little Hocking (current residents, 241.0 ng/ml; former residents regardless of years elapsed, 60.6 ng/ml) were much higher than in Lubeck (current residents, 69.4 ng/ml; former residents regardless of years elapsed, 31.0 ng/ml), due to the much higher drinking water PFOA concentrations in Little Hocking than Lubeck. The number of years elapsed since the former residents moved and thus stopped consuming contaminated water ranged from less than one year to almost 25 years. The data on the relationship between years elapsed since moving and serum PFOA levels suggest that PFOA elimination is biphasic and dependent on serum concentration. In former residents of Little Hocking, the half-life was 2.9 years for the first 4 elapsed years, and about 8.5 years after the first 4 elapsed years. In former residents of Lubeck, the half-life was about 8.5 years for the first 9 elapsed years, with no apparent decline in serum levels after 9 elapsed years.

PFOA levels in serum of exposed individuals were also studied in Arnsberg, Germany, where the Moehne River which is used a drinking water source was contaminated by runoff from PFOA-contaminated industrial waste applied to agricultural land (Brede et al., 2010). In a two-year study of 138 individuals before and after drinking water treatment removal was initiated, the geometric mean PFOA plasma levels declined by 39% in children and mothers, and by 26% in men; the geometric mean half-life was estimated as 3.26 years.

### **Isomer-specific kinetics**

PFOA exists as a mixture of linear and branched isomers, and the isomer profile varies depending on the manufacturing process used. PFOA made by the telomerization process is

primarily linear, while PFOA produced by electrochemical fluorination consists of a mixture of linear and branched isomers. Differences in the rates of elimination of the isomers have been investigated (Loveless et al., 2006; DeSilva et al., 2009). Loveless et al. (2006) reported that, after equivalent doses, serum levels of branched PFOA were lower than for linear PFOA in rats and mice. Similarly, after subchronic administration to rats, most branched isomers were eliminated more quickly than linear PFOA, with the exception of two minor unidentified branched isomers which had half-lives about twice that of linear PFOA (DeSilva et al., 2009). In humans, branched isomers were also more rapidly eliminated than linear isomers (Zhang et al., 2013; Gao et al., 2015). PFOA isomer profiles differed in maternal and cord serum within human infant-mother pairs, indicating that most branched isomers cross the placenta more efficiently than the linear forms (Beesoon et al., 2011). It has recently been reported that linear PFOA has a higher binding affinity than branched PFOA for human serum albumin and serum proteins in general, providing a potential explanation for the more rapid excretion of branched isomers of PFOA (Beesoon and Martin, 2015).

### **Toxicokinetics Relevant to Developmental Exposures**

#### **Summary**

It is important to consider toxicokinetics relevant to developmental exposures of PFOA in detail. Developmental effects are the most sensitive known endpoints for PFOA toxicity in experimental animals, and prenatal exposure is associated with decreased fetal growth in humans (see Toxicology and Epidemiology sections, below).

The toxicokinetics of PFOA during gestation and lactation have been studied in rats and mice but have not been evaluated in non-human primates. In rodents, PFOA is present in fetuses of dosed dams, as well as in the placenta and amniotic fluid. PFOA is also present in the breast milk of gestationally exposed dams.

Because it is excreted very quickly in female rats but very slowly in humans, the rat is not an ideal model for study of developmental effects of PFOA. In contrast, the mouse is a preferable model for evaluation of developmental effects because PFOA is excreted slowly in female mice. For this reason, many recent developmental studies have been conducted in mice.

In humans, PFOA has been measured in amniotic fluid, maternal serum, umbilical cord blood, and breast milk. PFOA concentrations are similar in maternal serum and umbilical cord blood serum, which is reflective of serum levels in the newborn. PFOA exposure in breast-fed infants is greatest during the first few months of life because both PFOA concentrations in breast milk and the rate of fluid consumption are highest during this time period. As a result, serum PFOA concentrations in breast-fed infants increase several fold from levels at birth within the first few months of life. Exposures to infants who consume formula prepared with contaminated water are also highest during this time period. These greatly elevated exposures during the first months

of life are of special concern because the neonatal period is a sensitive time period for the toxicological effects of PFOA.

### Rats

As discussed above, PFOA is excreted very rapidly by female rats (half-life of 2-4 hours). Because of its rapid excretion, PFOA is not continuously present in female rats dosed once daily, and the fetuses are thus not exposed continuously to PFOA from such a dosing regimen. Because PFOA is highly persistent in humans, the rat is not an ideal model for evaluation of developmental effects of PFOA.

In female rats given a single oral dose of PFOA, the maximum plasma concentration occurred about 1.25 hours after dosing (Kemper and Jepson, 2003). About 98.7% of an oral dose given to pregnant rats on gestation day (GD) 8 or 9 was excreted in the urine within 24 hours (Gibson and Johnson, 1983).

Kinetics of PFOA were studied in pregnant rats (strain not stated) dosed once daily by gavage with 3, 10, or 30 mg/kg/day on GD 4 to postnatal day (PND) 21, and their pups (Hinderliter et al., 2005). Because the gender difference in kinetics in rats develops at about 5 weeks of age (Hinderliter et al., 2006), plasma levels were similar in male and female pups until PND 21, the time period evaluated in this study. Plasma was taken from the dams 2 hours after dosing to allow maximum detection of PFOA, since virtually all PFOA is excreted within 24 hours after dosing of female rats. Mean PFOA concentrations in maternal plasma approximately 2 hours after doses of 3, 10, or 30 mg/kg were 11,000, 27,000, and 67,000 ng/ml, respectively. On GD 21, levels in fetal plasma were about half of those in the dams. Concentrations in milk were fairly constant on PND 3 through PND 21, and were about 10-fold lower than maternal serum levels. Pup plasma levels were about 4-fold lower than maternal plasma levels on PND 3, and about 10-fold lower at later time points. PFOA was also detected in the placenta and amniotic fluid. Interpretation of these data is complicated by the fact that maternal plasma levels varied widely during the course of the day between the daily doses, and milk and fetal/pup levels are compared to maternal plasma levels in samples taken near their daily peaks.

### Mice

In contrast to female rats, PFOA is slowly excreted in female mice with a half-life of several weeks (discussed above). Therefore, the fetus is continuously exposed when pregnant mice are dosed once daily. Because PFOA is persistent in humans, the mouse is a preferable model for evaluation of PFOA's developmental effects than the rat, and many recent developmental studies have been conducted in mice.

Fenton et al. (2009) studied the disposition of PFOA in pregnant CD-1 mice and their pups after a single oral gavage dose of 0.1, 1, or 5 mg/kg on GD 17. On GD 18 prior to delivery, the PFOA

concentration in the amniotic fluid was about half of the concentration in maternal serum. PFOA concentrations in the whole pup on GD 18 were similar to those in maternal serum. On PND 1 (the earliest time at which serum was measured in pups), pup serum PFOA concentrations were about 1.5 times the concentrations in maternal serum. The exposure of pups at this stage was thought by the authors to result primarily from *in utero* exposure rather than through lactation.

PFOA concentrations in maternal serum and in aspirated milk followed a U-shaped curve over time between PND 1 (serum) or PND 2 (milk) and PND 18, with decreases between the earliest time point (PND 1 or 2) and mid-lactation (PND 8, pups, and PND 11, milk), and increases from mid-lactation to PND 18. This increase between PND 8 and 18 was thought to result from decreased dilution of maternal serum and milk at PND 18. PFOA concentrations in pup serum and in whole pups decreased over time from postnatal day 1 to 18 on a ng/ml or ng/g basis, while the total PFOA pup body burden increased from GD 18 to PND 8, and decreased between PND 8 and 18, presumably because the intake of milk has decreased during this period. Milk concentrations were lower than maternal serum concentrations at all doses and time points, ranging from 11% to 56% of the serum concentration, with the higher percentages at early and late lactation time points.

Serum PFOA concentrations in lactating CD-1 mouse dams and their pups were also measured in a cross-fostering study of mammary gland developmental effects (White et al., 2009) in which dams were treated by gavage with 5 mg/kg/day from GD 8-17. After birth, litters of similar ages and exposures were mixed and fostered, resulting in four treatment groups: untreated dam with unexposed pup, treated dam with unexposed pup, untreated dam with pup exposed during gestation, and treated dam with pups exposed during gestation. Consistent with the single dose study (Fenton et al., 2009), serum PFOA levels on PND 1 in pups exposed *in utero* were about 50% higher than in treated dams. On PND 1, PFOA in the pups is primarily attributable to *in utero* exposure, rather than lactational exposure during the first postnatal day, since serum PFOA concentrations in pups not exposed *in utero* but nursing on treated dams were only about 3% of the concentrations in pups exposed *in utero*. Serum levels in treated dams and pups decreased between PND 1 and PND 10, with a greater decrease in pups exposed *in utero* that nursed from untreated dams than in pups exposed *in utero* that nursed from treated dams. Serum levels from pups not exposed *in utero* that nursed from treated dams rose over time, and by PND 10 serum levels in these pups were similar to levels in the treated dams they nursed from. Interestingly, serum levels in untreated dams that nursed pups exposed *in utero* increased between PND 1 and PND 10, presumably due to maternal behavior (grooming, and ingestion of urine and feces) resulting in ingestion of PFOA from the pups.

In a more recent study of effects on mammary gland development from lower doses of PFOA (Macon et al., 2011), pregnant CD-1 mice were dosed by gavage on GD 10 to 17 with 0, 0.01, 0.1, or 1 mg/kg/day. At PND 1, serum levels in the pups were 24, 285, 2304, and 16,306 ng/ml

in the untreated, 0.01, 0.1, and 1 mg/kg groups, respectively. The pup serum levels decreased over time to 3, 17, 132, and 2683 ng/ml at PND 21. Maternal serum levels were not measured by Macon et al. (2011). However, in other studies in which serum data are available for dams and pups within the same study (e.g. Fenton et al., 2009), serum levels on PND 1 were higher in pups than dams.

White et al. (2011) provide data on serum PFOA concentrations in CD-1 dams and female pups exposed to 5000 ng/L (5 µg/L) PFOA in drinking water over multiple generations. Results of this study are discussed in the section on Developmental Effects. Exposure began in P0 dams on GD 7 and continued throughout the F1 and F2 generations (except during F1 breeding and early gestation, to avoid exposing control males). Serum concentrations were 74.8 ng/ml in the P0 dams and 86.09 ng/ml in the F1 dams at weaning on PND 22, as compared to 4 and 2 ng/ml, respectively, in the corresponding control groups of dams. At this time point, the P0 dams had been exposed for about 32 days and the F1 dams had been exposed throughout their lifetimes beginning *in utero*, except during breeding and early gestation. As discussed below, serum concentrations in humans with ongoing exposure PFOA in drinking water are, on average, more than 100-fold higher than the concentration in drinking water. Thus, the serum PFOA concentrations in the mice exposed to 5000 ng/ml (5 µg/L) in drinking water are much lower than the average serum concentrations of more than 500 ng/ml expected in humans chronically exposed to this drinking water concentration.

Serum PFOA concentrations were similar in F1 and F2 pups, and concentrations in the pups were lower than in the dams at weaning. The serum concentrations in the F1 and F2 pups at PND 22 were 21.3 and 26.6 ng/ml, respectively; at PND 42, they were 48.9 and 57.4 ng/ml; and at PND 63, they were 66.2 and 68.4 ng/ml.

## Humans

### *Relationship between maternal and fetal/neonatal exposures*

PFOA has been detected in umbilical cord blood serum in studies of the general population worldwide. In seven studies reviewed by Post et al. (2012) in which both maternal and cord blood were analyzed, the mean cord blood serum:maternal serum (or plasma) ratio ranged from 0.68:1 to 1.26:1, with a mean ratio of less than 1:1 in all but one study. However, cord:maternal serum (or plasma) ratios for some individual neonate-maternal pairs within these studies were greater than 1:1. Since umbilical cord serum (or plasma) is reflective of neonatal serum (or plasma), these data indicate that serum (or plasma) levels are generally similar in the neonate and the mother. A more recent review by Kato et al. (2015) that evaluated 12 studies in total also concluded that the maternal:cord serum ratios for PFOA is approximately 1:1.

### Exposure to infants through breast milk and infant formula

PFOA is detected in human breast milk worldwide (reviewed by Liu et al., 2010; White et al., 2011a; Post et al., 2012). Factors which may potentially affect the concentration of PFOA in breast milk include whether the mother has previously nursed other infants and how soon after birth the sample is taken (Tao et al., 2008a; Haug et al., 2011; Thomsen et al., 2010). Thomsen et al. (2010) found that average breast milk concentrations were highest initially and decreased by about 7.7% per month, or about 94% during the first year of breast feeding, presumably due to decreased maternal body burden resulting from excretion into breast milk.

Breast milk PFOA concentrations were reported to be about 1% of mean general population serum levels by Tao et al. (2008a), and to be 2.5% and 9% of median maternal serum levels by Kim et al. (2011) and Liu et al. (2011), respectively. These data suggest a breast milk:maternal serum ratio of about 1:100 to 1:11. Based on a breast milk:maternal serum ratio of greater than or equal to 1:100 (Tao et al., 2008a; Kim et al., 2011; Liu et al., 2011) and a serum:drinking water ratio of greater than or equal to 100:1 (discussed below), the initial PFOA concentration in breast milk is expected to be greater than or equal to the concentration in the maternal drinking water source (Post et al., 2012).

Exposures to infants to PFOA from breast milk or formula are higher than in older individuals exposed to the same concentration of PFOA in drinking water. Mean breast milk consumption is 150 ml/kg/day during the first post-partum month when PFOA levels in breast milk are highest (Thomsen et al., 2010), and it is 83 ml/kg/day from 6-12 months of age (USEPA, 2008a). Similarly, the mean drinking water intakes in infants who consume drinking water (e.g. in formula prepared with water) are 137 ml/kg/day from birth to 1 month of age, and 53 ml/kg/day at 6-12 months of age (USEPA, 2011b). These fluid intakes are much higher than the mean drinking water consumption rates in lactating women, 26 ml/kg/day (USEPA, 2011b), and the general population (11 years of age or older), 13 ml/kg/day (USEPA, 2008a). Although breast milk or formula consumption on a body weight basis decreases as the infant gets older, it remains much higher than adult water consumption throughout infancy.

As noted above, serum PFOA levels are similar in newborns and in their mothers. Several studies, summarized below, have consistently demonstrated that serum PFOA concentrations in breast-fed infants increase by several fold during the first few months of life, presumably because both breast milk PFOA concentrations and intake of breast milk on a body weight basis are highest during this time period. Infants fed with formula prepared with contaminated drinking water also receive the greatest exposures during the first few months of life because the rate of fluid intake is highest then.

Serum PFOA levels were measured in umbilical cord blood at delivery and at 6 month and 19 months of age in infants from the German general population (Fromme et al., 2010). Average body burdens, as indicated by serum levels, were increased, typically by several-fold, from birth

to 6 months by exposure through breast milk. Levels declined between 6 months and 19 months, a time point at which breast feeding had stopped or was decreased, but remained higher at 19 months than at birth (Figure 5).

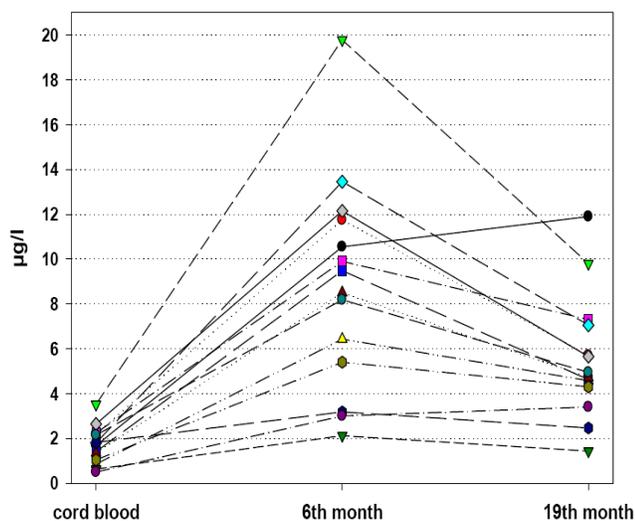


Figure 5. PFOA concentration in cord blood and blood collected in infants around six and nineteen months after birth (Fromme et al., 2010)

Duration of breastfeeding was also associated with higher serum PFOA concentrations in infants (n=49) from a community with PFOA-contaminated drinking water (the C8 Health Study). The increases were estimated as 6% per month of breastfeeding and 96% for one year of breastfeeding (Mondal et al., 2014). The authors noted that these values may underestimate the actual increases from exposure through breastfeeding, because they are based on comparisons to non-breastfed infants from the same communities who may also have had increased serum PFOA concentrations from exposures via formula prepared with contaminated drinking water.

Similarly, a study of Faroese infants (n= 80) with serum PFOA data at birth and 11, 18, and 60 months estimated an increase in serum PFOA concentrations of about 28% per month during the period of exclusive breast feeding (median of 4.5 months in the study group) and about 4% per month during the period of partial breast feeding (median of 4 additional months) (Mogensen et al., 2015). Serum PFOA concentration did not increase in non-breastfed (e.g. formula-fed) infants; presumably, the drinking water in this location was not contaminated with PFOA. Data for 12 infants from the study are shown in Figure 6.

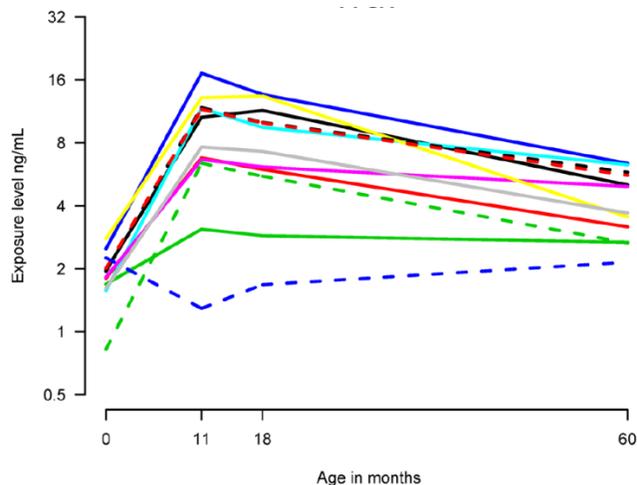


Figure 6. Serum PFOA concentrations over time in 12 infants from Mogensen et al. (2015).

Finally, Verner et al. (2016a,b) developed a pharmacokinetic model that predicts PFOA doses and plasma levels in breastfed infants and children, and their mothers. Monte Carlo simulations were used to predict the distribution of child:mother ratios for doses and plasma levels starting at birth (Figure 7). Doses (ng/kg/day) to infants were much higher than in their mothers during the first year of life. The infant:mother dose ratio peaked right after birth, with a median ratio of about 75:1 and a maximum of 231:1, and declined thereafter (Figure 7, right side). The infant:mother plasma level ratio peaked during the first year of life, with predicted ratios of 4.5-fold (median), 7.8-fold (95<sup>th</sup> percentile), and 15.3-fold (maximum) higher plasma PFOA concentrations in infants than in their mothers during the period of greatest infant exposure (Figure 7, left side).

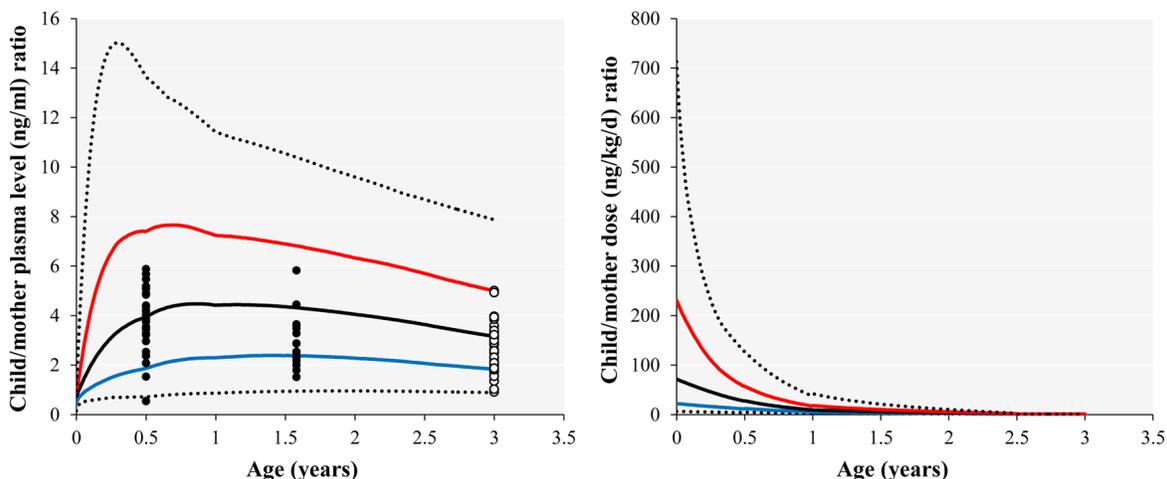


Figure 7. Monte Carlo simulations ( $n = 10\,000$ ) of child/mother ratios of plasma PFOA levels (ng/ml; right side of figure) and doses (ng/kg/day; left side of figure) for a breastfeeding period of 30 months. The black line represents the 50<sup>th</sup> percentile, the blue line represents the 5<sup>th</sup> percentile, the red line represents the 95<sup>th</sup> percentile, and the dotted lines represent minimum and maximum values (Verner et al., 2016a, b).

While peak serum PFOA concentrations occur during the first year of life, levels remain elevated for at least several additional years. Serum PFOA levels in children up to age 5 (or older) were higher than in adults in communities exposed through contaminated drinking water (Emmett et al., 2006a; Steenland et al., 2009a; Mondal et al., 2012, discussed above). In the study of Faroese children (Mogensen et al., 2015), serum PFOA levels declined after their peak in infancy but remained elevated above initial levels at birth until at least age 5 years, the last time point assessed. Similarly, the model developed by Verner et al. (2016a) predicts that plasma PFOA concentrations will remain several fold higher than at birth until at least age 3 years, the last time point modeled.

In summary, both breast-fed and formula-fed infants receive much greater exposures to PFOA from contaminated drinking water (directly or indirectly) than older individuals. Serum PFOA levels peak during the first year of life and remain elevated for several years. These elevated exposures during early life are of special concern because effects from neonatal exposure are sensitive endpoints for the toxicity of PFOA.

### **Relationship between administered dose and internal dose**

#### Repeated Dose Animal Studies

Information on kinetics in repeated dose animal studies was reviewed by Post et al. (2012). At higher doses, the kinetics of PFOA in rodents and primates (Griffith and Long, 1980; Ylinen et al., 1990; Mylchreest, 2003; Butenhoff et al., 2004a; Perkins et al., 2004; Loveless et al., 2006; Lau et al., 2006; Das et al., 2010) are not consistent with one-compartment or simple first-order models (Anderson et al., 2006; Clewell, 2009). Serum levels did not increase proportionally with increasing dose, except at lower doses in some studies. Additionally, steady-state was reached more rapidly at high doses than predicted by classical kinetics (4 to 5 half-lives).

However, at lower doses closer to those relevant to human environmental exposures, kinetics are consistent with first order processes, and serum levels are proportional to administered dose (Clewell, 2009; Lou et al., 2009; Loveless et al., 2006; Das et al., 2010). Available data indicate that serum levels in mice from doses below the administered range can be estimated by linear extrapolation from data on doses of 1 mg/kg/day or lower. The kinetics are consistent with the saturation of OATs responsible for renal reabsorption at high doses, resulting in a higher excretion rate at high doses than at low doses (Anderson et al., 2006; Clewell, 2009).

#### Human studies

##### *Relationship between drinking water and serum concentrations in exposed communities*

In communities with drinking water contaminated by PFOA, mean and median serum PFOA levels higher than in the general population. Variations in serum PFOA concentrations among individuals using the same source of drinking water arise from inter-individual differences in daily water consumption rates (L/kg/day) and/or toxicokinetic factors.

The relationship between drinking water concentration and serum concentration has been extensively evaluated for PFOA. It is well established that ongoing human exposure to PFOA in drinking water increases serum levels, on average, by at least 100 times the drinking water concentration. This conclusion is based on data from several studies of populations whose public water supplies or private wells were contaminated with a wide range of PFOA concentrations (60 ng/L to 13,300 ng/L).

In 282 residents of Little Hocking, Ohio at least six years of age exposed for two years or more, with occupationally exposed individuals excluded, the median ratio between the PFOA concentration in serum (371 ng/ml) and drinking water (3,550 ng/L) was 105:1 (25th-75th percentile range, 62:1-162:1), with a higher median ratio in young children (Emmett et al., 2006a).

This approximate 100:1 central tendency value for the serum:drinking water ratio was confirmed in communities with lower drinking water PFOA concentrations (Post et al., 2009a) based on data from approximately 70,000 residents of Little Hocking and five other Ohio and West Virginia water districts, when background serum levels found in the general population from non-water sources of exposure were taken into account. Drinking water levels in four of these districts were in the range of about 60 ng/L to 400 ng/L, while levels in a fifth district and Little Hocking were higher (Anderson-Mahoney et al., 2008).

Additionally, Hoffman et al. (2011) studied the relationship between drinking water concentrations and serum levels in the same region of Ohio and West Virginia in a study of 108 individuals using 62 private wells, with 1 to 4 participants using each well. Since the PFOA concentrations differed in each private well, this study included a greater range of drinking water concentrations than studies of the six affected public water districts in this vicinity. The median and mean PFOA levels in the wells were 200 ng/L and 800 ng/L, respectively, and the maximum concentration was 13,300 ng/L. An adjusted robust regression model of the serum and drinking water data provided an estimated serum:drinking water ratio of 141:1 (95% CI: 135:1 – 148:1), while a one-compartment pharmacokinetic model based on assumed water intake of 1.41 L/day and half-life of 2.3 years provided an estimated ratio of 114:1.

An approximate ratio of 100:1 or greater between serum and drinking water concentrations is also consistent with observations in 98 Minnesota residents tested 34 months after exposure to contaminated drinking water ended (MDH, 2009), when the expected post-exposure decline in serum levels is considered.

A lower serum:drinking water PFOA ratio of approximately 50:1 was observed in a German community whose drinking water source was contaminated with PFOA and other PFCs (Hölzer et al., 2008). Possible reasons for this difference are the use of bottled water by some

participants who were aware of the contamination for up to 6 months before their blood was sampled, uncertainty about the duration and time course of the water contamination, or differences in drinking water consumption patterns between German and U.S. residents.

*Pharmacokinetic modeling of the relationship between dose and serum concentration*

Biologically based pharmacokinetic modeling predicts a linear relationship in humans between external dose and internal dose, as measured by serum PFOA level, at doses relevant to environmental exposures such as from contaminated drinking water (Clewell, 2009). Increased serum levels that are linearly proportional to exposure levels have been observed in communities using contaminated drinking water (discussed above). However, non-linear kinetics such as observed in animals at higher doses, may occur at higher (occupational) human exposures (Clewell, 2009).

Lorber and Egeghy (2011) developed a simple single-compartment pharmacokinetic model that predicts the relationship in humans between PFOA dose (ng/kg/day) and serum concentration (ng/ml) based on volume of distribution and elimination rate, as follows:

$$\text{Serum concentration (ng/ml)} = \frac{\text{Dose (ng/kg/day)}}{\text{Volume of distribution (ml/kg)} \times \text{Elimination rate (day}^{-1}\text{)}}$$

This model uses a volume of distribution of 170 ml/kg (0.17 L/kg), based on a model calibrated with data from an Australian population exposed to PFOA through drinking water (Thompson et al., 2010), and an elimination rate of 0.0008 day<sup>-1</sup>, based on the human half-life of 2.3 years observed in the C8 Health Study population (Bartell et al., 2010a). The product of these two values provides a clearance factor that relates serum concentration (ng/ml) to dose (ng/kg/day) of 0.14 ml/kg/day (or 0.00014 L/kg/day).

The USEPA Office of Water (2016a) used the same values for volume of distribution and human half-life (elimination rate) selected by Lorber and Egeghy (2011) to derive the same clearance factor, 0.00014 L/kg/day. This value is very close to the clearance factor of 0.127 ml/kg/day (0.000127 L/kg/day) from the earlier unpublished model developed by Clewell (2006) that is discussed in Post et al. (2012).

USEPA (2016f) presents the following equation, which is equivalent to the equation presented by Lorber and Egeghy (2011) above:

$$\text{Serum Concentration (}\mu\text{g/L)} \times \text{Clearance (1.4} \times 10^{-4} \text{ L/kg/day)} = \text{Human Dose (}\mu\text{g/kg/day)}$$

The relationship between the concentration of PFOA in drinking water and serum predicted by the clearance factor of 0.00014 L/kg/day (Lorber and Egeghy, 2011; USEPA, 2016a) was

compared with the empirically observed average ratio of > 100:1 in communities with drinking water exposure to PFOA (discussed above) as follows:

The daily dose from a given concentration of PFOA in drinking water is:

$$\text{Human Dose } (\mu\text{g/kg/day}) = \text{Drinking Water Concentration } (\mu\text{g/L}) \times 0.016 \text{ L/kg/day}$$

Where: 0.016 L/kg/day is the mean daily water ingestion rate in the U.S. (USEPA, 2011b).

Therefore:

$$\text{Drinking Water Conc. } (\mu\text{g/L}) \times 0.016 \text{ L/kg/day} = \text{Serum Conc. } (\mu\text{g/L}) \times \text{Clearance } (1.4 \times 10^{-4} \text{ L/kg/day})$$

And:

$$\frac{\text{Serum Concentration } (\mu\text{g/L})}{\text{Drinking Water Concentration } (\mu\text{g/L})} = \frac{0.016 \text{ L/kg/day}}{1.4 \times 10^{-4} \text{ L/kg/day}} = 114:1$$

The serum:drinking water ratio of 114:1 based on the clearance factor and average daily water consumption is consistent with the observed ratios in communities exposed to contaminated drinking water. This calculation verifies that the clearance factor accurately predicts the relationship between human dose and human serum level. The clearance factor can therefore be used in the development of a Reference Dose (RfD) for PFOA from the Target Human Serum Level (RfD in terms of serum level).

#### *Increases in serum levels associated with PFOA in drinking water*

The increase in serum PFOA level, on average, expected from ongoing consumption of a given concentration of PFOA in drinking water can be predicted using the clearance factor, 0.00014 L/kg/day, and an assumed drinking water ingestion rate (L/kg/day).

The mean daily water ingestion rate in the U.S. is 0.016 L/kg/day (from above), and the daily water ingestion rate based on the upper percentile factors (2 L/day water consumption; 70 kg body weight) used to derive the ISGWQC is 0.029 L/kg/day. For each 10 ng/L in drinking water, ongoing exposure at the mean ingestion and upper percentile ingestion rates are predicted to increase serum PFOA by 1.2 ng/ml and 2.0 ng/ml, respectively. Increases in serum levels from various concentrations of PFOA in drinking water, and the percent increases from the most recent median serum level, 2.1 ng/ml, from NHANES (2011-12; CDC, 2015) are shown in Table 5 and Figure 8.

Table 5. Increase in serum PFOA concentrations predicted from various concentrations of PFOA in drinking water

Drinking Water Conc. (ng/L)	Mean Water Ingestion Rate (0.016 L/kg/day)			Upper Percentile Water Ingestion Rate (0.029 L/kg/day)		
	Increase in serum (ng/ml)	Total serum* (ng/ml)	% increase from drinking water*	Increase in serum (ng/ml)	Total serum* (ng/ml)	% increase from drinking water*
1	0.1	2.2	5%	0.2	2.3	10%
10	1.1	3.2	52%	2.0	4.1	95%
20	2.3	4.4	110%	4.0	6.1	190%
40	4.6	6.7	219%	8.0	10.1	381%
100	11.4	13.5	543%	20.0	22.1	952%
400	45.6	47.7	2171%	80.0	82.1	3810%

\*Total serum concentrations and % increases from drinking water are based on assumption of 2.1 ng/ml in serum (U.S. median value from NHANES, 2011-12; CDC, 2015) from non-drinking water exposures.

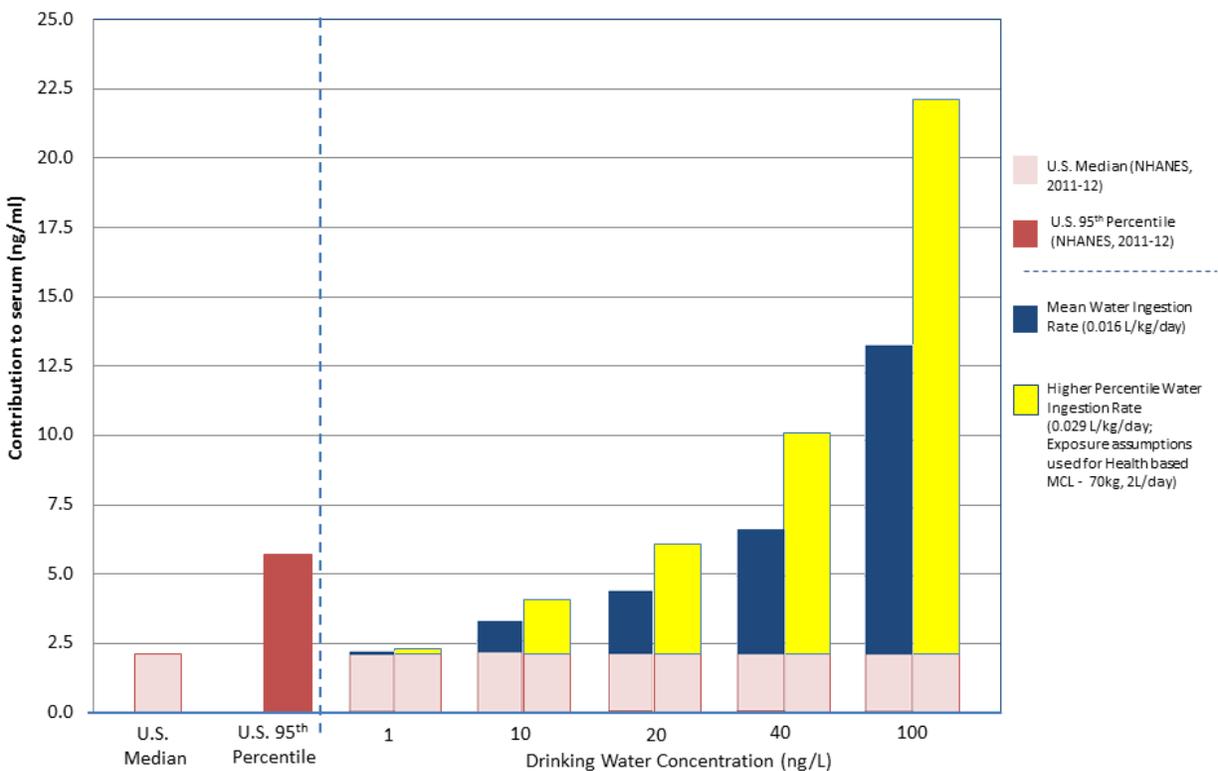


Figure 8. Increases in serum PFOA concentrations predicted from mean and upper percentile consumption of drinking water with various concentrations of PFOA, as compared to U.S. median and 95th percentile serum PFOA levels (NHANES, 2011-12).

It is evident from Table 5 and Figure 8 that relatively low concentrations of PFOA in drinking water are associated with substantial increases in serum PFOA concentrations; this has recently been observed in a study of serum PFOA levels in individuals served by PWS with PFOA detections in UCMR3 (median UCMR3 detection – 28 ng/L; Hurley et al., 2016). For example, ongoing exposure to 20 ng/L at the upper percentile ingestion rate is predicted to result in a serum concentration of 6.1 ng/ml, which is above the 95<sup>th</sup> percentile in the U.S population of 5.7 ng/ml (NHANES, 2011-12; CDC, 2015). With an average (mean) water ingestion rate, exposure to 40 ng/L is expected to result in an elevation in serum level to 6.7 ng/ml, also above the 95<sup>th</sup> percentile from NHANES. Additionally, it should be kept in mind that (as discussed above), the increases in serum levels in infants are expected to be several fold higher than those shown in Table 5 and Figure 8.

## **HEALTH EFFECTS - HUMAN STUDIES**

### **Overview**

The epidemiological database for PFOA is much larger than for most other drinking water and ground water contaminants, including those previously evaluated by NJDEP. Considering the large body of epidemiologic studies assessing associations with PFOA, it was decided to narrow and focus the human health effects section of this report. Studies of selected health endpoints were comprehensively reviewed, while information on other endpoints is summarized in the text. Conclusions of reviews of selected additional key health endpoints performed by other groups were also evaluated and are cited. This method allowed for a focus of available resources in development of this report, while maintaining a high level of scientific review.

The basis for selection of endpoints for comprehensive review was largely supported by a previous detailed evaluation of the scientific literature on PFOA by the DWQI Health Effects Subcommittee in 2009-2010, and a subsequent comprehensive review of PFOA as an emerging drinking water contaminant (Post et al., 2012). These efforts represent a large amount of work that had already been completed in reviewing information relevant to the development of a ISGWQC for PFOA and served as a starting point for the evaluation presented in this document.

Health endpoints evaluated comprehensively include: serum cholesterol/lipids, liver enzymes/bilirubin and liver disease, uric acid, thyroid function and thyroid disease, and antibody concentrations following vaccination. Some of the factors considered in selection of these endpoints were the extent and consistency of the data, whether the effect has been observed at exposures relevant to potential drinking water exposures, and evidence for reverse causality. Comprehensive evaluation involved the review of peer-reviewed studies identified through an *a priori* literature search and screening criteria. An individual study table summarizing the study design, location, study population characteristics, outcome and exposure assessment, study population exposure, statistical methods, results, major limitations which addresses risk of bias, and funding source for each reviewed study can be found in Appendix 4, and tables summarizing

all studies of each endpoint are found below. Two other critical endpoints, fetal growth following developmental exposure and cancer, were recently comprehensively reviewed by other authoritative scientific groups. Review reports by these groups are evaluated and summarized in this document.

In total, 54 epidemiological studies assessing associations with serum cholesterol/lipids, liver enzymes/bilirubin, uric acid, thyroid function and thyroid disease, and/or antibody concentrations following vaccination were evaluated in depth. The studies were conducted on populations in the U.S., Canada, and several European and Asian countries. The studies evaluated the general population, communities with drinking water contaminated with PFOA, and occupationally-exposed workers, thereby assessing health effects over a wide range of PFOA exposures and serum concentrations.

In human environmental health effect studies in general, confounding by co-exposure to contaminants other than the one being evaluated may be particularly important since it may bias results. In some instances, PFOA has been shown to be strongly correlated with other co-occurring PFCs which may not have been controlled for, and the same may be true for other environmental contaminants. This confounding bias could impact studies in any type of population, but may play a more important role in occupational populations which may be more likely than the general population to be exposed to co-occurring contaminants at meaningful levels. In general, co-exposure to other chemicals could also be more likely in communities where there are high levels of environmental contamination. However, this is not likely the case in the C8 Health Project, a large community study of populations with drinking water exposure to PFOA (discussed in more detailed below), since PFOA is the only contaminant that was reported to be present at elevated levels in drinking water or other environmental media.

As is the case for epidemiologic studies of environmental contaminants in general, the nature of these observational epidemiology studies, in contrast to experimental studies, limits our ability to definitively conclude that PFOA causes health effects. However, the findings from observational epidemiology studies are useful in assessing consistency, strength of association, exposure-response, temporality, specificity, and biologic plausibility - criteria which are useful in assessing causation.

### **Studies of Exposure Levels Found in the General Population**

For the endpoints that were comprehensively reviewed, the majority of studies evaluated the general population and/or study populations with general population-level exposures to PFOA. Twenty-nine (29) studies with general population, low-level exposures were identified. The serum PFOA concentrations (based on a measure of central tendency, which was presented as median, mean, or geometric mean) in these studies range from 0.9 to 7.1 ng/ml. A strength of the general population studies is their use of serum PFOA levels as the basis for exposure

assessment. Because of the long human half-life of PFOA, serum levels do not rapidly fluctuate with short term variations in exposure, and serum levels taken at a single time therefore reflect long-term exposures. Serum levels thus provide an accurate measure of internal exposure for each study participant, an advantage over studies based on external exposure metrics such as drinking water concentrations. Among these studies, the large majority are cross-sectional (23 studies, plus one which includes a cross-sectional component).

A general limitation of cross-sectional studies is that they evaluate information on both exposure and outcome at the same point in time, limiting their ability to establish temporality.

### **Studies in Exposed Communities**

For the endpoints selected for comprehensive evaluation, 15 studies evaluated highly-exposed individuals residing in communities with known PFOA drinking water contamination or in close proximity to a factory utilizing or producing PFOA. A large majority of these studies (14) occurred among communities in the Mid-Ohio Valley near the DuPont Washington Works plant in Parkersburg, WV. This industrial facility used large amounts of PFOA in the manufacturing of a fluoropolymer, polytetrafluoroethylene (PTFE), and discharged PFOA to the environment resulting in widespread drinking water contamination. Many of the studies in this population are the result of the settlement of a class-action lawsuit by residents exposed to PFOA-contaminated drinking water which mandated that DuPont fund a health study called the C8 Health Project. Additional epidemiologic studies of associations with PFOA and health endpoints in this population have also been published by other researchers.

The C8 Health Project is a community health study of approximately 70,000 Ohio and West Virginia residents of all ages (infants to very elderly) with at least one year of exposure to drinking water contaminated with PFOA at >50 ng/L to over 3000 ng/L (Frisbee et al, 2009; C8 Science Panel, 2014). The C8 Health Project was conducted by the C8 Science Panel, which consisted of three epidemiologists chosen jointly by the parties involved in the legal settlement. This study is notable because of its large size, the wide range of exposure levels, and the large number of parameters evaluated. Data collected included serum levels of PFOA and other PFCs, clinical laboratory values, and health histories. The median serum PFOA concentration in this population was 28 ng/ml (ppb), and serum concentrations in the lowest two deciles were within the U.S. general population range at the time (<10 ng/ml).

The C8 Science Panel was charged with determining if “probable links” exist between diseases and PFOA exposure in the C8 study population, based on the results of their studies and other information from the scientific literature. Probable links were defined as “.... given the scientific evidence available, it is more likely than not that a connection exists between C8 exposure and a particular human disease among class members...”. Probable links were established with PFOA exposure and six health endpoints (clinically defined high cholesterol, kidney and testicular

cancer, ulcerative colitis, thyroid disease, and pregnancy-induced hypertension). For a number of other endpoints, no probable link with PFOA exposure was reported. Associations were also found with additional health endpoints for which no probable link evaluation was conducted because they were not considered to be clinically defined diseases. These endpoints include increased serum levels of liver enzymes, uric acid, C-reactive protein, and others. C8 Science Panel reports and citations for peer-reviewed publications presenting the results of these studies are found at the C8 Science Panel website (C8 Science Panel, undated, b).

### **Occupational Studies**

There are 14 peer-reviewed occupational studies of the endpoints chosen for detailed evaluation, three of which also studied exposed community populations (Wang et al., 2012, Winquist and Steenland, 2014a, and Winquist and Steenland, 2014b). Eight of the 14 occupational studies are cross-sectional. Locations include industrial facilities in the U.S., Italy, Belgium, and China. Occupational studies are often considered useful for evaluating effects of environmental contaminants because exposure levels are generally higher than in general population or in communities exposed through site-specific environmental contamination. Mean or median serum PFOA levels in occupational studies reviewed in this report were generally over 1,000 ng/ml (ppb), several orders of magnitude higher than the median concentrations in the general population or in communities with drinking water exposure.

Associations of PFOA with some clinical parameters, including cholesterol, liver enzymes, and uric acid, exhibit a steep dose-response curve in the lower exposure range found in the general population, with a much flatter slope (approaching a plateau) at higher exposure such as those found occupationally (discussed in more detail below). For dose-response curves of this type, the associations found in populations with lower exposures may not be observed in workers because even the least exposed workers used as the comparison/reference group in occupational studies may have exposure levels that are high enough to fall on the much flatter upper portion of the dose-response curve.

Occupational studies may also have a selection bias from a “healthy worker effect” whereby workers usually have lower overall mortality and morbidity than individuals of the same age as a whole, since severely ill and disabled persons are typically not included in the workforce, especially in industrial settings (Shah, 2009). Longer duration of employment may also increase the effects of this bias, since sick people will be more likely to leave or change to safer work. Therefore, data based on duration of employment may not accurately reflect higher prevalence or larger magnitude of effects that are associated with longer exposures to the contaminant being evaluated.

Another issue with occupational studies of PFOA is the small number of exposed female employees which limits the ability of the occupational epidemiology to adequately address specific effects among women.

An additional issue is the possibility of effect modification due to exposure to other chemicals. Exposure to other PFCs, including PFOS at the 3M Decatur plant, may have played a role in the observed associations. Differences in exposures to other chemicals among manufacturing facilities may result in differences in degree of association with various effects.

### **Comprehensively Reviewed Endpoints**

#### **Serum lipids**

Associations of serum lipids and PFOA were evaluated in 24 studies, each of which included one or more of the following endpoints: total cholesterol, high density lipid cholesterol (HDL), non-HDL, ratio of total cholesterol to HDL, low-density lipid cholesterol (LDL), very low-density lipid cholesterol (VLDL), ratio of HDL to LDL, and triglycerides. There is also one additional study which only evaluated expression of genes related to cholesterol transport in humans (Fletcher et al., 2013). Study details are provided in the tables for individual studies (Appendix 4) and the summary table for serum lipids (Table 6A).

In total, 20 studies evaluated serum total cholesterol and two evaluated self-reported clinically defined high cholesterol (Steenland et al., 2015 and Winqvist and Steenland, 2014). Among the 20 serum total cholesterol studies, 15 were cross-sectional (Emmett et al., 2006b; Eriksen et al., 2013; Fisher et al., 2013; Frisbee et al., 2010; Fu et al., 2014; Geiger et al., 2014; Gilliland et al., 1996; Nelson et al., 2010; Olsen et al., 2000; Olsen and Zobel 2007; Sakr et al., 2007a; Starling et al., 2014; Steenland et al., 2009; Wang et al., 2012; and Zeng et al., 2015) and two studies included cross-sectional and other analyses (Costa et al., 2009; and Olsen et al., 2003). The cross-sectional studies include seven studies of the general population or individuals with low-level exposures (Eriksen et al., 2013; Fisher et al., 2013; Fu et al., 2014; Geiger et al., 2014; Nelson et al., 2010; Starling et al., 2014; and Zeng et al., 2015); four studies of residents of highly exposed communities (Emmett et al., 2006b; Frisbee et al., 2010; Steenland et al., 2009; Wang et al., 2012); and five studies of occupationally exposed individuals (Gilliland et al., 1996; Olsen et al., 2000; Olsen and Zobel 2007; Sakr et al., 2007a; and Wang et al., 2012). Five remaining studies evaluating serum total cholesterol and PFOA include an occupational case-control study (Costa et al., 2009), and four cohort studies including one study of residents of a highly-exposed community (Fitz-Simon et al., 2013) and three studies of occupationally exposed individuals (Olsen et al., 2003; Olsen et al., 2012; and Sakr et al., 2007b).

Six of seven cross-sectional studies of the general population or populations with low-level exposures found evidence of statistically significant positive associations with serum cholesterol and PFOA. These studies of general population level exposures include a study nested in a larger

cohort in Denmark of adults, aged 50 to 65 years, with mean serum PFOA concentration of 7.1 ng/ml (Eriksen et al., 2012); a general population study in Canada with a PFOA geometric mean of 2.5 ng/ml (Fisher et al., 2013); a small study of individuals randomly selected from attendees at a health check-up clinic with a median serum PFOA concentration of 1.4 ng/ml (Fu et al., 2014); a study of children in the U.S. general population with a serum PFOA mean concentration of 4.3 ng/ml (Geiger et al., 2014); a study of the general U.S. population aged 12 years older with a median PFOA concentration of 3.8 ng/ml (Nelson et al., 2010); and a study of subjects recruited from the control group of another study in Taiwan with median PFOA exposures of 1.1 ng/ml in boys and 0.9 ng/ml in girls (Zeng et al., 2015). A study of pregnant women recruited from a larger cohort in Norway, with a median serum PFOA concentration of 2.3 ng/ml, did not find a statistically significant positive association with PFOA and serum cholesterol; however, results showed a positive and increasing association of cholesterol with increasing quartiles of PFOA (Starling et al., 2014).

Two large cross-sectional studies evaluated individuals residing in communities located in the mid-Ohio Valley with drinking water contaminated with PFOA. One study included 12,476 children aged 1 to 17.9 years with a mean serum PFOA concentration of 69.2 ng/ml (Frisbee et al., 2010) and the other included 46,294 individuals aged 18 years or older with a median serum PFOA concentration of 27 ng/ml (Steenland et al., 2009). Both studies found a positive, statistically significant association of serum PFOA and cholesterol. A third smaller (n=371) cross-sectional study from the water district in the mid-Ohio Valley with the highest PFOA levels in its drinking water, with a much higher median serum PFOA concentration, 354 ng/ml, did not find a statistically significant association (Emmett et al., 2006b). A fourth study from China, which in addition to a study of 132 residents located near a plant utilizing PFOA with a median PFOA concentration of 284 ng/ml also included a worker study, did not find an association with serum cholesterol in either group (Wang et al., 2012).

Of the five occupational cross-sectional studies, only one U. S. occupational study (n=840) with a median serum PFOA concentration of 189 ng/ml found a positive statistically significant association with serum cholesterol (Sakr et al., 2007a). The remaining four occupational cross-sectional studies which did not find evidence of an association include two U.S. male only worker studies, one with a mean serum PFOA concentration of 3,300 ng/ml and a sample size of 115 (Gilliland et al., 1996), and one with a mean serum PFOA concentration of 1,190 ng/ml with a sample size of 265 (Olsen et al., 2000). The third study took place in both the U.S. and Belgium with a median PFOA concentration of 2,210 ng/ml and a sample size of 506 (Olsen and Zobel 2007) and the fourth cross-sectional study included 55 workers in China with a median PFOA concentration of 1,636 ng/ml (Wang et al., 2012).

Five of the 20 studies had study designs other than cross-sectional. A longitudinal analysis of workers from Belgium and U.S. with a range of PFOA means of 1,220 to 1,900 ng/ml (Olsen et

al., 2003), and another longitudinal worker cohort analysis from the U.S. with a range of PFOA exposure from 1,010 to 1,160 ng/ml (Sakr et al., 2007b), both found evidence of an association with PFOA and serum cholesterol. A third occupational cohort study utilizing matched-pair analysis of 98 to 179 workers (highly exposed of 881 ng/ml PFOA mean v. lower exposed of 28.9 ng/ml PFOA mean) did not find a statistically significant association (Olsen et al., 2012). None of these studies found evidence of a statistically significant inverse association with serum cholesterol and PFOA. An Italian male occupational case-control study with PFOA median concentration 4,400 among formerly exposed workers and a median of 5,700 ng/ml among currently exposed workers, with cross-sectional analysis, found evidence of a positive association (Costa et al., 2009). Among the cohort studies, a longitudinal study of individuals in highly-exposed mid-Ohio Valley communities, with geometric mean PFOA concentrations of 74.8 ng/ml at baseline and 30.8 ng/ml at follow-up, found evidence of a positive association (Fitz-Simon et al., 2013).

Although 15 of the 20 studies evaluating associations of PFOA and serum cholesterol were cross-sectional, thereby limiting the interpretation of temporality since exposures and outcomes are measured at the same point in time, four studies included longitudinal analyses (Fitz-Simon et al., 2013; Sakr et al., 2007b; Costa et al., 2009; Olsen et al., 2003). Each of these studies had multiple measurement data, and all four found a significant correlation over time between cholesterol and PFOA levels (Fitz-Simon et al., 2013; Steenland et al., 2010b). In summary, the epidemiologic data provide evidence of consistency, strength and dose-response, including some evidence of temporality, of PFOA and serum cholesterol.

Several of the studies mentioned above showed statistically significant trends for increased serum cholesterol with increasing serum PFOA. A decile analysis of PFOA with total cholesterol among a large study of residents of a highly exposed community showed an increasing effect of PFOA on cholesterol and additionally the odds of clinically defined hypercholesterolemia ( $\geq 240$  mg/dL) increased 40-50% from the lowest to the highest quartile of PFOA (Steenland et al., 2009). A statistically significant trend of increasing serum cholesterol with increasing PFOA was also reported in at least five other studies (Frisbee et al., 2010, Fu et al., 2014, Geiger et al., 2014; and Zeng et al., 2015).

In summary, general population level exposure studies (seven), found consistent evidence of a positive association between PFOA and serum cholesterol. Additionally, three very large studies (two cross-sectional and a cohort study) of highly exposed community populations found evidence of a positive association between PFOA and serum cholesterol. Two longitudinal occupational studies also found a positive association, along with one case-control occupational study. In contrast, results from two much smaller cross-sectional studies of highly exposed community populations (with higher median population exposures than the three larger studies) and a matched-pairs occupational study did not find an association. Although findings from the

occupational cross-sectional studies in general (four out of five) found no evidence of an association, they may be biased toward the null by a healthy worker effect. This is suggested by a similar pattern of inconsistency among these study's findings as compared to the findings from the corresponding database were also noted for other serum lipid endpoints (HDL and LDL – discussed below).

In general, studies of the general population, as well as large, mid-exposure range community studies and occupational studies with longitudinal designs, found consistent evidence of an association, while a few smaller, higher exposure range community and occupational studies found no evidence. None of the 20 studies evaluated found evidence of an inverse association.

A review by Steenland et al. (2010a) summarized and evaluated the epidemiologic literature on PFOA and cholesterol available at that time. The authors noted that the lower the range of PFOA that was studied, the greater the change in cholesterol per unit change in PFOA. They suggest that, as discussed in Occupational Studies (above), an exposure-response relationship that is steep at low PFOA concentrations and then flattens out (i.e. approaches a plateau) at higher serum PFOA concentrations is a possible explanation for the observed differences in effect magnitudes. Therefore, studies of populations with high serum PFOA concentrations may not detect an association of PFOA with serum cholesterol if there is a steep dose-response curve for the association in the lower exposure ranges. For dose-response curves of this type, associations may not be evident in populations with higher exposures since even the least exposed individuals in the comparison group may have exposures that fall on the much flatter (approaching a plateau) portion of the exposure/response curve.

Associations of PFOA and high-density lipid cholesterol (HDL), non-HDL, ratio of total cholesterol to HDL, low-density lipid cholesterol (LDL), very low-density lipid cholesterol (VLDL), ratio of HDL to LDL, and/or triglycerides were evaluated in 20 studies. All but two of these 20 studies also evaluated serum cholesterol.

HDL and PFOA were evaluated in 19 studies. It should be noted that an increase in HDL is considered to be beneficial, as compared to increases in total cholesterol, LDL, and non-HDL, which are considered to be undesirable. None of these studies found an association with increased HDL, while four of the 19 studies found evidence of statistically significant decreased association with HDL (Gilliland et al., 1996; Olsen et al., 2000; Olsen and Zobel 2007; and Wang et al., 2012). Interestingly, these four studies are all occupational cross-sectional studies which also did not find evidence of an association with PFOA and increased serum cholesterol (described above), whereas the only other additional occupational cross-sectional study found no evidence of an association with HDL but did find a statistically significant positive association between PFOA and cholesterol (Sakr et al., 2007a). These differences in findings suggest that these occupational cross-sectional studies may be biased from a healthy worker effect. There was no evidence of statistically significant associations with HDL in any of the other 15 studies

(Costa et al., 2009; Fisher et al., 2013; Fitz-Simon et al., 2013; Frisbee et al., 2010; Fu et al., 2014; Geiger et al., 2014; Lin et al., 2011; Nelson et al., 2010; Olsen et al., 2003; Olsen et al., 2012; Sakr et al., 2007a; Sakr et al., 2007b; Starling et al., 2014; Steenland et al., 2009; Wang et al., 2012 [resident study]; and Zeng et al., 2015).

Non-HDL was evaluated in four studies: two general population cross-sectional studies (Fisher et al., 2013; and Nelson et al., 2010), a U.S. occupational longitudinal study (Olsen et al., 2012), and a large cross-sectional study of residents in highly exposed communities (Steenland et al., 2009). Three of the studies found statistically significant positive associations with non-HDL and PFOA (Fisher et al., 2013; Nelson et al., 2010; and Olsen et al., 2012), while the occupational longitudinal study had a negative association with non-HDL which was not statistically significant (Olsen et al., 2012).

The ratio of total cholesterol to HDL was evaluated in three studies with inconsistent findings. A general population study in Canada did not find evidence of a statistically significant association (Fisher et al., 2013), U.S. occupational longitudinal study found a statistically significant negative association (Olsen et al., 2012), and a large study of residents from a highly exposed community found a statistically significant positive association (Steenland et al., 2009).

Associations of LDL and PFOA were evaluated in 16 studies. Fourteen of the studies are cross-sectional, which includes seven low level exposure populations (Fisher et al., 2013; Fu et al., 2014; Geiger et al., 2014; Lin et al., 2013; Nelson et al., 2010; Starling et al., 2014; and Zeng et al., 2015), three studies of residents from a highly exposed community (Frisbee et al., 2010; Steenland et al., 2009; Wang et al., 2012), and five studies of occupationally exposed individuals (Gilliland et al., 1996; Olsen et al., 2000; Olsen and Zobel 2007; Sakr et al.; 2007a ; and Wang et al., 2012). The other two studies of LDL and PFOA include an occupational longitudinal study (Sakr et al., 2007b) and a cohort study of residents from the highly exposed community, mid-Ohio Valley (Fitz-Simon et al., 2013).

Among the cross-sectional studies of populations with low level exposure, three found evidence of statistically significant positive associations with LDL (Fu et al., 2014; Geiger et al., 2014; and Zeng et al., 2015) and four found no statistically significant evidence of an association (Fisher et al., 2013; Lin et al., 2013; Nelson et al., 2013; and Starling et al., 2014). Of the three cross-sectional studies of residents from a highly exposed community; the two large studies in the mid-Ohio Valley, one which included children and the other of adults, found evidence of statistically significant positive association (Frisbee et al., 2010, and Steenland et al., 2009); while the third smaller study of 132 residents in China found no evidence of an association (Wang et al., 2012). Four of the five occupational cross-sectional studies found no association (Gilliland et al., 1996; Olsen et al., 2000; Olsen and Zobel, 2007; and Wang et al., 2012) while only one of the studies found evidence of a statistically significant association with both LDL

and VLDL (Sakr et al., 2007a). Additionally, an occupational longitudinal study found a positive, non-statistically significant association with LDL (Sakr et al., 2007b) while a cohort study of residents from a highly exposed community found a statistically significant positive association (Fitz-Simon et al., 2013). Finally, the ratio of HDL to LDL was evaluated in a cross-sectional study which assessed both occupational and highly exposed residential populations and found a negative association with the worker population and no evidence of a statistically significant association with the residential population (Wang et al., 2012).

In summary, positive associations with PFOA and LDL were inconsistent among low level exposure populations, and largely unassociated in occupational studies, but there is consistent evidence of an association with PFOA and LDL among larger studies of the highly exposed mid-Ohio Valley communities: two cross-sectional studies one among children and another among adults, and a longitudinal study.

Sixteen studies evaluated triglycerides with inconsistent findings. Four of the studies found evidence of positive statistically significant association (Frisbee et al., 2010; Olsen et al., 2003; Olsen and Zobel, 2007; and Zeng et al., 2015), one found evidence of a negative statistically significant association (Lin et al., 2013), and 11 studies found no evidence of a statistically significant association (Costa et al., 2009; Fisher et al., 2013; Fitz-Simon et al., 2013; Fu et al., 2014; Geiger et al., 2014; Lin et al., 2011; Olsen et al., 2000; Sakr et al., 2007a; Sakr et al., 2007b; Starling et al., 2014; and Wang et al., 2012).

Selection bias may be an issue in Fu et al. (2014) since the study included only individuals attending a health clinic check-up such that individuals concerned with existing health issues may be more likely to be included. Selection bias may also be an issue in Lin et al. (2013), which included individuals with an abnormal urinalysis from a population-based screening program in which the final study population was made up of 246 (37%) individuals with elevated blood pressure. Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. In contrast, some occupational studies relied on medical record abstraction of clinical parameters. Other limitations of occupational studies include small sample size that may limit power to detect associations, possibility of healthy worker effect, inclusion of few or no women, and the possibility that exposure in the least exposed groups may be well above the population exposure range in occupationally exposed individuals.

The biological plausibility of the association of PFOA and serum cholesterol was investigated in a study of associations of serum PFOA and changes in expression of genes involved in cholesterol metabolism. In this cross-sectional study, expression of 13 genes involved in cholesterol metabolism (cholesterol biogenesis, peroxisome proliferation, cholesterol transport, downstream transcriptional activation of PPAR-alpha, and mobilization of cholesterol) was

evaluated in whole blood from 290 subjects from a highly exposed community (geometric mean serum PFOA, 32.2 ng/ml). Statistically significant associations between genes involved in cholesterol transport and mobilization and PFOA were found, and the affected genes differed in men and women. The authors state that these change in gene expression “appear consistent with PFOA promoting a hypercholesterolemic environment” (Fletcher et al., 2013).

Effects of PFOA on serum lipids in laboratory animals are considered in evaluating the biological plausibility of the associations of PFOA and cholesterol found in humans. Serum cholesterol was not affected by PFOA in a 6-month study of cynomolgus monkeys (Butenhoff et al., 2002), while triglycerides were significantly increased compared to controls at several time points in the monkeys in the mid- and high dose groups (10 and 20/30 mg/kg/day). As discussed above, the dose-response curve for increased cholesterol in humans appears steepest at serum PFOA levels below about 40 ng/ml, with a much flatter dose-response at higher serum levels. In the monkey study, the mean serum PFOA level in the control group was 134 ng/ml. If the dose-response curve is similar in monkeys such that effects on serum cholesterol are steepest at lower serum PFOA levels, effects may not be observable because the control exposure levels could be high enough to fall on the much flatter portion of the dose-response curve.

Although PFOA is consistently associated with increased serum cholesterol in humans, serum cholesterol and triglycerides are generally decreased by PFOA in rodents. This effect in rodents is attributed to PPAR-alpha activation (Lau, 2013). As discussed in detail in the Mode of Action section below, PPAR-alpha activators typically reduce serum lipids in both rodents and humans, and this is the basis for the use of fibrates as hypolipidemic drugs in humans. It is well established that the effects of PFOA in rodents differ from those of other PPAR-alpha activators, and that PFOA affects rodents through both PPAR-alpha independent and PPAR-alpha dependent pathways. It is possible that the dissimilar effects of PFOA on serum lipids in humans and rodents could arise from a different balance of PPAR-alpha dependent and independent processes with opposite effects on this endpoint.

Two recent studies, discussed in detail in the Mode of Action section, suggest the important possibility that the contrasting effects on serum cholesterol observed in humans and animals may result from differences in dietary fat content, rather than intrinsic interspecies biochemical or physiological differences (Tan et al., 2013; Rebholz et al., 2016). In these studies, mice were fed a diet with a high fat content, similar to that of a typical U.S. diet, instead of standard lower fat laboratory chow. Serum cholesterol was either increased or unaffected in mice fed the high fat diets. In contrast, serum cholesterol was decreased, as is typically seen in mice fed the regular diet in Tan et al. (2013), while Rebholz et al. (2016) did not include a regular diet group. These results suggest that effects of PFOA on cholesterol may be similar in rodents and humans consuming the same dietary fat content, and they provide evidence for biological plausibility for PFOA's effects on serum cholesterol in humans.

In summary, the epidemiologic database for serum cholesterol and PFOA, which included twenty studies, provides evidence of consistency, strength and dose-response, including some evidence of temporality. Associations with clinically defined hypercholesterolemia were reported in some studies. These findings provide evidence supporting a causal relationship between PFOA and serum cholesterol. Overall, the epidemiologic evidence suggests no evidence of an association with HDL and PFOA. There were a limited number of epidemiologic studies evaluating an association with non-HDL or the ratio of total cholesterol to HDL and PFOA. The epidemiologic database for PFOA and LDL appears inconsistent. Although there is some evidence of an association with LDL, it remains limited due to the interpretation of other studies which found no evidence of an association. There is limited epidemiologic evidence evaluating associations of VLDL, the ratio of HDL to LDL, and triglycerides with PFOA.

#### Liver enzymes/bilirubin

A total of 16 studies evaluated associations between PFOA and clinical biomarkers used in the diagnosis and/or evaluation of treatment of liver function or metabolic disease. These biomarkers include the following liver enzymes: alanine aminotransferase (ALT /SGPT), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST /SGOT), and alkaline phosphatase (ALP), as well as total bilirubin or unspecified bilirubin (TB) and direct bilirubin (DB). Additionally, two studies assessed PFOA and liver disease (LD). Study details are provided in the tables for individual studies (Appendix 4) and the summary table for liver enzymes/bilirubin (Table 6B).

Two larger cross-sectional general population studies utilizing different survey cycles of the U.S. National Health and Nutrition Examination Survey (NHANES) both found evidence of statistically significant positive associations with PFOA and the liver enzyme ALT (Gleason et al., 2015 and Lin et al., 2010). Two other low cross-sectional studies of a population with low-level exposure cross-sectional studies have also evaluated this association. A study that was based on a population recruited from a larger cohort in Taiwan (n=608) found a positive statistically significant correlation (Yamaguchi et al., 2013), while a small study (n=141) of pregnant women in China did not find a significant correlation between PFOA and ALT (Jiang et al., 2014). Of the three cross-sectional studies of mid-Ohio Valley residents, the smaller study (n=371) with a higher median and narrower range of PFOA exposure found no evidence of an association (Emmett et al., 2006b), while the two larger studies (n=47,092) with a wider range of exposures found a consistent positive statistically significant association with ALT and PFOA (Gallo et al., 2012; and Gallo et al., 2016). Nine additional occupational studies investigated associations of ALT and PFOA with inconsistent findings. Among these studies only one cross-sectional study found evidence of a positive association (Olsen et al., 2007), one found evidence of a negative association (Gilliland et al., 1996) and four cross-sectional studies found no consistent evidence of an association (Olsen et al., 2000; Olsen et al., 2003; Sakr et al., 2007a; and Wang et al., 2012). An occupational case-control study, with cross-sectional components, found some evidence of a positive association (Costa et al., 2009); one longitudinal occupational

study found evidence of a negative association (Olsen et al., 2012), and a second longitudinal occupational study found no evidence of an association (Sakr et al., 2007b).

Although results of occupational studies were inconsistent, both cross-sectional general population studies found evidence of an increasing trend (Gleason et al., 2015 and Lin et al., 2010). The much larger studies of a highly-exposed community also found increasing levels of ALT with increasing serum concentrations of PFOA (Darrow et al., 2016; Gallo et al., 2012). Further, the associations noted by Gallo et al. (2012) were consistent both between water districts and among individuals within the same district, which also increased the strength of evidence. Additionally, the modeled serum PFOA exposure assessment used by Darrow et al. (2016) complements evidence from previous studies because these estimates are not affected by reverse causation.

Thirteen studies evaluated associations of PFOA and GGT: six studies found evidence of a positive statistically significant association (Costa et al., 2009; Gallo et al., 2012; Gleason et al., 2015; Lin et al., 2010; Olsen and Zobel, 2007; and Sakr et al., 2007a) and the remaining seven studies found no statistically significant evidence of an association (Darrow et al., 2016; Emmett et al., 2006b; Gilliland et al., 1996; Olsen et al., 2000; Olsen et al., 2003; Sakr et al., 2007b; and Yamaguchi et al., 2013). Twelve studies also evaluated the association of PFOA and AST; three found evidence of a positive statistically significant negative association (Gleason et al., 2015; Sakr et al., 2007b; Yamaguchi et al., 2013); two studies found some evidence of a negative association (Gilliland et al., 1996 and Wang et al., 2012) and seven other studies found no evidence (Emmett et al., 2006; Jiang et al., 2014; Olsen et al., 2000; Olsen et al., 2003; Olsen and Zobel 2007; Olsen et al., 2012; and Sakr et al., 2007a).

Eight studies evaluated the association of PFOA and the liver enzyme ALP. Only one found some limited evidence of a positive statistically significant association (Costa et al., 2009), while the other seven studies found no evidence of an association (Emmett et al., 2006b; Gleason et al., 2015; Olsen et al., 2000; Olsen et al., 2003; Olsen and Zobel, 2007; Olsen et al., 2012; and Sakr et al., 2007b).

Thirteen studies evaluated the association of PFOA and either total or direct bilirubin. A component of total bilirubin is direct bilirubin, a product of hemoglobin metabolism for which increased serum concentrations reflect increases in liver and bile duct disease. Therefore, total bilirubin serves only as an inferential measure of liver function. Among studies of total bilirubin, three studies found evidence of a statistically significant association (Costa et al., 2009; Olsen and Zobel, 2007; and Sakr et al., 2007b); one study found a positive statistically significant association (Gleason et al., 2015); and seven found no association with total bilirubin (Emmett et al., 2006b; Jiang et al., 2014; Lin et al., 2010; Olsen et al., 2000; Olsen et al., 2003; Olsen et al., 2012; and Sakr et al., 2007a). Two additional studies found no association with direct bilirubin

(Gallo et al., 2012; and Darrow et al., 2016), and Olsen et al. (2000) also found no association with total or direct bilirubin.

Three studies investigated association with PFOA and clinical liver disease. Melzer et al., 2011 found no statistically significant association of PFOA and current liver disease in a cross-sectional study of the U.S. general population (NHANES). Also, Darrow et al. (2016) found no evidence of an association with modeled serum PFOA and medically-validated liver disease when categorized as either any liver disease or restricted to enlarged liver, fatty liver, or cirrhosis among the highly exposed C8 Health Study community. A retrospective cohort of a U.S. occupational population also found no association with self-reported liver disease and estimated serum PFOA concentration (Steenland and Zhao et al., 2015).

As previously described, cross-sectional studies limit interpretation of temporality. Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. Small sample sizes in some studies may have limited their power to detect associations (Emmett et al., 2006b; Jiang et al., 2014; Wang et al., 2012; and Yamaguchi et al., 2013). In addition to small sample size, some occupational studies relied on abstraction of clinical parameters from medical records. Other limitations of occupational studies include the possibility of healthy worker effect, inclusion of few or no women, and the possibility that exposure in the least exposed groups may be well above the population exposure range in occupationally exposed individuals.

Toxicological and mode of action data support the biological plausibility of hepatic effects of PFOA in humans. As discussed in detail in the Toxicology and Mode of Action sections below, it is well established that hepatic toxicity is a sensitive effect of PFOA in experimental animals. Based on studies in non-human primates, standard strains of rodents, and rodents lacking a functional PPAR-alpha receptor, hepatic effects of PFOA are considered relevant to humans.

In summary, the evaluation of epidemiologic studies provides evidence of some inconsistencies among the group of studies evaluated. However, there was consistency among the larger non-occupational studies, as well as evidence of specificity, exposure-response, strength, and biologic plausibility for PFOA and ALT. These findings provide evidence supporting a causal relationship between PFOA and ALT. The epidemiologic evidence of an association with PFOA and GGT, AST, and bilirubin is inconsistent, while there was no evidence of an association with PFOA and ALP. There is also limited epidemiologic evidence of a causative relationship with PFOA and liver disease, and the available studies did not find an association.

### Thyroid hormones, TSH, and thyroid disease

Twenty studies were identified as evaluating thyroid hormones, TSH, hypo- and hyperthyroidism, thyroid disease in general, and/or other thyroid conditions. Study details are provided in the tables for individual studies (Appendix 4) and the summary table for thyroid effects (Table 6C).

Thyroid stimulating hormone (TSH) was the most commonly evaluated thyroid endpoint, and there was limited evidence of a positive statistically significant relationship with PFOA. Three general population studies which include a cross-sectional U.S. population study (Jain, 2013), a South Korean prospective birth cohort (Kim et al., 2011), and a prospective cohort study in Canada (Webster et al., 2014) found some evidence of a positive statistically significant association of elevated TSH and PFOA. The remaining 12 studies found limited or no evidence of a positive association. These 12 studies are all cross-sectional study design, which include six general population studies (Bloom et al., 2010; Ji et al., 2012; Lin et al., 2013; Shrestha et al., 2015; Wang et al., 2014; and Wen et al., 2013), three studies of residents in a highly exposed community (Emmett et al., 2006b; Knox et al., 2011; Lopez-Espinosa et al., 2012), and three occupational studies (Olsen et al., 1998; Olsen et al., 2003; Olsen and Zobel 2007). Three of the 12 studies also included components of other study designs in addition to the cross-sectional design: birth cohort (Lopez-Espinosa et al., 2012), longitudinal (Olsen et al., 1998), and prospective birth cohort (Wang et al., 2014)

Additionally, total thyroxine (TT4) has been extensively evaluated with little evidence of a positive statistically significant association. Only two studies found some evidence of statistically significant positive association (de Cock et al., 2014, and Knox et al., 2011), while 11 others found no evidence of a statistically significant association (Jain 2013; Ji et al., 2012; Kim et al., 2011; Lin et al., 2013; Lopez-Espinosa et al., 2012; Olsen et al., 2003; Olsen and Zobel 2007; Shrestha et al., 2015; Wang et al., 2014; Webster et al., 2014; and Wen et al., 2013). A case-control study of hypothyroxemic pregnancy matched with non-hypothyroxemic pregnant women in Canada evaluated the association of PFOA and maternal hypothyroxemia, a common condition in pregnant women characterized by low maternal free thyroid hormone (fT4) and normal TSH levels, and found no evidence of a statistically significant association (Chan et al., 2011).

Eight studies evaluated PFOA and associations with total triiodothyronine (TT3). Four of these studies found some evidence of a statistically significant positive association, including two larger (n=1,540 and 1,180) cross-sectional studies of the U.S. general population (Jain 2013 and Wen et al., 2013, respectively) as well as both of the occupational studies (Olsen et al., 2003; Olsen and Zobel 2007). Three studies did not find any statistically significant evidence of an association (Kim et al., 2011; Shrestha et al., 2015; and Wang et al., 2014), while a large (n=50,113) cross-sectional study of the mid-Ohio Valley which found some evidence of an inverse association (Knox et al., 2011). Two of these studies also evaluated free triiodothyronine

(FT3) and neither found evidence of a statistically significant association (Jain, 2013; and Wen et al., 2013). These same two studies also evaluated associations of PFOA and thyroglobulin and found no evidence of a statistically significant association (Jain, 2013 and Wen et al., 2013).

Three studies evaluated the association of PFOA and hypo- and hyperthyroidism, with mixed results. Hypothyroidism is a condition in which the thyroid gland is under-active and is characterized by elevated TSH serum levels combined with low serum FT4. Hyperthyroidism is a condition involving an over-active thyroid gland and is characterized by very low TSH hormone and raised FT4. Lopez-Espinosa et al. (2012) found a borderline statistically significant positive association with measured PFOA concentrations and self-reported subclinical hypothyroidism, but found non-statistically significant results for modeled PFOA, including modeled *in utero* exposure to PFOA, and subclinical measures of hypothyroidism. Odds ratio for PFOA and hyperthyroidism were mixed and not statistically significant. A study by Wen et al. (2013) of the U.S. adult general population found a statistically significant positive association of hypothyroidism among women but not men, and a statistically significant negative association of hyperthyroidism among men and not women. Winqvist and Steenland (2014b) found increasing hazards with increasing PFOA exposure for hypothyroidism, although the trend was not statistically significant, while retrospective and prospective analyses were statistically significantly positively associated among men. A statistically significant trend of hyperthyroidism and increasing PFOA exposure was found overall and for women.

Five studies evaluated thyroid disease in general, which may also include hypo- and hyperthyroidism. Three studies found some evidence of a statistically significant positive association with PFOA and thyroid disease. A large study of highly exposed children in the mid-Ohio Valley found a positive statistically significant association among measured PFOA concentrations, median of 29 ng/ml, and parent-reported thyroid disease, but this association was not statistically significant with modeled PFOA (Lopez-Espinosa et al., 2012). A cross-sectional study of the U.S. general population found increasing odds ratio of self-reported thyroid disease, both ever and current, with increasing quartiles of PFOA among women but not men (Melzer et al., 2011). A large retrospective cohort study with prospective analyses found evidence of a positive association with thyroid disease and increasing quintiles of PFOA which was strongest among women for retrospective analyses, but prospective analyses found no clear associations with PFOA and thyroid disease (Winqvist and Steenland, 2014b). The remaining two studies, a small study in a highly exposed community with median serum PFOA concentration of 354 ng/ml and a relatively narrow range of exposures (Emmett et al., 2006b), and a retrospective occupational cohort with a median PFOA exposure of 113 ng/ml (Steenland et al., 2015), found no evidence of a statistically significant association with thyroid disease and PFOA.

As discussed above, the C8 Science Panel concluded there was a probable link with PFOA and thyroid disease. The C8 Science Panel summarized the epidemiologic evidence of an association

with PFOA and thyroid function or disease and, although the evidence was deemed inconsistent, concluded that "...the presence of some independent pieces of evidence indicative of an association was not easily dismissed, despite a lack of coherence among them." The C8 Science Panel determined the strongest evidence for an association was increased occurrence of medically validated thyroid disease (hyperthyroidism in women, hypothyroidism in men) with increasing measured PFOA exposure (2005-2006) in the prospective analyses (2005-2010). Therefore, despite inconsistencies in the evidence, the Panel concluded that there was evidence of a probable link between C8 and thyroid disease (C8 Science Panel, 2012).

Selection bias may be an issue in Lin et al. (2013b) which included individuals with an abnormal urinalysis from a population-based screening program. Information bias is unlikely to have had an impact in these studies, as they relied mostly on serum concentrations of exposure and outcomes. Although serum thyroid function measures are collected at a single time point in many studies, these thyroid function measures are maintained over time in the body. Also, reliance on recall for studies assessing thyroid disease, hypo-, and hyperthyroidism may bias results (Lopez-Espinosa et al., 2012). Small sample sizes in some studies may have limited their power to detect associations (Bloom et al., 2010; Kim et al., 2011b; Mundt et al., 2007; and Webster et al., 2014).

Although thyroid endpoints were not evaluated in most toxicology studies of PFOA, data from a limited number of studies, reviewed in the Toxicology section below, support the biological plausibility of effects of PFOA on human thyroid function. Although no effects on thyroid hormones were seen in a very small 29-day study of cynomolgus monkeys, changes in TSH, T3, and T4 occurred at multiple time points in a 6-month study in this species. Thyroid hormones were significantly decreased in rats in a short duration exposure study using a high dose of PFOA. Thyroid endpoints have not been evaluated in longer duration, lower dose, or rodent studies.

Overall, studies evaluating thyroid hormones, TSH, and thyroid disease provide inconsistent evidence of any associations with PFOA. The C8 Science Panel concluded that, despite the inconsistencies of findings among studies, compelling evidence for associations with thyroid disease could not be dismissed, and a probable link with PFOA and thyroid disease was determined.

#### Uric acid

Uric acid is a product of purine metabolism with both oxidant and antioxidant properties, and elevated levels are a marker of kidney disease. Additionally, some studies have shown that elevated uric acid is associated with cardiovascular disease and may trigger hypertension (Klein et al., 1973; Fang et al., 2000; and Freedman et al., 1995). Seven studies evaluated the association of uric acid and serum PFOA concentrations; three of these also assessed clinically

defined hyperuricemia. Study details are provided in the tables for individual studies (Appendix 4) and the summary table for uric acid (Table 6D).

These studies include six cross-sectional studies and one occupational study which was a case-control study with cross-sectional components. All of these studies found strong, positively statistically significant associations of uric acid and PFOA (Costa et al. 2009; Geiger et al., 2013; Gleason et al., 2015; Sakr et al., 2007a; Shankar et al., 2011; and Steenland et al., 2010) with the exception of Lin et al., 2013 which did not find a statistically significant association. Additionally, all three studies which evaluated clinically defined hyperuricemia found strong evidence of a positive statistically significant association (Geiger et al., 2013; Shankar et al., 2011; and Steenland et al., 2010b).

Although the six studies with evidence of statistically significant association are mainly cross-sectional, they represent the general population, residents from a highly exposed community, and an occupationally exposed population. These studies therefore evaluated a wide range of serum PFOA concentrations - about 4 ng/ml in the general population studies (Geiger et al., 2013; Gleason et al., 2015; Shankar et al., 2011), a median of about 28 ng/ml in a highly exposed community population (Steenland et al., 2010b), and a median serum PFOA concentration range from 428 ng/ml (Sakr et al., 2007a) to 4,400 -5,700 ng/ml (Costa et al., 2009) among occupationally exposure populations. Also, importantly, these studies evaluated a wide range of age groups as well, including children less than 19 years of age (Geiger et al., 2013), adolescents and adults greater than 11 years of age (Gleason et al., 2015), and adult populations 20 years or older (Costa et al., 2009, Shankar et al., 2011; and Steenland et al., 2010b).

The general population studies which evaluated associations of uric acid with increasing serum concentrations of PFOA found strong exposure-response relationships (Geiger et al., 2013; Gleason et al., 2015; and Shankar et al., 2011). Additionally, Steenland et al. (2010b) found a significant trend and some evidence of increased changes in uric acid with high level of serum PFOA concentrations among residents from a highly exposed community. Lin et al. (2013) did not find evidence of a statistically significant trend in mean uric acid across categories of PFOA exposure but there does appear to be a small, non-significant increase in uric acid level as serum PFOA concentrations increase.

Information bias is unlikely to have an impact in the general population studies which relied on serum concentrations and clinical biomarkers. Selection bias may have impacted findings in Lin et al. (2013), which included individuals with an abnormal urinalysis from a population-based screening program in which the final study population was made up of 246 (37%) individuals with elevated blood pressure.

Reverse causality is a potential explanation for increased uric acid with increasing PFOA. It has been proposed that PFOA could be higher in individuals with reduced excretion due to reduced kidney function, and that this would also result in increased uric acid (Kataria et al., 2015). Also, Kataria et al., (2015) reviewed toxicology evidence and suggests that PFOA and other PFCs can adversely impact renal function. Unfortunately, a hypothesis of reverse causality cannot be assessed because the six studies which evaluated uric acid and PFOA are limited by their cross-sectional design in which exposures and outcomes are measured at the same point in time.

Epidemiologic evidence provides evidence of consistency among findings, strength of findings with clinically defined outcomes, and exposure-response with PFOA and uric acid. These findings provide evidence supporting a causal relationship between PFOA and uric acid. However, there are limitations in use of the epidemiologic evidence to draw conclusions regarding temporality and there remain some questions of biologic plausibility due to possible reverse causality explanation.

#### Antibody concentrations following vaccination

Five studies evaluated associations of serum PFOA concentrations and antibody concentrations following six types of vaccines. Study details are provided in the tables for individual studies (Appendix 4) and the summary table for this effect (Table 6E). Only one type of vaccine (tetanus) was evaluated in three of the five studies, each of four vaccine types were evaluated in two studies each, and one (mumps) was evaluated in only one study. These five studies include a prospective cohort following 656 singleton births recruited from birth and followed to seven years of age (n=587) in the Faroe Islands (Grandjean et al., 2012). In this group of children with a median serum PFOA concentration at 5 years of age of 4.1 ng/ml, researchers found strong statistically significant evidence of a decrease in antibody concentrations following a tetanus or diphtheria vaccination at age 5 and 7 years. Another prospective birth cohort study in Norway collected blood samples at delivery from 99 pregnant women with a subsequent follow-up sample of 56 children at 3 years of age (Granum et al. 2013). The median PFOA maternal serum concentration was 1.1 ng/ml. Investigators found strong evidence of decreased rubella-induced antibodies with increasing PFOA maternal serum concentrations, but associations with PFOA and responses to tetanus, measles, and influenza vaccines were not statistically significant. A prospective cohort of 12 adults recruited from hospital staff in Denmark with median serum PFOA concentration of 1.7 ng/ml found no statistically significant associations with antibody response to tetanus or diphtheria vaccines (Kielsen et al., 2015). A larger prospective cohort of 411 adults from the mid-Ohio valley with a high median serum PFOA concentration of 31.5 ng/ml found evidence of decreasing antibody concentrations following Influenza A H3N2 vaccination; however no statistically significant associations were found with responses to vaccines for Influenza Type B or Influenza Type A H1N1 (Looker et al., 2014). The largest of the studies includes a sample size of 1,191 adolescents aged 12 to 19 years of age from the U.S. general population with a geometric mean serum PFOA concentration of 4.1 ng/ml. This cross-

sectional study found some evidence of a statistically significant decrease of rubella- and mumps vaccine induced antibody concentration, but a decreased antibody response to measles vaccine was not statistically significant (Stein et al., 2016).

Associations between decreased antibody concentration and increasing PFOA concentration may be related to a threshold such that limited evidence of associations was found among the two studies with median serum PFOA concentrations below 2 ng/ml (Granum et al, 2013 and Kielsen et al., 2015). Both of these studies also had small sample sizes which may have restricted the power of the study to detect a statistically significant decrease.

Specificity of the observed association may also be difficult to interpret since responses to many different vaccines were evaluated, with each type of vaccine included only in a few (and often in only one or two) studies. Unlike many of the other outcomes evaluated in studies of the human health effects of PFOA, four of the studies that assessed associations with antibody concentrations following vaccination had a prospective study design, allowing temporality assessment. Since the exposures and outcomes were followed over time, it can be concluded that exposures preceded the outcome. There was limited evidence or exploration of exposure-response relationships.

Data from other human studies and toxicology studies provide support for biological plausibility of decreased immune system response to vaccines in humans. As discussed in the Toxicology and Mode of Action sections, PFOA suppressed the immune system in studies of both non-human primates and rodents. Fletcher et al. (2009) reported several statistically significant associations between several markers of immune function (decreased IgA; decreased IgE in females only; increased anti-nuclear antibody; decreased C-reactive protein) and serum PFOA levels in communities with drinking water exposure to PFOA in a C8 Science Panel status report (Fletcher et al., 2009). As yet, only the information on C-reactive protein has been published (Genser et al., 2015). Genser et al. (2015) found consistent and significant associations of serum PFOA with this effect, both within each of the six water districts included in the study and on an aggregated basis. They concluded that these within- and between-district associations strengthen the evidence of causality for this effect.

Review of epidemiologic studies provides evidence of consistent findings among studies of decreased antibody concentrations following vaccination and PFOA. However, there are a limited number of comparisons across the same vaccination types, making consistency/specificity difficult to evaluate. While there is epidemiologic evidence of temporality, evidence of an exposure-response is limited.

Additionally, the National Toxicology Program (NTP) recently completed a systematic review of immunotoxicity of PFOA, based on consideration of human and animal studies, along with

mechanistic data (NTP, 2016). The NTP assessment concluded that PFOA is presumed to be an immune hazard to humans based on (1) a high level of evidence from animal studies and a moderate level of evidence from human studies that PFOA suppresses antibody response, and (2) a high level of evidence from animal studies and a low level of evidence from human studies that PFOA increases hypersensitivity-related outcomes. NTP also considered additional, although weaker, evidence primarily from epidemiological studies that PFOA reduced infectious disease resistance and increased autoimmune disease. NTP states that the evidence for effects on multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans.

### **Endpoints Evaluated by Other Researchers:**

#### **Fetal growth**

A collaborative team of scientists developed a methodology for the systematic review of environmental health data titled the “Navigation Guide.” The Navigation Guide is intended as a “systematic and rigorous approach to research synthesis” that will “reduce bias and maximize transparency in the evaluation of environmental health information” (Woodruff and Sutton, 2014). The Navigation Guide methodology utilizes a three-step process in which a study question is specified, evidence is selected, and the quality and strength of the evidence is evaluated. Developmental exposure to PFOA and fetal growth was selected by the research group as a proof-of-concept of the methodology. This effort included evaluation of human epidemiologic data (Johnson et al., 2014), animal toxicology data (Kousta et al., 2014), and a synthesis of both types of data to develop overall conclusions (Lam et al., 2014).

The application of the Navigation Guide methodology to human studies required that each identified study was evaluated for risk of bias, and that the quality and strength of the evidence across all studies was rated. The authors reviewed 19 datasets available from 18 studies which met the inclusion criteria. Additionally, a meta-analysis of 10 studies with combinable study attributes was performed. Investigators concluded there was a low risk of bias across the studies. The meta-analyses of birth weight resulted in an overall continuous regression estimate per ng/ml serum PFOA of -18.9 g (95% CI -29.8, -7.9), an estimate of -0.06 cm (95% CI -0.1, -0.02) in birth length, a -0.01 (95% CI 0.03, 0.01) reduction in ponderal index (birth weight/length<sup>3</sup> x 100), and a -0.03 cm (95% CI -0.08, 0.01) reduction in head circumference. The quality of the human evidence was rated as “moderate”. By evaluating the quality of studies, direction of effect estimate, confidence of effect, and other possible compelling attributes, it was concluded that there is “sufficient” human evidence, the strongest descriptor for strength of evidence, that developmental exposure to PFOA reduces fetal growth in humans (Johnson et al., 2014).

The conclusions of another recent meta-analysis of human data (Verner et al., 2015) were generally consistent with Johnson et al. (2014). Verner et al. (2015) estimated a decrease in birth weight per ng/ml PFOA in maternal or cord blood serum of -14.7 g (95% CI -21.7, -7.8). They

note that their analysis was less formal than the one conducted by Johnson et al. (2014) and that it did not include two studies included in the analysis of Johnson et al. (2014).

The animal evidence was also reviewed by the Navigation Guide research group, and it was concluded that there is “sufficient” evidence that exposure to PFOA adversely affects fetal growth in animals (Koustas et al., 2014). The human and non-human evidence was integrated to develop an overall conclusion on whether developmental exposure to PFOA affects fetal growth in humans. The human and non-human mammalian evidence were both rated as being of “moderate” quality and “sufficient” strength. The authors concluded that there is “sufficient” evidence that developmental exposure to PFOA adversely affects human health based on decreased fetal growth in both human and non-humans (Lam et al., 2014).

Although previous research in the C8 Health Study population did not find an association with clinically defined birth weight and PFOA (Stein et al., 2009, C8 Science Panel, 2011), a subsequent study in this population found associations with low birth weight evaluated as a continuous variable (Savitz et al., 2012). Thus, findings from the C8 Health Study do not contradict conclusions reached by the Navigation Guide.

Evidence supporting biological plausibility for decreased fetal growth from PFOA is provided by studies (discussed in the Toxicology section) showing that PFOA adversely effects prenatal and postnatal growth and development. As discussed in the Mode of Action section, cellular receptors that play a role in the developmental toxicity of PFOA are found in human tissues, and developmental effects observed in laboratory animals are believed to be relevant to humans.

#### Potential impact of glomerular filtration rate on association of PFOA and fetal growth

Glomerular filtration rate (GFR) is the flow rate of fluids being filtered through the kidneys. GRF increases during the first half of pregnancy and declines slightly during the second half (Gibson 1973). Decreased GFR has been associated with lower infant birth weight in some studies (reviewed by Lam et al., 2014; Morken et al., 2014). Because decreased GFR might also result in higher serum PFOA levels due to slower excretion, it has been hypothesized that lower GFR could be a confounding factor for the association of PFOA and decreased fetal growth.

Lam et al. (2014) considered the evidence for reverse causality related to decreased GFR for the association of PFOA with decreased fetal growth. They concluded that the available data did not justify revision of their conclusion of sufficient human evidence for association of PFOA and decreased fetal growth. Vesterinen et al. (2015) subsequently adapted and applied the Navigation Guide methodology, described above, to assess the evidence of an association between fetal growth and GFR. The authors identified 35 relevant studies (31 human observational, two non-human observational, and two non-human experimental), all of which were rated as either “low” quality or “very low” quality. All three of these evidence streams were classified as

“inadequate,” indicating that the association between GFR and fetal growth was “not classifiable.” Based on their review, Vesterinen et al. (2015) concluded that the current evidence is insufficient to support the plausibility of a reverse causality hypothesis for the associations between environmental chemicals during pregnancy and fetal growth. In an additional recent study not included in the review of Vesterinen et al. (2015), Morken et al. (2014) analyzed data from 953 pregnant Norwegian women (470 with pre-eclampsia and 483 without pre-eclampsia) and estimated GFR in the second trimester based on plasma creatinine. The association of estimated GFR and infant birth weight was not significant at  $p < 0.05$  when women with pre-eclampsia, which is associated with decreased kidney function, were excluded from the analysis. When these women with pre-eclampsia were included, the association was significant.

Verner et al. (2015) used data from three studies that provide data on maternal GFR and birth weight on an individual basis to modify a physiologically based pharmacokinetic (PBPK) model for PFOA during pregnancy (previously developed by Loccisano et al., 2013) to include the relationship between GFR and birth weight. A Monte Carlo simulation using the modified model was used to estimate the population distribution of PFOA levels in maternal and cord blood plasma during pregnancy and at delivery. Based on an assumed PFOA half-life of 3.8 years, the model simulation predicted a decrease in birth weight per ng/ml PFOA of -7.9 g (CI -9.4, -6.4) based on maternal plasma at term and -7.1 g (95% CI -8.5, -5.8) based on cord plasma at delivery. When a shorter half-life of 2.3 years (which is likely more appropriate, discussed in Toxicokinetics section) was assumed, greater effects on birth weight per ng/ml PFOA were predicted: -9.6 g (95% CI -11.0, -8.2) based on maternal plasma at delivery and -8.1 g (95% CI -9.4, -6.8) based on cord plasma at delivery. The data from the simulation were compared to the results of a meta-analysis of the effect of maternal or cord plasma PFOA on birth weight (-14.7 g per ng/ml, 95% CI -21.7, -7.8) to estimate the effect of GFR on this association. Results of this study suggests that GFR may confound a portion (less than 50%) of the association between PFOA and decreased birth weight, and that it would be desirable to control for GFR in studies of PFOA and birth weight to account for potential confounding by GFR.

Based on review of the relevant information, it was concluded that confounding by GFR does not account for the major portion of the decrease in fetal growth that is associated with PFOA.

### Cancer

The USEPA Science Advisory Board (USEPA, 2006) described PFOA as “likely to be carcinogenic to humans” according to the criteria provided in the USEPA Guidelines for Carcinogen Risk Assessment (2005b). This determination was made prior to the publication of the epidemiology studies summarized below and was based on the toxicology and mode of action data available at that time.

The International Agency for Research on Cancer (IARC) is the specialized cancer agency of the

World Health Organization (WHO). One of IARC's major activities is the evaluation of the evidence of human carcinogenicity of specific exposures, including environmental contaminants, by international expert working groups which it convenes. IARC considers human, animal, and mechanistic data in making its determinations of evidence for cancer risk to humans. It has classified PFOA as "possibly carcinogenic to humans" (Group 2B) based on "limited evidence that PFOA causes testicular and renal cancer, and limited evidence in experimental animals" (IARC, 2016). The human data considered by IARC (2016) in making this determination included increases in kidney cancer among workers in the DuPont Washington Work plant in Parkersburg, WV (Steenland and Woskie, 2012) and in kidney and testicular cancer among highly exposed members of the C8 Health Project study population (Barry et al., 2013 and Vieira et al., 2013). IARC (2016) also notes that a second occupational study (Raleigh et al., 2014) from another location did not find evidence for increased incidence of kidney cancer. The epidemiological studies cited by IARC (2016) as the basis for its conclusion are presented in more detail below.

More recently, the USEPA Office of Water (2016a) concluded that PFOA has suggestive evidence of carcinogenic potential for PFOA based on the human studies mentioned above that found an association of serum PFOA with kidney and testicular tumors in communities with drinking water exposure and increased incidence of tumors in one more organs in two chronic rat bioassays.

Steenland and Woskie (2012) studied the mortality of 5,791 workers at the DuPont chemical plant in Parkersburg, West Virginia from 1952-2008. Exposure to PFOA in workers was estimated using a job exposure matrix developed from serum data available for a smaller set of workers. Standardized Mortality Ratios (SMRs) were calculated using other DuPont workers in the region and the U.S. population as referent groups. PFOA exposure was categorized into quartiles. The SMR for kidney disease increased with increasing quartiles of exposure (Quartile 1: SMR=1.07, 95% CI 0.02, 3.62 (based on 1 death); Quartile 2: SMR=1.37, 95% CI 0.28, 3.99 (based on 3 deaths); Quartile 3: SMR=0.00, 95% CI 0.00, 1.42 (based on 0 deaths); Quartile 4: SMR=2.66, 95% CI 1.15, 5.24 (based on 8 deaths). Mesothelioma and chronic renal disease (malignant and non-malignant) also both had exposure-response relationships with PFOA. When investigators lagged models by 10-years and 20-years, kidney cancer and chronic renal disease remained most elevated in the highest quartile of exposure compared to the lower quartiles. An important potential confounder in this occupational cohort could be tetrafluoroethylene (TFE) which was also used in the manufacture fluoropolymers at the Parkersburg, WV facility and has been identified as a rodent kidney carcinogen (NTP, 1997). However, the authors believe that appreciable exposures would have been unlikely, since TFE exposure would have been well controlled due to its explosive and volatile nature.

Vieira et al. (2013) performed an ecological study in 13 counties which encompass six PFOA-contaminated water districts in Ohio and West Virginia located near the DuPont chemical plant in Parkersburg, West Virginia. Cases of 18 types of cancer were obtained from the Ohio and West Virginia cancer registries, in which the final data set included 7,869 Ohio cases and 17,328 West Virginia cases. To calculate adjusted odds ratios (OR) with logistic regression, controls were selected from the state cancer registries as all other cases of cancers (excluding cases of kidney, pancreatic, testicular, and liver cancers, the types which have been associated with PFOA in animal or human studies). Exposure estimates were based on estimated 1995 median serum PFOA concentrations in the six water districts. The analysis was adjusted for age, sex, diagnosis year, smoking status, and insurance provider. The first of two analyses, using water district of residence as the exposure of interest and including both Ohio and West Virginia cases, found the odds of testicular cancer was statistically significantly increased (OR=5.1, 95% CI 1.6-15.6) and the odds of kidney, non-Hodgkin lymphoma, and prostate cancer were also increased, in the highest exposed water district of Little Hocking, although not statistically significant. Kidney cancer was also elevated in the third highest exposed water district of Tupper's Plain (OR=2.0, 95% CI 1.3-3.1). Lung cancer was statistically significantly associated with PFOA in the total exposed group and in two of the water districts. For the second analysis, serum PFOA levels were estimated at a finer geographic scale and restricted to Ohio-only cases. Kidney cancer was elevated in both the very highly exposed category (serum concentration of 110-665 ng/ml; OR=2.0, 95% CI 1.0, 3.9) and the highly exposed category (serum concentration of 30.8-109 ng/ml; OR=2.0, 95% CI 1.3, 3.2) groups.

Barry et al. (2013) evaluated cancer incidence among individuals exposed to PFOA through contaminated drinking water in the mid-Ohio Valley, as well as subjects who worked at the local chemical plant where PFOA was used (n=32,254). Associations between self-reported cancers and cumulative (based on retrospective yearly estimates for each individual) serum PFOA concentrations were evaluated using a proportional hazards model. The model was adjusted for time-varying smoking, time-varying alcohol consumption, sex, education, and 5-year birth year period. Analyses were restricted to the 21 types of reported primary cancers validated through medical records and state cancer registries (n=2,507, 70% validated). Among the cancer types, only testicular cancer was statistically significantly associated with cumulative serum PFOA concentration (OR=1.34, 95% CI 1.00, 1.79), and the effect was stronger when restricted to community members only (OR=1.73 95% CI 1.24,2.40). Thyroid cancer was statistically significantly associated with cumulative serum PFOA concentrations among occupational workers (OR=1.93, 95% CI 1.00, 3.71) but not among the community study population. Results for cumulative serum PFOA concentrations by quartiles show that estimated risk ratios for kidney cancer (p-value=0.10) and testicular cancer (p-value=0.05) generally increased across quartiles. This pattern was less consistent for thyroid cancer (p-value=0.20), except when restricted to the occupational study population (p-value=0.04). Since this is largely a survivor cohort, differences in cancer survival rates could cause cancer types with shorter than 5-year

survival to be likely to be captured by the study. Additionally, if individuals with cancers caused by high exposure were more likely to die before being captured in the cohort, results could be biased towards the null (less likely to find an association). Therefore, results about these cancer types should be interpreted with caution.

A comprehensive review of PFOA as an emerging drinking water contaminant (Post et al., 2012) found evidence that occupational studies showed some consistency of increased cancer mortality and/or incidence for bladder, kidney, and prostate cancer. Post et al. (2012) also reviewed studies showing that white blood cell neoplasms (Leonard, 2003; Leonard et al., 2008), thyroid cancer (Leonard et al., 2008), and carcinoid tumors (Morel-Symons et al., 2007) were also increased at one industrial facility.

A recent review of PFOA's carcinogenic potential focusing on human epidemiological studies (Chang et al., 2014) concludes that the "existing epidemiological evidence does not support the hypothesis of a causal association between PFOA exposure and cancer in humans. However, further research on this topic is warranted." Specifically, Chang et al. (2014) suggest that quantitative exposure assessment at industrial facilities in Asia that continue to produce or use PFOA (now phased out in the U.S.) could be used as the basis for future cohort studies with sufficient follow up time. Additionally, continued follow-up of existing cohorts with use of cancer incidence data from cancer registries could provide further information about human cancer risk of PFOA.

Information on human breast cancer and PFOA is of interest because toxicological findings provide some evidence suggesting biological plausibility, although the available toxicological data do not support firm conclusions on this topic. Developmental exposures to PFOA cause delayed mammary gland development in mice. Several researchers have stated that, in general, such disruptions of mammary gland development may result in adverse effects later in life resulting in increased cancer risk, although no information specific to PFOA is available on this question (Fenton 2006; Rudel and Fenton, 2009; IOM, 2011; Rudel et al., 2011; Fenton et al., 2012). Developmental exposures to PFOA in mice also caused increased numbers of darkly stained foci (described in detail in the Toxicology section) that persisted into adulthood and are considered permanent (White et al., 2009). Gore et al. (2015) notes that abnormalities of this type can be associated with increased breast cancer risk. However, chronic carcinogenicity studies have not been conducted in mice.

Epidemiological studies of associations of PFOA with breast cancer did not evaluate early life exposures. Studies of workers with occupational exposures to PFOA include very few women and are thus not informative about disease incidence in females. The incidence of breast cancer was not increased in a study of many types of cancer in the C8 Health Study population in Ohio and West Virginia communities with drinking water exposure to PFOA that included about

17,000 women (Barry et al., 2013). A small study (31 cases and 98 controls) in the Inuit population in Greenland found significantly increased risk of breast cancer associated with PFOA, perfluorooctane sulfonic acid (PFOS), total perfluorinated carboxylates, and total perfluorinated sulfonates (Bonefeld-Jorgensen et al., 2011). A subsequent study of this small group of cases and controls found a greatly elevated odds ratio for breast cancer in women with both high PFC levels and specific polymorphisms of xenobiotic metabolizing enzymes that affect levels of hormones such as estrogens, suggesting that inter-individual variations in these polymorphisms may affect sensitivity to the effects of PFCs on breast cancer risk (Ghisari et al., 2014). Because this was the only such study and it is of small size, these data are considered preliminary and suggest the need for further research on this topic.

In regard to biological plausibility for human carcinogenicity, PFOA caused hepatic, testicular Leydig cell, and pancreatic acinar cell tumors in two chronic studies in male rats (discussed in Toxicology section). Potential human relevance of these tumors is discussed in the Mode of Action section. Although liver tumors in rodents caused by compounds that activate PPAR-alpha are generally not considered relevant to humans, activation of this receptor may not be the sole mode of action for liver tumors caused by PFOA. Additionally, a PPAR-alpha mode of action may be involved in hepatic effects in the fetus, infants, and children. Therefore, the potential for human relevance of the tumors observed in rodents cannot be dismissed. The mode of action for the testicular and pancreatic tumors has not been established.

### **Additional endpoints**

In addition to the epidemiologic endpoints reviewed above, there remain a large number of peer-reviewed studies that have evaluated other endpoints. Associations with PFOA have been found in some of these studies and not in others. Although these endpoints are not evaluated in this document, other organizations and publications may provide a more comprehensive review

Additional endpoints evaluated in studies but not reviewed in this report include biomarkers of kidney function or damage such as blood urea nitrogen, serum creatinine, renal glomerular filtration rate (eGFR) and chronic kidney disease; heart disease and hypertension; cerebrovascular disease including stroke, diabetes, immune function (with the exception of immune response following vaccination which was reviewed above) including asthma and other allergies, autoimmune disease including osteoarthritis, lupus, juvenile diabetes, rheumatoid arthritis, multiple sclerosis, and Crohn's disease; osteoporosis and bone mineral density; neurological and neurodegenerative disorders including self-reported memory impairment and Parkinson's disease; cognitive and behavioral developmental milestones; performance testing, and attention deficit hyperactivity disorder (ADHD) in children; reproductive and developmental outcomes (with the exception of lower birth weight and birth size of neonates), including decreased sperm count, longer time to pregnancy, birth defects, miscarriage and stillbirth, and overweight and obesity measured by BMI and waist circumference in offspring. It is noted that the C8 Science Panel concluded that there are "probable links" with PFOA exposure in the C8

Health Study population for two adverse health conditions, pregnancy-induced hypertension and ulcerative colitis, that were not reviewed above.

Health effects that occur later in life from prenatal and early life exposures to environmental contaminants are a current focus of research in toxicology and epidemiology (Barouki et al., 2012; Heindel and Vandenberg, 2015). Exposures of developing tissues to toxic substances during sensitive time periods can result in increased risk or severity of later disease or dysfunction (Heindel and Vandenberg, 2015). Recent studies have found that developmental exposures to PFOA cause toxicological effects that persist into adulthood and/or become evident later in life, including hepatic toxicity, delayed mammary gland development, and bone morphology and mineral density (Macon et al., 2011; White et al., 2009; Filgo et al., 2015; Quist et al., 2015; Koskela et al., 2016). However, the effects of developmental exposures to PFOA on these endpoints have not been evaluated in humans.

### **Summary of conclusions for epidemiologic information**

Of the endpoints that were evaluated comprehensively, the evidence for association with PFOA is strongest for serum cholesterol which demonstrates consistency, strength, dose-response and some evidence of temporality. PFOA was associated with clinically defined hypercholesterolemia in a community exposed through drinking water. There was also consistency among the larger non-occupational studies, as well as evidence of specificity, exposure-response and strength for associations between PFOA and both serum cholesterol and the liver enzyme ALT. These findings provide evidence supporting a causal relationship between PFOA and both cholesterol and ALT. There is evidence of consistency, strength, and exposure-response with PFOA and uric acid as well, but interpretation for temporality is limited.

In general, the human data for PFOA are notable because of the consistency between results among human epidemiologic studies in different populations for some endpoints, the concordance with toxicological findings from experimental animals, the use of serum concentrations as a measure of internal exposure, the potential clinical importance of the endpoints for which associations are observed, and the observations of associations within the exposure range of the general population.

As mentioned above, the epidemiologic databases for cholesterol, the liver enzyme ALT, and other endpoints are sufficient to support one or more of the criteria for causality. The sufficiency of the database to support these criteria is particularly evident when compared to the human epidemiologic databases for numerous other environmental contaminants for which quantitative risk assessment is based on animal toxicological studies in the absence of substantial support from human epidemiologic data (for example, 1,2,3-trichloropropane and methyl tertiary butyl ether [MTBE]).

For some endpoints, studies of populations from varied locations including the U.S., Canada, and several European and Asian countries, with study populations including children, young adults, adults, the elderly, and pregnant women, demonstrate a congruence of consistent findings. Although the human epidemiologic database is largely driven by cross-sectional studies, consistency is supported from studies with stronger study designs such as cohort and/or case-control, especially in studies of the general populations and populations exposed to drinking water contaminated with PFOA.

A particular strength of epidemiologic studies of PFOA and other PFCs is the availability of serum concentrations, a direct and accurate measure of internal dose in individuals. In contrast, human studies of most other environmental contaminants usually rely on estimates of external exposure. Exposure assessment tends to be susceptible to misclassification that may lead to biased findings. However, information bias is unlikely to have an impact in most general population studies of PFOA because of their reliance on serum concentrations and clinical biomarkers

Application of the Navigation Guide systematic review methodology to epidemiologic data resulted in the conclusion that there is sufficient evidence that developmental exposure to PFOA adversely affects fetal growth. The methodology developed and utilized to assess the relationship of PFOA and fetal growth was substantive and robust such that conclusions reached through application of the Navigation Guide are reasonable and supportable.

Reviews by several authoritative groups concluded that there is evidence of carcinogenic potential in humans for PFOA. The USEPA SAB (2006) described PFOA as “likely to be carcinogenic to humans” based on the criteria provided in USEPA (2005b) cancer risk assessment guidance. More recently, IARC (2016) concluded that, based on its evaluation criteria, PFOA is possibly carcinogenic to humans. Finally, the USEPA Office of Water (2016a) concluded that there is suggestive evidence of human carcinogenicity for PFOA.

For some other endpoints, limited evidence of an association with PFOA was found. There is consistent evidence of decreased antibody concentrations following vaccination, with evidence of temporality. However, most of the vaccine types were evaluated in only one or two studies, limiting the ability to determine specificity, and there is limited evidence of exposure-response. The epidemiologic database for PFOA and LDL appears inconsistent, although there is some evidence of an association. Epidemiologic evidence for other serum lipid endpoints is limited. The epidemiologic evidence of a casual association with PFOA and GGT, AST, and bilirubin lacks consistency, and there is a limited evidence of associations with PFOA and liver disease. Evidence of thyroid disease remains limited.

There was no evidence of an association with PFOA and the liver enzyme ALP. Overall the epidemiologic evidence suggests no evidence of an association with HDL and PFOA. Overall studies evaluating thyroid hormones and TSH provide limited or no evidence of any associations with PFOA.

Although the magnitude of change for the parameters associated with PFOA is generally relatively small, they are of public health concern because population-level changes of this magnitude in parameters such as serum cholesterol or liver enzymes will result in a shift in the overall distribution of values such that the numbers of individuals with clinically abnormal values is increased. Additionally, small changes in a clinical biomarker may be an indicator of other effects which were not assessed. For example, birth weight represents a gross measure of development, and relatively small decreases in birth weight may be an indication of changes in other subtler developmental parameters which were not assessed.

The steepest dose-response for associations of some health endpoints with PFOA has been observed within the lower range of serum PFOA concentrations. Epidemiologic studies of smaller numbers of highly exposed participants may not be inclusive of these low level exposure ranges and thus may not detect associations. Therefore, an important limitation of studies of participants with extremely high serum PFOA concentrations may be that there are an insufficient number of study participants with low(er) level exposures who can serve as a comparison.

To illustrate this point, Figures 9A and 9B show changes in total serum cholesterol across deciles of serum PFOA concentrations in adults (Steenland et al., 2009) and children (Frisbee et al., 2010) from the mid-Ohio Valley. In both children and adults, associations increased steeply at low ranges of serum PFOA concentrations, with a much flatter dose-response at higher levels. In the absence of data from the lower range of serum PFOA concentrations, modeling techniques might not be able to detect statistically significant changes of this effect with increasing serum PFOA concentrations.

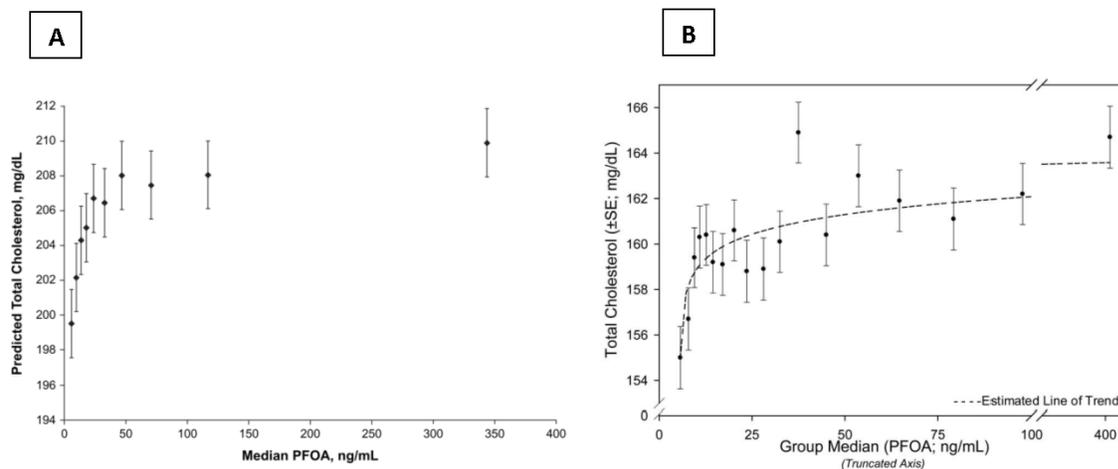


Figure 9. Adjusted-predicted total cholesterol change with increasing group median deciles. (A). Adults, Steenland et al., 2009 (B). Adolescents, Frisbee et al., 2009

For instance, in Emmett et al. (2006b), almost all participants had highly elevated serum PFOA levels (25<sup>th</sup> percentile value was 184 ng/ml), and comparisons were made only within the study population. Most or all of the lowest exposed individuals in this study may have had serum levels that fall on the flatter portion of the dose-response curve for serum cholesterol, limiting the ability to detect an association. Additionally, Olsen and Zobel (2007) provide data on serum cholesterol and other lipids in workers with PFOA exposure which indicate that the median serum PFOA level in the lowest exposed group, 60 ng/ml, lies on the flatter portion of the dose-response curve in Figure 9A and 9B above.

In summary, the consistency between results in different populations, the concordance with toxicological findings from experimental animals, the potential clinical importance of the endpoints for which associations are observed, and the observation of associations within the exposure range of the general population justify concerns about exposures to PFOA through drinking water. Although there is evidence to support causality for some epidemiological endpoints, the epidemiological data have limitations and therefore are not used as the quantitative basis for the ISGWQC. Instead, the human data are considered as part of the weight of evidence for the health effects of PFOA and are used to support a public health-protective approach in development of a ISGWQC based on animal toxicology data.

Table 6A. Summary of findings from epidemiologic studies of PFOA and serum lipids

Citation	Study Population	Study Details	TC	HDL	Non-HDL	TC/HDL	LDL	VLDL	HDL/LDL	TG	Genes
1. Costa et al., 2009	Italy., Occupational: Cases – Male workers engaged in the PFOA production department; Controls – Male workers never exposed to PFOA	* <i>Study Design:</i> Exposure case-control & Cross-sectional * <i>Study Size:</i> n=160 * <i>Study Population Age:</i> 20-63 years * <i>Exposure Range (Median):</i> 4400 – 5700 ng/ml	↑	—						—	
2. Emmett et al., 2006b	U.S., Highly exposed community	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=371 * <i>Study Population Age:</i> 2.5-89 years * <i>Exposure Range (Median):</i> 354 ng/ml	—								
3. Eriksen et al., 2013	Demark, nested in a larger cohort, General population	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=753 * <i>Study Population Age:</i> 50-65 years * <i>Exposure Range (Mean):</i> 7.1 ng/ml	↑								
4. Fisher et al., 2013	Canada, General population	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=5,604 * <i>Study Population Age:</i> 6-79 years * <i>Exposure Range (Geo Mean):</i> 2.46 ng/ml	↑-	—	↑-	—	—			—	
5. Fitz-Simon et al., 2013	U.S., Highly exposed community	* <i>Study Design:</i> Cohort * <i>Study Size:</i> n=560 * <i>Study Population Age:</i> 20-60 years * <i>Exposure Range (Geo Mean):</i> 30.8 – 74.8 ng/ml	↑-	—			↑			—	
6. Fletcher et al., 2013	U.S., Highly exposed community	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=290 * <i>Study Population Age:</i> 20-60 years * <i>Exposure Range (Median):</i> 30.1 ng/ml									<sup>1</sup> ↑-
7. Frisbee et al., 2010	U.S., Highly exposed community	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=12,476 * <i>Study Population Age:</i> 1-17.9 years * <i>Exposure Range (Mean):</i> 69.2 ng/ml	↑	—			↑			↑-	
8. Fu et al., 2014	China, random selection of attendees to health check-up clinic (General population)	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=133 * <i>Study Population Age:</i> 0-88 years * <i>Exposure Range (Median):</i> 1.43 ng/ml	↑	—			↑			—	

Citation	Study Population	Study Details	TC	HDL	Non-HDL	TC/HDL	LDL	VLDL	HDL/LDL	TG	Genes
9. Geiger et al., 2014	U.S., General population	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=815 * <i>Study Population Age:</i> ≤ 18 years * <i>Exposure Range (Mean):</i> 4.3 ng/ml	↑-	—			↑			—	
10. Gilliland et al., 1996	U.S., Occupational - men	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=115 * <i>Study Population Age:</i> Mean 39.2 years * <i>Exposure Range (Mean):</i> 3300 ng/ml	—	↓-			—				
11. Lin et al., 2011	Taiwan, Recruited from hypertension cohort (General population)	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=287 * <i>Study Population Age:</i> 12-30 years * <i>Exposure Range (Median):</i> 2.39 ng/ml		—						—	
12. Lin et al., 2013	Taiwan, Individuals with abnormal urinalysis results from population-based screening program in Taiwan	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> 664 (246 w/ elevated blood pressure and 398 w/ normal blood pressure) * <i>Study Population Age:</i> 12-30 years * <i>Exposure (Median):</i> 3.49 ng/ml					—			↓	
13. Nelson et al., 2010	U.S., General population	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=416 to n=860 * <i>Study Population Age:</i> 12-80 years * <i>Exposure Range (median):</i> 3.8 ng/ml	↑-	—	↑		—				
14. Olsen et al., 2000	U.S., Occupational – male workers	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=265 * <i>Study Population Age:</i> Adults * <i>Exposure Range (Median):</i> 1190 ng/ml	—	↓-			—			—	
15. Olsen et al., 2003	Belgium & U.S., Occupational	* <i>Study Design:</i> Cross-sectional & Longitudinal * <i>Study Size:</i> n=421 & 174 * <i>Study Population Age:</i> Adults * <i>Exposure Range (Mean):</i> 1220-1990 ng/ml	↑	—						↑	
16. Olsen and Zobel 2007	Belgium & U.S., Occupational - male	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=506 * <i>Study Population Age (Mean):</i> 37-41 * <i>Exposure Range (Median):</i> 2210 ng/ml	—	↓-			—			↑-	
Citation	Study Population	Study Details	TC	HDL	Non-HDL	TC/HDL	LDL	VLDL	HDL/LDL	TG	Genes

17. Olsen et al., 2012	U.S., Occupational, direct employed and contract workers	* <i>Study Design:</i> Longitudinal * <i>Study Size:</i> n=179 * <i>Study Population Age:</i> Adults * <i>Exposure Range (Mean):</i> Baseline/mean change - direct employed (881/-218.3 ng/ml), contract (28.9/32.1 ng/ml)	—	—	—	↓						
18. Sakr et al., 2007a	U.S., Occupational	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=840 * <i>Study Population Age:</i> Adults * <i>Exposure Range (Median):</i> 189 ng/ml	↑	—			↑	↑			—	
19. Sakr et al., 2007b	U.S., Occupational	* <i>Study Design:</i> Longitudinal * <i>Study Size:</i> n=454 * <i>Study Population Age:</i> Adults * <i>Exposure Range (Median):</i> 1040/1160 ng/ml	↑	—			—				—	
20. Starling et al., 2014	Norway, Pregnant women recruited from larger cohort	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=891 * <i>Study Population Age:</i> not stated - Adults * <i>Exposure Range (Median):</i> 2.25 ng/ml	—	—			—				—	
21. Steenland et al., 2009	U.S., Highly exposed community	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=46,294 * <i>Study Population Age:</i> ≥ 18 years * <i>Exposure Range (Median):</i> 27 ng/ml	↑	—	↑	↑	↑					
22. Steenland et al., 2015	U.S., Occupational	* <i>Study Design:</i> Retrospective cohort * <i>Study Size:</i> n=3,713 * <i>Study Population Age:</i> Adult (mean year of birth 1951) * <i>Exposure Range (Median):</i> 113 ng/ml	<sup>2</sup> —									
23. Wang et al., 2012	China, Occupational (male) and Highly exposed community	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> Workers: n=55 / Residents: n=132 * <i>Study Population Age:</i> Adult * <i>Exposure Range (Median):</i> 1636 / 284 ng/ml	—/ —	↓/ —			—/ —			↓/ —	—/ —	
24. Winquist and Steenland, 2014a	U.S., Occupational (male) and Highly exposed community	* <i>Study Design:</i> Retrospective cohort (prospective analyses) * <i>Study Size:</i> Workers: n=3,713/ Residents: n=28,541 * <i>Study Population Age:</i> Adult * <i>Exposure Range (Median):</i> 112/ 24 ng/ml	<sup>2</sup> ↑									

Citation	Study Population	Study Details	TC	HDL	Non-HDL	TC/HDL	LDL	VLDL	HDL/LDL	TG	Genes
25. Zeng et al., 2015	Taiwan, recruited from control group of another study	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=255 * <i>Study Population Age:</i> 12-15 years * <i>Exposure Range (Median):</i> Boys=1.1, Girls=0.9 ng/ml	↑	—			↑			↑	
<p>↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at <math>\alpha=0.05</math>]</p> <p>TC= total cholesterol, HDL= high density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol, TG=triglycerides</p> <p>1. Changes in the expression of genes 2. Self-reported high cholesterol</p>											

Table 6B. Summary of findings from epidemiologic studies of PFOA and liver enzymes/bilirubin									
Citation	Study Population	Study Details	ALT	GGT	AST	ALP	TB	DB	LD
1. Costa et al., 2009	Italy, Occupational (male)	*Study Design: Exposure case-control & Cross-sectional *Study Size: n=160 *Study Population Age: 20-63 years *Exposure Range (Median): 4400 – 5700 ng/ml	↑-	↑-		↑-	↓-		
2. Darrow et al., 2016	U.S., Highly exposed community	*Study Design: Cross-sectional *Study Size: up to n=32,254 including 3,713 workers *Study Population Age: >20 years of age *Exposure Range (Median): 16.5 ng/ml	↑	—				↓	— <sup>1</sup>
3. Emmett et al., 2006b	U.S., Highly exposed community	*Study Design: Cross-sectional *Study Size: n=371 *Study Population Age: 2.5-89 years *Exposure Range (Median): 354 ng/ml	—	—	—	—	—		
4. Gallo et al., 2012	U.S., Highly exposed community	*Study Design: Cross-sectional *Study Size: n=47,092 *Study Population Age: ≥ 18 years *Exposure Range (Median): 23.1 ng/ml	↑	↑-				—	
5. Gilliland et al., 1996	U.S., Occupational (male)	*Study Design: Cross-sectional *Study Size: n=115 *Study Population Age: Mean 39.2 years *Exposure Range (Mean): 3300 ng/ml	↓-	—	↓-				
6. Gleason et al., 2015	U.S., General population	*Study Design: Cross-sectional *Study Size: n=4,333 *Study Population Age: ≥ 12 years *Exposure Range (Median): 3.7 ng/ml	↑	↑	↑-	—	↑		
7. Jiang et al., 2014	China, pregnancy women	*Study Design: Cross-sectional *Study Size: 141 *Study Population Age: Not stated - adults *Exposure Range (Median): 4.2 ng/ml	—		—		—		
8. Lin et al., 2010	U.S., General population	*Study Design: Cross-sectional *Study Size: n=2,216 *Study Population Age: ≥ 18 years *Exposure Range (Mean): 4.5 ng/ml	↑	↑			—		
9. Melzer et al., 2011	U.S., General population	*Study Design: Cross-sectional *Study Size: n=3,974 *Study Population Age: ≥ 20 years *Exposure Range (Geo Mean): Men=4.9, Women=3.8 ng/ml							—

Citation	Study Population	Study Details	ALT	GGT	AST	ALP	TB	DB	LD
10. Olsen et al., 2000	U.S., Occupational – (male)	*Study Design: Cross-sectional *Study Size: n=265 *Study Population Age: Adults *Exposure Range (Median): 1190 ng/ml	—	—	—	—	—	—	
11. Olsen et al., 2003	Belgium & U.S., Occupational	*Study Design: Cross-sectional & Longitudinal *Study Size: n=421 & 174 *Study Population Age: Adults *Exposure Range (Mean): 1220-1990 ng/ml	—	—	—	—	—	—	
12. Olsen and Zobel 2007	Belgium & U.S., Occupational (male)	*Study Design: Cross-sectional *Study Size: n=506 *Study Population Age (Mean): 37-41 *Exposure Range (Median): 2210 ng/ml	↑-	↑-	—	—	↓-		
13. Olsen et al., 2012	U.S., Occupational	*Study Design: Longitudinal *Study Size: n=98-179 *Study Population Age: Adults *Exposure Range (Mean): Baseline/mean change -direct employed (881/-218.3ng/ml), contract (28.9/32.1ng/ml)	↓-		—	—	—		
14. Sakr et al., 2007a	U.S., Occupational	*Study Design: Cross-sectional *Study Size: n=840 *Study Population Age: Adults *Exposure Range (Median): 189 ng/ml	—	↑	—	—	—		
15. Sakr et al., 2007b	U.S., Occupational	*Study Design: Longitudinal *Study Size: n=454 *Study Population Age: Adults *Exposure Range (Median): 1040/1160 ng/ml	—	—	↑	—	↓		
16. Steenland et al., 2015	U.S., Occupational	*Study Design: Retrospective cohort *Study Size: n=3,713 *Study Population Age: Adult (mean year of birth 1951) *Exposure Range (Median): 113 ng/ml							—
17. Wang, et al., 2012	China, Occupational (male) and Highly exposed community	*Study Design: Cross-sectional *Study Size: Workers: n=55 / Residents: n=132 *Study Population Age: Adults *Exposure Range (Median): 1636 / 284 ng/ml	—/ —		—/ ↓				
18. Yamaguchi et al., 2013	Taiwan, recruited from larger cohort	*Study Design: Cross-sectional *Study Size: n=608 *Study Population Age: 16-76 years *Exposure Range (Median): 2.1 ng/ml	↑	—	↑				

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at  $\alpha=0.05$ ], — findings from some analyses are negative, positive, and non-significant. ALT (SGPT)=alanine aminotransferase, GGT=gamma-glutamyl transferase, AST (SGOT)=aspartate aminotransferase, ALP=alkaline phosphatase, TB=total bilirubin or unspecified bilirubin, DB=direct bilirubin, LD=liver disease. <sup>1</sup>Includes findings from both any liver disease and second category of liver disease which includes enlarged liver, fatty liver, and cirrhosis



Citation	Study Population	Study Details	TSH	TT4	FT4	TT3	FT3	TG	TD	Hypo	Hyper	OC
1. Bloom et al., 2010	U.S., Subgroup of sportfish anglers	*Study Design: Cross-sectional *Study Size: n=31-38 *Study Population Age: 31-45 years *Exposure Range (Geo Mean): 1.3 ng/ml	—		—							
2. Chan et al., 2011	CAN, Cases – hypothyroxemic pregnant women; Controls – nonhypothyroxemic pregnant women	*Study Design: Matched case-control *Study Size: n=271 *Study Population Age: Adults *Exposure Range (Median): Cases -3.9, Controls – 3.6 ng/ml										— <sup>a</sup>
3. de Cock et al., 2014	The Netherlands, Mother-child pairs	*Study Design: Cross-sectional *Study Size: n=83 pairs *Study Population Age: Newborns *Exposure Range (Median): 0.9 ng/ml		<sup>1</sup> ↑-								
4. Emmett et al., 2006b	U.S., Highly exposed community	*Study Design: Cross-sectional *Study Size: n=371 *Study Population Age: 2.5-89 years *Exposure Range (Median): 354 ng/ml	—						—			
5. Jain 2013	U.S., General population	*Study Design: Cross-sectional *Study Size: n=1,540 *Study Population Age: > 12 years *Exposure Range (Median): 4.1 ng/ml	↑	—	—	↑	—	—				
6. Ji et al., 2012	South Korea, Recruited from cohort study	*Study Design: Cross-sectional *Study Size: n=633 *Study Population Age: > 12 years *Exposure Range (Median): 2.7 ng/ml	—	—								
7. Kim et al., 2011	South Korea, Mother-Infant pairs	*Study Design: Prospective birth cohort *Study Size: Mothers – n=44, Pairs - n=26 *Study Population Age: > 25 years *Exposure Range (Median): Maternal prepartum blood– 1.5, Cord Blood- 1.2 ng/ml	<sup>3</sup> ↑-	—		—						
8. Knox et al., 2011	U.S., Highly exposed community	*Study Design: Cross-sectional *Study Size: n=50,113 *Study Population Age: >20 years *Exposure Range (Mean): 86.6 ng/ml	—	↑-		↓-						
Citation	Study Population	Study Details	TSH	TT4	FT4	TT3	FT3	TG	TD	Hypo	Hyper	OC
9. Lin et al., 2013	Individuals with abnormal urinalysis	*Study Design: Cross-sectional	—	—								

	results from population-based screening program in Taiwan	*Study Size: 664 (246 w/ elevated blood pressure and 398 w/ normal blood pressure) *Study Population Age: 12-30 years *Exposure (GM): 2.7 ng/ml										
10. Lopez-Espinosa et al., 2012	U.S., Highly exposed community	*Study Design: Cross-sectional and birth cohort *Study Size: n=10,725 *Study Population Age: 1-17 years *Exposure Range (Median): 29 ng/ml	—	—					↑-	↑-	—	
11. Melzer et al., 2011	U.S., General population	*Study Design: Cross-sectional *Study Size: n=3,974 *Study Population Age: ≥ 20 years *Exposure Range (Geo Mean): Men=4.9 and Women=3.8 ng/ml							↑-			
12 Olsen et al., 1998	U.S., Male, Occupational	*Study Design: Cross-sectional *Study Size: 111 (1993) and 80 (1995) *Study Population Age: Not presented *Exposure Range (Range) 0 to 80,000 (1993) and 0 to 115,000 (1995) ng/ml	—									
13. Olsen et al., 2003	Belgium & U.S., Occupational	*Study Design: Cross-sectional & Longitudinal *Study Size: n=421 & 174 *Study Population Age: Adults *Exposure Range (Mean): 1220-1990 ng/ml	—	—	—	↑						
14. Olsen and Zobel 2007	Belgium & U.S., Occupational - male	*Study Design: Cross-sectional *Study Size: n=506 *Study Population Age (Mean): 37-41 *Exposure Range (Median): 2210 ng/ml	—	—	↓-	↑-						
15. Shrestha, et al., 2015	U.S., Community-based	*Study Design: Cross-sectional *Study Size: n=87 *Study Population Age: 55-74 years *Exposure Range (Median): 9.3 ng/ml	—	—	—	—						
<b>Citation</b>	<b>Study Population</b>	<b>Study Details</b>	<b>TSH</b>	<b>TT4</b>	<b>FT4</b>	<b>TT3</b>	<b>FT3</b>	<b>TG</b>	<b>TD</b>	<b>Hypo</b>	<b>Hyper</b>	<b>OC</b>
16. Steenland et al., 2015	U.S., Occupational	*Study Design: Retrospective cohort *Study Size: n=3,713 *Study Population Age: Adult (mean year of birth 1951)							—			

		* <i>Exposure Range (Median):</i> 113 ng/ml										
17. Wang et al., 2014	Taiwan, pregnant women recruited from cohort	* <i>Study Design:</i> Cross-sectional and prospective birth cohort * <i>Study Size:</i> n=285 women and n=116 neonates * <i>Study Population Age:</i> Adult and newborns * <i>Exposure Range (Median):</i> maternal=2.4 ng/ml	—	—	—	—						
18. Webster et al., 2014	Canada, Euthyroid pregnant women recruited from study	* <i>Study Design:</i> Prospective cohort study * <i>Study Size:</i> n=152 * <i>Study Population Age:</i> >18 years * <i>Exposure Range (Median):</i> 1.7 ng/ml	<sup>4</sup> ↑-	—	—							
19. Wen et al., 2013	U.S., General population	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=1,180 * <i>Study Population Age:</i> > 20 years * <i>Exposure Range (Geo Mean):</i> 4.2ng/ml	—	—	—	<sup>1</sup> ↑-	—	—		<sup>1</sup> ↑-	<sup>2</sup> ↓-	
20. Winquist and Steenland, 2014b	U.S., Highly exposed community and Occupational	* <i>Study Design:</i> Retrospective cohort, prospective analyses * <i>Study Size:</i> Resident, n=28,541; Worker, n=3,713 * <i>Study Population Age:</i> > 20 years * <i>Exposure Range (Median):</i> 26.1 ng/ml							<sup>1</sup> ↑-	<sup>2</sup> ↑-	↑-	
<p>↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at <math>\alpha=0.05</math>]</p> <p>TSH=thyroid stimulating hormone, TT4=total thyroxine, FT4=free thyroxine, TT3=total triiodothyronine, FT3= free triiodothyronine, TG=thyroglobulin, TD=thyroid disease, Hypo=hypothyroidism, Hyper=hyperthyroidism, OC=other thyroid conditions (a. Maternal hypothyroxemia)</p> <p>1. In girls/women only. 2. Men only 3. Outcome is fetal thyroid concentrations. 4.Sensitivity analysis indicates association in High TPOAb (marker of autoimmune hypothyroidism) only</p>												

Citation	Study Population	Study Details	Uric Acid	Hyper
1. Costa et al., 2009	Italy, Occupational: Cases – Male workers engaged in the PFOA production department; Controls – Male workers never exposed to PFOA	* <i>Study Design:</i> Exposure case-control & Cross-sectional * <i>Study Size:</i> n=160 * <i>Study Population Age:</i> 20-63 years * <i>Exposure Range (Median):</i> 4400 – 5700 ng/ml	↑	
2. Geiger et al., 2013	U.S., General population	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=1,772 * <i>Study Population Age:</i> ≤ 18 years * <i>Exposure Range (Median):</i> 4.3 ng/ml	↑	↑
3. Gleason et al., 2015	U.S., General population	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=4,333 * <i>Study Population Age:</i> ≥ 12 years * <i>Exposure Range (Median):</i> 3.7 ng/ml	↑	
4. Lin et al., 2013	Taiwan, Individuals with abnormal urinalysis results from population-based screening program in Taiwan	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> 664 (246 w/ elevated blood pressure and 398 w/ normal blood pressure) * <i>Study Population Age:</i> 12-30 years * <i>Exposure (Median):</i> 3.5 ng/ml	—	
5. Sakr et al., 2007a	U.S., Occupational	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=840 * <i>Study Population Age:</i> Adults * <i>Exposure Range (Median):</i> 189 ng/ml	↑	
6. Shankar et al., 2011	U.S., General population	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=3,883 * <i>Study Population Age:</i> > 20 years * <i>Exposure (Median):</i> Women, 3.5; Men 4.6 ng/ml	↑	↑
7. Steenland et al., 2010b	U.S., Highly exposed community	* <i>Study Design:</i> Cross-sectional * <i>Study Size:</i> n=54,591 * <i>Study Population Age:</i> > 20 years * <i>Exposure (Median):</i> 27.9 ng/ml	↑	↑
<p>↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at <math>\alpha=0.05</math>] Hyper= hyperuricemia</p>				

Table 6E. Summary of findings from epidemiologic studies of PFOA and antibody concentrations (following vaccination)								
Citation	Study Population	Study Details	Tetanus	Diphtheria	Rubella	Measles	Mumps	Influenza
1. Grandjean et al., 2012	Faroe Islands, singleton births following through age 7 years	* <i>Study Design</i> : Prospective birth cohort * <i>Study Size</i> : n=656 → 587 * <i>Study Population Age</i> : Birth to 7 years * <i>Exposure Range (Median)</i> : Children (age 5) 4.1 ng/ml	↓	↓				
2. Granum et al., 2013	Norway, Sub-cohort recruited from cohort	* <i>Study Design</i> : Prospective birth cohort * <i>Study Size</i> : n=99 → 56 * <i>Study Population Age</i> : Birth to 3 years * <i>Exposure Range (Median)</i> : 1.1 ng/ml	—		↓	—		<sup>1</sup> —
3. Kielsen et al., 2015	Denmark, recruited from hospital staff	* <i>Study Design</i> : Prospective cohort * <i>Study Size</i> : n=12 * <i>Study Population Age</i> : 23-66 years * <i>Exposure Range (Median)</i> : 1.7 ng/ml	—	—				
4. Looker et al., 2014	U.S., Highly exposed community	* <i>Study Design</i> : Prospective cohort * <i>Study Size</i> : n=411 * <i>Study Population Age</i> : Adult * <i>Exposure Range (Median)</i> : 31.5 ng/ml						<sup>2</sup> ↓-
5. Stein et al., 2016	U.S., General population	* <i>Study Design</i> : Cross-sectional * <i>Study Size</i> : n=1,191 * <i>Study Population Age</i> : 12-19 years * <i>Exposure Range (Geo Mean)</i> : 4.1 ng/ml			↓-	—	↓-	

↑ = statistically significant increased association, ↓ = statistically significant decreased association, ↑- = inconsistent positively associated finding (findings from different models resulted in both statistically and non-statistically significant associations), ↓- = inconsistent negatively associated finding, — = not statistically significant, [statistical significant determined at α=0.05]

Tetanus=tetanus antibody concentrations; diphtheria=diphtheria antibody concentrations; Rubella = rubella antibody concentrations; Measles = measles antibody concentrations; Influenza= Influenza antibody titer

1. Haemophilus influenza type B (HiB) antibody concentrations 2. Influenza A H3N2 virus and an increased risk of not attaining the antibody threshold considered to offer long-term protection, other virus studied included Influenza Type B and Influenza Type A H1N1.

## **HEALTH EFFECTS - ANIMAL TOXICOLOGY**

### **Overview**

The toxicological database for PFOA includes studies of numerous effects in non-human primates and rodents. As discussed in the Introduction, a literature search was performed to identify publications relevant to health effects of PFOA including toxicological studies. The review focused on toxicological endpoints identified as sensitive and potentially relevant for use in risk assessment. The effects selected for detailed review were hepatic toxicity, developmental effects, immune system toxicity, and carcinogenicity. As discussed in the Epidemiology section, effects relevant to these endpoints have been associated with PFOA in humans. Studies related to these endpoints were summarized in summary tables and/or individual study tables, as described in the sections on each endpoint below. Additionally, information is presented on general toxicity in non-human primates, as well as thyroid, neurobehavioral, and male reproductive effects.

### **General issues**

In reviewing the toxicology data, it should be kept in mind that female rats excrete PFOA much more quickly than males. Therefore, assuming that both genders are equally sensitive based on internal dose, effects are expected from lower administered doses of PFOA in male rats than female rats.

Another general issue for some of the animal toxicology studies is that, using sensitive analytical methods, measurable levels of PFOA were detected in the serum of animals in the untreated control groups. This exposure was likely due to a combination of two factors. First, there is likely some level of unavoidable background exposure to PFOA in laboratory animals, just as in the general human population, due to the ubiquitous presence of PFOA at low levels in the environment. Second, in some studies, the controls may have experienced some level of inadvertent exposure to the PFOA used to dose the treated animals. Serum levels of PFOA in control groups from some of the studies discussed below were: Butenhoff et al. (2004a) cynomolgus monkey – 134 ng/ml; Loveless et al. (2006) rat and mouse – 100-400 ng/ml; DeWitt et al. (2008) mouse – 25-600 ng/ml; Abbott et al. (2007) mouse developmental: adults – 28-131 ng/ml, pups – 17-29 ng/ml; Macon et al. (2011) low dose mouse developmental: pups – 4-23 ng/ml.

Such low level exposure in laboratory control groups from sources such as diet and bedding also occurs for many other chemicals that are ubiquitous environmental contaminants. However, this issue is of particular relevance to PFOA because, unlike most other environmental contaminants, the dose-response curves for several of the associations seen in human epidemiology studies appear to be steepest at serum levels below about 40 ng/ml (discussed in Human Studies, above). Quantitation levels for serum PFOA in animal toxicology studies (e.g. 5 ng/ml, Reiner et al., 2009) are higher than in studies of human populations (e.g. 0.1 ng/ml in NHANES; CDC, 2015),

and serum levels in the control groups in some animal studies are within or above the range in which associations are found in the general population. Thus, the shape of the dose-response curve within the lower range of serum levels (at which associations were observed in some epidemiology studies) cannot be determined in these animal studies.

Finally, it should be noted that ammonium perfluorooctanoate (APFO, the ammonium salt of PFOA) was administered in some studies. Because APFO dissociates to PFOA in the body, results of studies of APFO are applicable to the evaluation of PFOA's toxicity.

### **Acute Toxicity**

Oral LD<sub>50</sub> values of 680 mg/kg (95% CI 399-1157 mg/kg) and 430 mg/kg (95% CI 295-626 mg/kg) were reported for male and female albino rats, respectively, after a single gavage doses of APFO and observation for 14 days (Griffith and Long 1980). In this study, five rats per sex per dose group were administered 100, 215, 464, 1000, or 2150 mg/kg. One or more deaths occurred in all groups except the 215 mg/kg group, and all animals in the 2150 mg/kg group died on day 1. Ptosis, piloerection, hypoactivity, decreased limb tone, ataxia, and corneal opacity were reported. These signs were intermittent, and there was no apparent dose-response relationship for them. Changes in the lungs (congestion, pitting, red foci) and the stomach (distension, hyperemic and thickened mucosa) were commonly seen in animals that died during the study, and many survivors had mottled kidneys and stomach changes similar to those in the animals that died. Other oral acute toxicity studies, as well as dermal and inhalation studies, are summarized by Kennedy et al. (2004).

### **Subchronic Studies in Non-Human Primates**

#### **90-day rhesus monkey study (Goldenthal, 1978)**

Goldenthal (1978) dosed rhesus monkeys (2/sex/group) with 0, 3, 10, 30 or 100 mg/kg/day APFO by gavage for 90 days (13 weeks). All 3 and 10 mg/kg/day animals survived the study. Soft stools, diarrhea, and frothy emesis were seen in the 3 mg/kg/day group, and one monkey in the 10 mg/kg/day group was anorexic during week 4, had a pale and swollen face in week 7, and had black stools for several days in week 12. In the 3 and 10 mg/kg-day groups, body weight gains were similar to controls, and no treatment related hematology or clinical chemistry changes were reported. Serum PFOA data are not provided in this study.

Mortality occurred in the two higher dose groups. In the 30 mg/kg/day group, 3 of 4 monkeys died during the study, one male during week 7 and the two females during weeks 12 and 13. All 30 mg/kg/day animals showed decreased activity, beginning in week 4. One monkey in this group had emesis and ataxia, swollen face, eyes, and vulva, as well as pallor of the face and gums. Beginning in week 6, two monkeys in this group had black stools, and one monkey had slight to moderate dehydration and ptosis of the eyelids.

All monkeys in the 100 mg/kg/day group died by week 5, with the first death occurring during week 2. These animals showed signs and symptoms that first appeared during week 1 including

anorexia, frothy emesis, pale face and gums, swollen face and eyes, decreased activity, prostration and trembling. Weight loss occurred in the two higher dose groups after week 1.

No treatment related gross pathological lesions were reported. Histopathological examination revealed effects on the adrenal gland, bone marrow, spleen, and lymph nodes in the two higher dose groups. Notably, all animals that died during the study had marked diffuse lipid depletion in the adrenal glands. All animals (including the one 30 mg/kg/day survivor) in the two higher dose groups had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen, and one 30 mg/kg/day female and all 100 mg/kg/day animals had moderate atrophy of the lymphoid follicles in the lymph nodes.

Statistically significant changes in absolute or relative organ weights at either 3 or 10 mg/kg/day in either males or females (but not both) were reported for heart, brain, and pituitary. The biological significance of these changes was stated to be unknown.

Relative liver weight data are of interest because this endpoint was affected by PFOA in cynomolgus monkeys and rodents (see below). Since each dose group consisted of only two animals, a large change would be needed to reach statistical significance for this effect. Although there were no statistically significant effects on this endpoint, the data presented in Table 7 suggests that PFOA caused increased relative liver weight at 30 and 100 mg/kg/day.

Dose (mg/kg/day)	Relative Liver Weight	
	Males (2 per group)	Females (2 per group)
0	1	1
3	1.1	1
10	1.1	1
30	1.4 (1.5)	(1.9)
100	(1.6)	(1.4)

(Numbers in parentheses include dead animals)

The LOAEL for this study was 3 mg/kg/day based on the toxicity described above in this group, and no NOAEL was identified.

Because serum PFOA levels were not measured in this study, it is not possible to compare the internal dose at which effects occurred in the rhesus monkeys with the results from cynomolgus monkeys and rodents (detailed below).

#### 4 week cynomolgus monkey study (Thomford, 2001a)

In a range finding study, the toxicity of APFO was studied in male cynomolgus monkeys (young adult to adult) dosed daily by capsule for 4 weeks (Thomford, 2001a). Dose levels were 0 (n=2), 2 mg/kg/day (n=3), and 20 mg/kg/day (n=3). Parameters evaluated in blood (prior to treatment and on Day 30) included serum PFOA (although data were not reported in Thomford et al., 2001a), hematology, clinical chemistry (also evaluated on Day 2, about 24 hours after first dose), and hormones (estradiol, estrone, estriol, thyroid stimulating hormone, total and free triiodothyronine, and total and free thyroxin). Of the parameters measured in blood, estrone was notably lower in both treated groups

All animals survived until the end of the study, no clinical signs of toxicity were observed in the treated groups, and body weight was not affected by treatment. Low or no food consumption occurred in one animal dosed with 20 mg/kg-day. Microscopic evaluation of the adrenals, liver, pancreas, spleen, and testes found minimal mineralization in the adrenals of one animal given 2 mg/kg/day and immature testes in one animal in each of the two dosed groups (2 of 6 dosed animals). The authors stated that these findings were not related to PFOA treatment. There was no evidence of peroxisome proliferation (assessed by palmitoyl CoA oxidase activity; PCO) in the liver, or increased cell proliferation (assessed by the proliferating cell nuclear antigen; PCNA) in the liver, testes, and pancreas of treated monkeys. The authors concluded that NOAEL was 20 mg/kg, and no LOAEL was established.

#### 6 month (26 week) cynomolgus monkey study (Thomford, 2001b; Butenhoff et al., 2002)

The toxicity of APFO was studied in male cynomolgus monkeys dosed daily by capsule for 6 months (Thomford, 2001b; Butenhoff et al., 2002). This study is described in detail because it has been used as the partial or entire basis for several earlier final and draft PFOA risk assessments (USEPA, 2005a; MDH, 2008; Post et al., 2009a; Tardiff et al., 2009; NC SAB, 2010; ATSDR, 2015), and because it is considered in evaluating the human relevance of hepatic effects of PFOA (See Mode of Action section, below). Concerns related to use of this study as the basis for risk assessment and the reasons why the data do not support Benchmark Dose modeling based upon it (Butenhoff et al., 2004a) are discussed in detail in Appendix 3 of this document. A table summarizing this study, with a focus on hepatic effects, is included in Appendix 5. Data for increased liver weight from this study are included in Table 10.

In this study as originally designed, male monkeys, age 3 to 9 years, were to be administered doses of 0, 3, 10, or 30 mg/kg/day by capsule, 7 days per week for 26 weeks. Six animals were dosed per group, except for the 3 mg/kg/day group which had four animals. However, as detailed below, the high dose (30 mg/kg/day) was reduced to 20 mg/kg/day due to overt toxicity. Additionally, only three of the four low dose (3 mg/kg/day) and two of the six high dose (30 mg/kg/day) monkeys tolerated the administered dose well enough to complete the study, while all six animals in the mid-dose group (10 mg/kg/day) completed the study. Two animals from the control and 10 mg/kg/day groups were observed for delayed effects for 13 weeks following the 6 months of dosing.

One low dose (3 mg/kg/day) monkey was sacrificed on day 137 (week 19) after loss of about 10% of its weight in one week accompanied by low food consumption, few feces, hind-limb paralysis, ataxia, and lack of response to pain. No specific organ pathology or clinical chemistry changes explaining the morbidity were found, and the study authors stated that it was unclear whether or not these effects resulted from exposure to PFOA. It is notable that the liver-to-body weight ratio in this animal, 2.44, was much higher than for other animals in this group and was comparable to the ratios in the high dose group. Also, the liver-to-brain weight ratio in this animal, 1.66, was the highest of any animal in the study. However, the serum and liver PFOA levels in this animal were not higher than for other animals in the same treatment group. These findings suggest that this animal may have been particularly susceptible to the hepatic effects of PFOA.

Dosing of the high dose (30 mg/kg/day) group was stopped on day 12 due to toxicity in the first week, including low food consumption, weight loss, and few or no feces. The dosing of this group was restarted at 20 mg/kg/day on day 22, after a 10-day break to allow for recovery from the toxicity in first week.

One high dose monkey was sacrificed on day 29 due to decreased body weight, lack of eating, hypoactivity, and coldness to touch. The liver of this animal had lesions including mid-zonal and centrilobular hepatocellular degeneration and necrosis, diffuse hepatocellular vacuolation, and centrilobular hepatocyte basophilia indicative of liver regeneration. Other effects observed in this monkey included necrosis, erosions, and ulcerations of the esophagus and stomach, and involution of the thymus. This animal had increased serum enzymes indicative of substantial liver and muscle injury, and a marked decrease in serum cholesterol to about 10% of control levels. The study authors state that the liver lesions were likely related to treatment.

Dosing of three high dose (30/20 mg/kg/day) monkeys was stopped on days 43, 66, and 81 due to low or no food consumption, dramatic weight loss (18-23% of body weight), and few or no feces. These three monkeys were monitored without dosing for the rest of the study, and their body weights increased to above pre-dosing levels by the end of the study.

In the study, serum PFOA levels were analyzed every 2 weeks, including in animals for which dosing was stopped. Steady state was reported to have been reached after 4-6 weeks. The complete set of serum data was reported in a separate publication (Butenhoff et al., 2004a). Means and standard deviations for serum levels in the control, 3, 10, and 30/20 mg/kg/day groups, excluding animals for which dosing was stopped, were reported as 134±113 ng/ml, 80,000±40,000 ng/ml, 99,000±49,000 ng/ml, and 155,000±103,000 ng/ml. It is evident from the data that there was a wide variation in serum levels within the same treatment group, and that serum levels did not increase proportionally with dose. The ratios between mean serum levels at steady-state in the 3, 10, and 30/20 mg/kg/day groups were approximately 1:1:2 (i.e. serum

levels were similar in the 3 and 10 mg/kg/day groups), and the mean serum levels at 3 mg/kg/day and 10 mg/kg/day did not differ significantly at the  $p < 0.05$  level (Butenhoff et al., 2002).

Hepatic PFOA concentrations at sacrifice did not differ between the 3 and 10 mg/kg/day groups ( $15,000 \pm 3600$  ng/g and  $14,000 \pm 7600$  ng/g, respectively) and were very disparate in the two high dose (20/30 mg/kg/day) animals that completed the study (16,000 and 83,000 ng/g). It is notable that the highest liver concentration by far (154,000 ng/g) was found in the high dose animal sacrificed due to treatment-related morbidity at week 5.

Average body weight gains during the dosing period in the 0, 3, and 10 mg/kg/day groups were 19, 20, and 20%, while the weight changes in the two 30 mg/kg/day animals that completed the study were  $-8\%$  and  $18\%$ .

Serum cholesterol was not affected by PFOA treatment in this study, while triglycerides were significantly increased compared to controls at several time points in the mid- and high dose group. These observations are notable, since decreases in serum cholesterol have been observed in rodent studies with PFOA, consistent with the effects of other PPAR-alpha activators, and since human PFOA exposure has been associated with increases in cholesterol (see Epidemiology section, above). As discussed above, the dose-response curves for increased cholesterol in humans appears steepest at serum PFOA levels below about 40 ng/ml, with a much flatter dose-response between about 40 and 350 ng/ml PFOA in serum (Nelson et al., 2010; Steenland et al., 2009b). Since the mean serum PFOA level in the control monkeys was 134 ng/ml, the shape of the dose response curve for cholesterol at serum levels relevant to effects observed in humans is not known.

In the high dose group, including the three monkeys in which treatment was stopped, total neutrophils, total serum protein, and albumin were decreased. In the two monkeys in this group that were dosed until the end of the study, the changes were not significant but were consistent over time. In one of the monkeys whose dosing was stopped, serum liver enzyme levels and elevated serum bile acids were greatly increased three days prior to the stopping of the dosing. Additionally, the high dose monkey that was sacrificed in moribund condition on Day 29 had markedly elevated liver enzymes and markedly low cholesterol.

Changes in thyroid hormone levels occurred in treated groups; these are described in detail in the section on thyroid effects below. Although estradiol levels in the high dose group appeared to decrease with treatment, the authors did not attribute these findings to treatment.

Absolute liver weight and liver-to-body weight ratios were increased in all treated groups (Butenhoff et al., 2002). The increase in absolute liver weight was statistically significant ( $p < 0.01$ ) in all groups, but was only significant for relative liver weight in the high dose group. However, this analysis does not include the animals that were sacrificed in moribund condition during the study. The absolute and relative liver weight of the sacrificed low dose monkey was

far higher than the others in its group, and inclusion of data for this animal increases the mean value for these parameters (Table 10). Liver-to-brain weight ratio is considered to be a reliable measure of effects on liver weight, because brain weight tends to remain stable when body weight changes. This ratio was increased in all treated groups compared to controls but did not increase with administered dose or serum level (Butenhoff et al., 2004c). In the control, 3, 10, and 30/20 mg/kg/day groups, the liver-to-brain weight ratios were 0.934, 1.34, 1.30, and 1.22, respectively.

No gross or microscopic pathological changes were seen in the organs examined from animals that completed the study. However, as mentioned above, multiple pathological changes were found in the high dose monkey sacrificed in moribund condition on Day 29. These included edema and inflammation of the esophagus and stomach (attributed to dosing injury); liver lesions including mid-zonal and centrilobular hepatocellular degeneration and necrosis, diffuse hepatocellular vacuolation, and hepatocyte basophilia in centrilobular areas indicative of liver regeneration; involution of the thymus (noted as a common stress response); and degeneration and necrosis of the heart (noted as likely an agonal change).

Two monkeys from the control group and two from the 10 mg/kg/day group were observed for 13 weeks following the 6-month treatment period. Weight gain during the recovery period was lower in the 10 mg/kg/day animals than in the controls. During the recovery period, the control group monkeys gained 10 and 11% of their body weight, while one treated monkey gained 5% and the other lost 3%.

Studies of hepatic biochemical markers in this study are discussed in the Mode of Action section, below.

The LOAEL in this study is 3 mg/kg/day based on mortality possibly related to treatment (25%) and increased liver weight, and the NOAEL is unknown. Mean serum levels at this dose were reported by Butenhoff et al. (2004a) as  $80,600 \pm 40,000$  ng/ml. It is important to note that 6 months represents less than 2% of the lifespan of about 30 years in this species of monkey. It is not known whether additional or more severe effects would have occurred with continued dosing of the monkeys that tolerated dosing for the full 6 months of the study. Additionally, this study did not include female monkeys and, because exposure was initiated at age 3 to 9 years, effects specific to exposures during development or at younger ages would not be evident.

Comparison of the results of this study with chronic rat studies discussed below suggests that cynomolgus monkeys are more sensitive to the overt toxicological effects of PFOA than are rodents. As discussed above, four of the six high dose monkeys exhibited severe toxicity that necessitated removal from the study. The serum PFOA level in the high dose monkey that was sacrificed on Day 29 with severe multi-organ toxicity was 822,000 ng/ml. The serum levels of the other three high dose monkeys removed from the study on days 43, 66, and 81 due to overt toxicity (low or no food consumption, dramatic weight loss of 18-23%, and few or no feces)

were 102,000 ng/ml, 467,000 ng/ml, and 235,000 ng/ml, respectively, at the times when dosing was stopped. These severe toxic effects occurred after dosing for about 0.3-0.7% of the monkeys' lifespans. In contrast, survival was unaffected or increased compared to controls, and no overt toxicity was observed, in male rats with lifetime (2 year) exposure to PFOA doses that were estimated to result in serum PFOA levels of almost 600,000 ng/ml (Sibinski 1987; Biegel et al., 2001; USEPA, 2005a). Thus, severe toxicity resulted from relatively short exposures to PFOA in primates at serum levels below those that were well tolerated chronically by rats.

### **Hepatic Effects in Rodents and Non-Human Primates**

PFOA causes liver toxicity in experimental animals, including rodents and non-human primates (reviewed by Kennedy et al., 2004; Lau, 2012). As discussed in the Epidemiology section, increased liver enzymes are associated with exposure to PFOA in humans.

Increased liver weight is a sensitive toxicological endpoint for PFOA that has been observed in many toxicological studies. Although studies of PFOA that report increased liver weight do not always include evaluation of other hepatic endpoints, numerous studies of PFOA have demonstrated that increased liver weight co-occurs with and/or progresses to more severe hepatic effects including increased serum liver enzymes, hepatocellular necrosis, fatty liver, and/or hyperplastic nodules. Additionally, recent studies show that cellular damage indicative of liver toxicity persists until adulthood following developmental exposure to PFOA.

This section first reviews data indicative of liver damage from toxicological studies of PFOA. This is followed by a presentation of data on increased relative liver weight from PFOA. Numerous toxicological studies of PFOA have evaluated liver weight, and a separate discussion of each of these is beyond the scope of this document. Because studies of increased liver weight that include relatively low doses of PFOA are most relevant to quantitative risk assessment, they are reviewed in detail and are summarized in Table 10.

Chronic exposure to PFOA also caused hepatic adenomas in rats (See Chronic Studies, below). The mode of action for hepatic effects, particularly as related to human relevance, is discussed in detail in the Mode of Action section.

### **Histopathological changes in the liver**

Hepatocellular hypertrophy is a consistently reported histopathological finding for PFOA that accompanies increased absolute and relative liver weight. A number of studies in both non-human primates and rodents also report other histopathological changes that are indicative of liver injury and/or lipid accumulation in the liver. These effects occurred at doses similar to those causing increased liver weight. Notably, prenatal exposure to a very low dose (0.01 mg/kg/day) of PFOA caused liver toxicity that persisted until adulthood at doses below those which caused increased liver weight (Quist et al., 2015).

International harmonization of diagnostic criteria and terminology for histopathologic lesions has recently been developed to provide consistency and to reflect current knowledge of the biochemical and cellular processes involved in changes in the liver and other organs (Mann et al., 2012). The terminology and current definitions that are recommended by the international harmonization group to describe hepatic damage were applied where appropriate to describe the histopathological findings discussed below.

Information from some studies that reported histopathological effects indicative of liver damage is summarized below. It should be noted that this summary does not represent a complete compilation of all studies such effects. The significance of these effects as related to the mode of action of PFOA is discussed in the Mode of Action section.

As discussed above, the high dose (30/20 mg/kg/day) male cynomolgus monkey sacrificed in moribund condition on Day 29 of the 90-day study had liver lesions including mid-zonal and centrilobular hepatocellular degeneration and necrosis, diffuse hepatocellular vacuolation, and centrilobular hepatocyte basophilia indicative of liver regeneration (Butenhoff et al., 2002).

In a 28-day study of male and female Chr-CD albino rats with dietary exposure to 30-1000 ppm APFO, degeneration and/or necrosis of hepatocytes and focal bile duct proliferation occurred in all treated groups (Kennedy et al., 2004). The dose from 30 ppm in the diet was estimated as 1.5 mg/kg/day. Similar changes were seen in livers of rats dosed with 3 to 1000 ppm in the diet for 90 days, and were stated to be more frequent in males, and most pronounced at the highest dose (Griffith and Long, 1980).

Individual cell and focal necrosis, increased hepatocellular mitotic figures, fatty changes, and bile duct hyperplasia were observed in male CD-1 mice dosed by gavage with linear APFO for 29 days (Loveless et al., 2008). The LOAEL for individual cell and focal necrosis was reported by the authors as 1 mg/kg/day, and the NOAEL as 0.3 mg/kg/day. Focal necrosis occurred in one of ten mice at 0.3 mg/kg/day but was stated by the authors to not be related to PFOA treatment. Increased mitotic figures, fatty changes, and bile duct hyperplasia occurred at  $\geq 10$  mg/kg/day.

Multiple histopathological changes were observed in the livers of three strains (wild type, PPAR-alpha null, and humanized PPAR-alpha) of male Sv/129 mice dosed with 1 or 5 mg/kg/day PFOA for 6 weeks (Nakagawa et al., 2011). PFOA caused a dose-dependent increase in macrovesicular steatosis in the PPAR-alpha null mice, while this effect was not seen in the wild type mice. Additionally, microvesicular steatosis occurred at both doses in the PPAR-alpha null and humanized PPAR-alpha mice. Lobular inflammation was observed only in the PPAR-alpha null mice treated with PFOA. Single cell necrosis occurred in all three strains of PFOA treated mice and appeared to be most severe in the wild type mice. Hydropic degeneration of hepatocytes was seen in the PPAR-alpha null and humanized PPAR-alpha strains treated with PFOA, but not the wild type strain.

Adverse histological changes including deranged liver architecture, severe edema, vacuolar degeneration, focal necrosis, and obvious inflammatory infiltration were observed in livers of male Kunming (KM) mice dosed by gavage with 2.5 to 10 mg/kg/day PFOA for 14 days (Yang et al., 2014). These changes were more severe with increasing dose and were not seen in the control mice.

Bile duct injury, which was much more severe in PPAR-alpha null mice than in wild type mice (male 129S4 strain), occurred after 4 weeks of gavage dosing with 4.4, 10.8, or 21.6 mg/kg/day APFO (Minata et al., 2010). Microvesicular steatosis was also more prominent in treated PPAR-alpha null than similarly treated wild type mice, and focal necrosis occurred at the highest dose in the null mice.

Focal and multi-focal hepatic necrosis was observed at sacrifice on PND 109-120 in F1 male offspring in the 3, 10, and 30 mg/kg/day dose groups (but not at 1 mg/kg/day) in a two-generation rat reproductive developmental study that is discussed in detail below (Butenhoff et al., 2004b).

Histopathological changes occurred in the livers of male Sprague-Dawley rats sacrificed one day after the end of dietary exposure to 300 ppm APFO for 1, 7, or 28 days (Elcombe et al., 2010). This study was conducted twice, and the daily doses were estimated as 19 mg/kg/day and 23 mg/kg/day in the two studies. In both studies, PFOA caused periportal glycogen depletion after 1, 7, and 28 days of exposure to PFOA, hepatocellular hypertrophy after 7 and 28 days, and hepatocellular hyperplasia in all treated rats after 28 days.

In a chronic rat study discussed in detail below (Sibinski, 1987; Butenhoff et al., 2012), focal hepatocellular necrosis occurred in 6/15 high dose (14.2 mg/kg/day) males but not in any of the 15 controls at the one-year sacrifice. (The low dose group was not evaluated at one year). At the two-year sacrifice, the incidence of focal hepatocellular necrosis was similar in control and treated groups, but the incidence of hepatic hyperplastic nodules was increased in the high dose males compared to the control and low dose groups. Butenhoff et al. (2012) concluded that the increased incidence of focal necrosis and vacuolation at one year, but not at later time points, may have been due to the higher background incidence of these changes in older rats.

Additionally, PFOA caused hepatocellular adenomas in chronically exposed male rats in one of the two chronic rat studies that have been conducted (Biegel et al. 2001). Although these tumors were not increased in the other chronic rat study (Sibinski et al., 1987), the EPA Science Advisory Board (2006) concluded that the increased in hepatic hyperplastic nodules in Sibinski et al. (1987) may have been part of the continuum of proliferative lesions in the hepatic carcinogenic process. More recently, Butenhoff et al. (2012) suggested that the observations at one year and two years in Sibinski et al. (1987) suggest a progression of lesions “from hepatocellular hypertrophy to fatty degeneration to necrosis followed by regenerative

hyperplasia.” Butenhoff et al. (2012) also note that the diagnostic criteria for these nodules, which indicate a regenerative process, have changed since the study was evaluated in 1986.

PFOA caused hepatic triglyceride accumulation (fatty liver) in male Wistar rats after 7 days of dietary exposure (Kawashima et al., 1995). Hepatic triglyceride levels were significantly increased at the lowest dose (25 ppm) and were 3.5 times control levels in the 100 ppm dose group.

Several studies have evaluated hepatic effects of PFOA in mice that were fed diets containing specific types of lipids or higher general fat content. Kudo and Kawashima (1997) studied the effect of PFOA (2.5 - 10 mg/kg/day by intraperitoneal injection for 7 days) on hepatic triglyceride accumulation in male mice that had been fed diets containing soy bean oil, perilla oil, or fish oil for 4 weeks. Fish oil, but not the other two types of oil, was known to generally decrease hepatic triglycerides. PFOA caused a dose-dependent increase in hepatic triglycerides in mice exposed to perilla oil and soybean oil, but had no effect in mice exposed to fish oil.

Additionally, necrotic cell death, lipid droplet accumulation, and inflammatory cell infiltration were found in male C57BL/6N mice dosed with 5 mg/kg/day PFOA in a liquid diet for 3 weeks (Tan et al., 2013). These effects of PFOA were more severe in mice receiving a high fat diet than a regular diet. PFOA caused increased hepatic triglyceride levels in mice receiving regular diets or high fat diets, and relative white adipose tissue weight was decreased by PFOA treatment.

Finally, PFOA (5, 10, or 20 mg/kg/day by gavage for 14 days) caused lipid accumulation in the livers of male Balb/C mice fed either a regular diet or a high fat diet (Wang et al., 2013). Consistent with Tan et al. (2013), PFOA caused decreased relative weight of adipose tissue in mice receiving either diet. The authors concluded that lipids were both being released from the adipose tissue and accumulating in the liver. Evaluation of hepatic ultrastructural changes showed that PFOA caused a dose-dependent increase in the accumulation of lipid droplets in the nucleus of hepatic cells; the dose-response for this effect was similar in the regular and high fat diet groups. In the regular diet mice dosed with 10 and 20 mg/kg/day, dilation of the endoplasmic reticular was observed. More severe changes were seen in high fat diet mice at these doses including mitochondrial swelling, irregular nuclei, and condensed chromatin suggesting apoptosis.

Two recent studies found persistent hepatic damage in female mouse offspring after maternal gestational exposure to low doses of PFOA. Quist et al. (2015) evaluated hepatic effects in female CD-1 mouse offspring from dams dosed with 0.01 to 1 mg/kg/day PFOA on GD 1-17. Livers from offspring were evaluated on PND 21 (weaning) and PND 91. On PND 21, relative liver weight was significantly increased at 0.3 and 1 mg/kg/day, with no effect at 0.01 or 0.1 mg/kg/day, and PFOA treatment did not cause hepatocellular hypertrophy at any dose at this time point. In contrast, hepatocellular hypertrophy was significantly increased ( $p < 0.01$ ) at PND 91 in all dosed groups (0.01 mg/kg/day; NOAEL not identified), although the increased relative

liver weight observed at higher doses on PND 21 had been resolved. Additionally, dose-related hepatic periportal inflammation occurred in treated offspring on PND 21 and 91, and was more severe on PND 21 than on PND 91. On PND 21, the severity score for this effect was significantly ( $p < 0.05$ ) increased at all doses ( $\geq 0.01$  mg/kg/day) in a dose-related fashion, with no NOAEL identified. In this study, periportal inflammation was a more sensitive indicator of toxicity from prenatal exposure to PFOA than increased liver weight in female CD-1 mice.

Quist et al. (2015) also evaluated ultrastructural hepatic changes in control and highest dose (1 mg/kg/day) mice using transmission electron microscopy. Mitochondrial proliferation and abnormal mitochondrial morphology occurred in PFOA-treated mice at both PND 21 and PND 91 and was more severe at the later time point. Peroxisomes were present only in a single liver section from one treated animal and were closely associated with areas of mitochondrial proliferation in this section.

In summary, hepatocellular hypertrophy was observed by Quist et al. (2015) at low doses ( $\geq 0.01$ ) on PND 91, a time point when PFOA had almost completely been eliminated from the body and the transient increased relative liver weight observed at the higher PFOA doses on PND 21 had resolved. Although hepatocellular hypertrophy from PFOA in adult rodents has typically been associated with proliferation of peroxisomes or smooth endoplasmic reticulum, these changes were not seen in adult (PND 91) female mice after prenatal exposure to PFOA (1 mg/kg/day). Instead, hepatocellular hypertrophy was associated with mitochondrial proliferation and abnormal mitochondria. The significance of these observations is further discussed in the Mode of Action section.

Filgo et al. (2015) evaluated histopathological effects in the liver at age 18 months in female offspring of dams dosed during gestation. The study evaluated three strains of mice: CD-1, 129/Sv wild type, and 129/Sv PPAR-alpha null. CD-1 dams were dosed with PFOA at 0, 0.01, 0.1, 0.3, 1, and 5 mg/kg/day, and 129/Sv wild type and PPAR-alpha null dams were dosed with 0, 0.1, 0.3, 1, and 3 mg/kg/day. Findings related to non-neoplastic hepatic changes in CD-1 mice included significant dose-related trends for increased incidence of centrilobular hepatocyte hypertrophy, Ito cell hypertrophy, and oval cell hyperplasia, and for increased severity of chronic active inflammation. In the 129/Sv strains, non-neoplastic hepatic changes caused by PFOA were primarily observed in the PPAR-alpha null mice. These included increased incidence of bile duct hyperplasia, hyaline droplet accumulation in the bile duct, hematopoietic cell proliferation, and centrilobular hepatocyte hypertrophy. In the wild type 129/Sv mice, only severity of centrilobular hepatocyte hypertrophy was increased by PFOA treatment.

The incidence of liver tumors was also evaluated by Filgo et al. (2015) because of the unexpected finding of hepatic tumors in some animals that died before the scheduled end of the study. However, the authors emphasize that the study was not designed or intended to be a carcinogenicity bioassay. In CD-1 mice, single or multiple hepatocellular adenomas were found in one or more animals in each of the treated groups ( $n=21$  to 37 per group) except for at the

lowest dose (0.01 mg/kg/day), but were not found in controls (n=29). In total, adenomas occurred in 4.9% (7 of 144) treated CD-1 mice, compared to a historic control incidence of 0.4% in untreated female CD- mice. Hepatocellular carcinomas occurred in two treated CD-1 mice (0.3 and 5 mg/kg/day) but not in controls. In 129/Sv wild type mice, hepatocellular adenomas did not occur in control or treated groups (n=6 to 10 per group). In PPAR-alpha null mice of this strain, in contrast, there were no adenomas in the controls (n=6), one adenoma in the 0.1, 0.3, and 1 mg/kg/day groups (n=9 or 10), and two adenomas at 3 mg/kg/day (n=9). These tumors occurred in 13.2% of all treated PPAR-alpha null mice. The significance of these findings is further discussed in the Mode of Action section below.

#### Serum liver enzymes and bile acids

Most toxicology studies of PFOA's effects on liver weight and histopathology did not assess serum levels of hepatic enzymes or bile acids. However, several studies in rodents have reported that these parameters were increased by PFOA, and these data are summarized below. As above, this summary does not represent a complete compilation of all data on these endpoints. These endpoints are of interest because they are indicative of hepatic damage, and because increased serum liver enzymes are associated with PFOA in human studies of the general population, communities with drinking water exposure, and workers (see Epidemiology section, above).

In the 90-day cynomolgus monkey study, ALT and AST were greatly elevated (about 10 to 50 times control levels) in two of the high dose animals that experienced toxicity from PFOA prior to the end of the scheduled dosing period (Butenhoff et al., 2002).

The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were increased in all dose groups ( $\geq 0.49$  mg/kg/day) in a study of mice dosed with PFOA in drinking water for 21 days (Table 8). These increases were statistically significant ( $p < 0.05$ ) at  $\geq 2.64$  mg/kg/day for AST and  $\geq 17.62$  mg/kg/day for ALT (Son et al., 2008).

Table 8. Liver enzymes (relative to control) in serum of mice exposed to PFOA in drinking water for 21 days (Son et al., 2008)		
Dose (mg/kg/day)	ALT	AST
0	1	1
0.49	1.5	2.0
2.64	2.9*	1.5
17.62	4.2*	3.3*
47.21	5.2*	4.0*

\* $p \leq 0.05$ .

Numerical data for liver enzymes (mean  $\pm$  SD) are presented in Son et al. (2008).

PFOA caused dose-related increases in serum levels of four liver enzymes (ALT, AST, alkaline phosphatase, and lactic dehydrogenase [LDH]), as well as total bile acids, in male mice (Yang et al., 2014). The LOAEL for increased ALT was 2.5 mg/kg/day (the lowest dose in the study), while the LOAEL for increases in the other liver enzymes and total bile acids was 5 mg/kg/day. All parameters were increased to several-fold above control levels at 5 and/or 10 mg/kg/day.

Dose-related increases in ALT, AST, total bilirubin, and total bile acids occurred in both wild type and PPAR-alpha null mice treated with PFOA (Minata et al., 2010). For all of these parameters, the maximum increase at the highest dose was much greater in the PPAR-alpha null mice than in the wild type mice.

Nakagawa et al. (2011) also reported small but statistically significant increases in ALT in all three strains of mice (wild type, PPAR-alpha knockout, humanized PPAR-alpha) studied. The greatest increase was 2.3-fold in wild type mice given 5 mg/kg/day PFOA, with smaller increases in the PPAR-alpha null and humanized PPAR-alpha strains.

Liver enzymes (AST, ALT, GGT, and LDH) were significantly elevated on GD 18 in pregnant ICR mice dosed with 10 mg/kg/day PFOA on GD 1-17. AST and ALT were elevated at 5 mg/kg/day, but this effect was not significant, while no changes were observed at 1 mg/kg/day. (Yahia et al., 2010).

In a chronic rat study discussed below, ALT, AST, and alkaline phosphatase were increased in males in both low (1.3 mg/kg/day) and high dose (14.2 mg/kg/day) groups between 2 and 18 months of dosing, and in the high dose group after 24 months of dosing (Sibinski, 1987; Butenhoff et al., 2012). Butenhoff et al. (2012) conclude that these increases may result from the hepatic hypertrophy and/or “borderline chronic liver toxicity” caused by PFOA.

#### Detailed review of selected data for increased relative liver weight

Numerous studies have consistently reported that PFOA causes increased absolute and relative liver weight in laboratory animals. Increased relative liver weight is a sensitive endpoint for PFOA that co-occurs and/or can progress to more severe manifestations of hepatic toxicity. Data on increased relative liver weight from rodent studies that used relatively low doses (1 mg/kg/day or less), as well as the 90-day cynomolgus monkey study (Butenhoff et al., 2002), are summarized in Table 10. This group of studies includes five studies that provide data on serum PFOA levels at the end of the dosing period (Butenhoff et al., 2002; Lau et al., 2006; Loveless et al., 2006; Perkins et al., 2004; Macon et al., 2011) and six additional studies that do not include such serum data. Studies providing serum PFOA data at the end of the dosing period are most appropriate for dose-response evaluation in risk assessment, because serum levels are highest at this time point and thus represent the maximum internal doses that could have caused the observed effect. Detailed individual study tables are found in Appendix 5 for the five studies that provide relevant serum PFOA levels.

### Rodent data on increased relative liver weight

Three studies (Perkins et al., 2004; Loveless et al., 2006; Loveless et al., 2008) evaluated effects in male rats exposed to APFO. Perkins et al. (2004) and Loveless et al. (2006) provide serum PFOA data useful for dose-response modeling, while Loveless et al. (2008) does not include serum PFOA levels. Individual study tables for the two rat studies that include serum data (Perkins et al., 2004 and Loveless et al., 2006) are found in Appendix 5.

APFO formulations containing differing isomeric compositions (linear/branched, linear, or branched) were used in the three male rat studies (Perkins et al., 2004; Loveless et al., 2006; Loveless et al., 2008). Both linear/branched and linear PFOA were produced industrially, while information on effects of the branched form, which was not produced industrially, contributes to the understanding of the toxicology and mode of action of PFOA. Perkins et al. (2004) evaluated effects of linear/branched PFOA for 4, 7, and 13 weeks, while Loveless et al. (2006) studied the effects of 2 weeks of exposure to the linear isomer, branched isomers, or a mixture of linear/branched isomers. Both of these studies evaluated absolute and relative liver weight, hepatic palmitoyl CoA oxidase activity (PCO; a marker of peroxisome proliferation), and serum PFOA levels at sacrifice. The third male rat study, Loveless et al. (2008) evaluated the linear isomer of PFOA and reported relative liver weight data after exposure for 29 days, but did not report serum PFOA levels or PCO activity. (Both Loveless et al., 2006, and Loveless et al., 2008, also evaluated male mice, discussed below.)

In Perkins et al. (2004), serum PFOA levels in rats reached steady state by 4 weeks. The dose-response curves for increased liver weight relative to body weight were almost identical at all three time points (4, 7, and 13 weeks), indicating that relative liver weight did not continue to increase over time with exposures longer than 4 weeks.

In the study evaluating the three PFOA formulations with differing isomer content (Loveless et al., 2006), serum PFOA levels from branched PFOA were lower than from the same doses of linear/branched or linear PFOA. This difference is likely to result from the more rapid excretion of the branched isomers (Benskin et al., 2009; DeSilva et al., 2009). The serum level LOAELs for male rats after 2 weeks of exposure to linear/branched PFOA reported by Loveless et al. (2006) are consistent with those from the longer exposure periods in Perkins et al. (2004). The serum level dose response curves for increased relative liver weight from linear/branched PFOA in these two male rat studies with differing exposure durations are also consistent at the lower serum PFOA levels (less than 75,000 ng/ml) and are generally similar at the higher serum PFOA levels. Additionally, the dose-response curves for male rats based on administered doses of linear PFOA in the 2-week study (Loveless et al., 2006) and the 29-day study (Loveless et al., 2008) are generally consistent; serum PFOA data are not provided in the 29-day study.

Effects in male mice from exposure for 2 weeks to the three isomeric formulations of PFOA were also studied by Loveless et al. (2006). The same parameters that were evaluated in rats were studied in the mice, including absolute and relative liver weight, hepatic PCO activity, and

serum PFOA levels at sacrifice. The dose-response curves for male mice based on administered doses of linear PFOA in the the 2-week study (Loveless et al., 2006) and the 29-day study (Loveless et al., 2008) were generally similar. However, as above, serum PFOA data are not provided in the 29-day study.

The LOAEL for increased in liver weight in male mice was 0.3 mg/kg/day (the lowest dose in the study) for the linear form and the branched form. The serum levels at this dose were 13,000 ng/ml for the linear form and 14,000 ng/ml for the branched form. These are the lowest serum level LOAELs that were identified for increased relative liver weight. The relative liver weight at this dose was 1.17 for the linear form and 1.19 for the branched form, compared to the controls. For linear/branched PFOA, the serum level at 0.3 mg/kg/day was 10,000 ng/ml, and relative liver weight was 1.19 compared to the controls (similar to the increase at 0.3 mg/kg/day for the other isomeric forms). However, this change was not reported to be statistically significant, and the authors reported 0.3 mg/kg/day as the NOAEL and 1 mg/kg/day as the LOAEL for the linear/branched mixture.

PFOA increased hepatic peroxisome proliferation (as indicated by PCO activity) in rats in Perkins et al. (2004) and in rats and mice in Loveless et al. (2006). Evaluation of these PCO data reveals that, in the standard strains of rodents used in these experiments, increased liver weight did not correlate well with this biochemical marker of hepatic peroxisome proliferation, particularly in mice. Additionally (as mentioned above), PFOA caused increased hepatic peroxisome proliferation (as indicated by PCO activity) in primates as well as rodents. These PCO data are informative in evaluating the role of PPAR-alpha activation in the increased liver weight caused by PFOA and are discussed in detail in the Mode of Action section below.

In another study that includes serum PFOA data from the end of the dosing period, pregnant mice were dosed on GD 1-17 with 1, 3, 5, 10, 20, or 40 mg/kg/day PFOA (Lau et al, 2006; Table 10; Appendix 5A). At sacrifice on GD 18, the serum level LOAEL for increased liver weight was 22,000 ng/ml (from an administered dose of 1 mg/kg/day) and a NOAEL was not identified (Lau et al., 2006). (Numerical data are not shown in publication and were provided by the investigator.)

A second study of pregnant mice evaluated liver weight and serum PFOA levels on PND 21 in wild type and PPAR-alpha null mice dosed throughout gestation (Abbott et al., 2007). The NOAELs and LOAELs for increased liver weight based on serum PFOA from this study are not comparable to those from the other studies because serum PFOA was evaluated 3 weeks after dosing ended.

Two additional studies in male (Son et al., 2008) or female (DeWitt et al., 2008) adult mice exposed to PFOA in drinking water for 15 or 21 days that did not report serum PFOA data are also included in Table 10. As shown in the table, LOAELs based on administered dose from

these studies are generally consistent with the other mouse studies discussed above. NOAELs were not identified in these studies.

Three studies included in Table 10 evaluated increased liver weight in mouse pups after developmental exposures to PFOA. One of these studies (Macon et al., 2011) evaluated liver weight in offspring on PND 1, 4, 7, 14, and 21 after maternal exposure on GD 10-17 (late gestation). This study provides serum PFOA data from PND 1, one day after gestational exposure ended. As shown in the detailed table for this study in Appendix 5B and in Table 12, delayed mammary gland development (LOAEL – 0.01 mg/kg/day) was a much more sensitive endpoint than increased liver weight (LOAEL – 1 mg/kg/day) in this study. In an additional component of this study with full gestational exposure (GD 1-17) to PFOA, serum PFOA levels were not measured. The LOAEL for increased liver weight on PND 7 was lower with the longer exposure period (GD 1-17) than for the late gestational (GD 10-17) exposure discussed above, consistent with the greater total dose from longer exposure.

Another study (Tucker et al., 2015) assessed relative liver weight on PND 21 and later time points in CD-1 and C57/Bl mouse pups after maternal exposure to 0.01 – 1 mg/kg/day PFOA on GD 1-17. As in Macon et al. (2011), delayed mammary gland development was a more sensitive endpoint than increased liver weight in this study.

Finally, Abbott et al. (2007) evaluated relative liver weight in wild type and PPAR-alpha null offspring, as well as maternal relative liver weight (above), on PND 21. In this study, the LOAEL for increased relative liver weight in wild type pups (0.1 mg/kg/day) was 10-fold lower than the maternal LOAEL (1 mg/kg/day), while the LOAEL for this effect in PPAR-alpha null mice was the same in pups and dams (3 mg/kg/day).

#### *Cynomolgus monkey data on increased relative liver weight*

Liver weight (absolute and relative to body weight) was increased in all treated groups in the 90-day study of cynomolgus monkeys (Appendix 5; Butenhoff et al., 2002). The increase in absolute liver weight was statistically significant ( $p < 0.01$ ) in all groups, but relative liver weight was significantly increased only in the high dose group. However, this analysis did not include the animals sacrificed during the study due to overt toxicity. The absolute and relative liver weight in the sacrificed low dose monkey was far higher than the others in its group, and inclusion of data for this animal increases the mean value for these parameters in the low dose group. Liver-to-brain weight ratio is considered to be a reliable measure of effects on liver weight, because brain weight tends to remain stable when body weight changes. This ratio was increased in all treated groups compared to controls, with statistical significance at the two lower dose levels, but there was no dose-response based on either administered dose or serum level. As discussed above, serum PFOA levels did not differ significantly between the low and mid dose groups (Butenhoff et al., 2004c).

The data from Butenhoff et al. (2002) are not informative as to the dose-response curve or the NOAEL for PFOA's effects on increased liver weight in this species of monkey, because this effect did not increase with either administered or internal dose. Additionally, mortality of one of four animals at the lowest dose was possibly treatment-related, and four of the six high dose monkeys did not complete the study because of overt toxicity or mortality.

Comparison of the relative liver weight and serum PFOA level data from the 90-day cynomolgus monkey study and the rat studies (Perkins et al., 2004; Loveless et al., 2006) reveals similar increases in liver weight at comparable serum levels in both species. Additionally, comparable increases in PCO activity occurred at similar serum PFOA levels in monkeys and rats (discussed further in Mode of Action section). These data suggest that cynomolgus monkeys and rats have similar sensitivity to the hepatic effects of PFOA.

### **Immune system effects in rodents and non-human primates**

PFOA causes suppression of the immune response in experimental animals. As discussed in the Epidemiology section, decreased response to vaccinations has been associated with PFOA in humans. Toxicological studies that evaluated effects of oral exposure to PFOA on the immune system in rodents are summarized in Table 13. Sixteen publications of such studies were identified. Of these, 14 publications report only studies of mice, one publication reports only studies in rats, and one publication includes studies in both species. Both of the rat studies, and 11 of the 15 mouse studies, were conducted in males, while two of the mouse studies evaluated adult females, and two of the mouse studies evaluated effects of developmental exposures on offspring. Additionally, effects on the immune system were reported in the 90-day oral study of rhesus monkeys (Goldenthal, 1978). Studies of effects on the immune system from dermal exposure to PFOA are not reviewed in this document. Toxicological effects on the immune system are of particular interest because PFOA and other PFCs have been associated with decreased vaccine response in humans (see Epidemiology section).

#### Non-human primates

In the 90-day rhesus monkey study (Goldenthal, 1978), histopathological changes related to the immune system occurred in all animals in the two higher dose groups (30 and 100 mg/kg/day). All animals in these two dose groups had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen, and one 30 mg/kg/day female and all 100 mg/kg/day animals had moderate atrophy of the lymphoid follicles in the lymph nodes. These changes were not seen in the lower dose groups (3 and 10 mg/kg/day). Because serum PFOA levels are not provided in this study, it cannot be used for dose-response in risk assessment.

#### Rodents

PFOA consistently suppressed the immune system in studies of mice (Table 13). Effects in mice include decreased absolute and relative spleen and thymus weights, decreased thymocyte and splenocyte counts, decreased immunoglobulin response, and changes in total numbers and/or

specific populations of lymphocytes in the spleen, thymus, peripheral blood, and bone marrow. The available data indicates that rats are less sensitive than mice to immunotoxic effects of PFOA, since immune system effects were not observed in the two studies that have been conducted in rats. These rat studies included doses higher than those which generally caused immune effects in mice.

Relative liver weight was evaluated along with immune parameters in nine of the 13 studies of adult mice. In all of these nine studies, the LOAEL for increased relative liver weight was the same or lower than the LOAEL for immune system effects. As shown in Table 13, the lowest dose at which immune effects were clearly demonstrated in mice is 0.49 mg/kg/day in a 21-day drinking water study, with no NOAEL reported (Son et al., 2009). Increased liver weight also occurred at this dose in this study, as reported in the accompanying paper (Son et al., 2008). Therefore, the data available at this time suggest that increased relative liver weight is an endpoint that is as sensitive as or more sensitive than immune system effects in rats and mice. For this reason, immune system effects from toxicology studies were not used as the basis for the dose-response in this risk assessment, and individual study tables are not provided for these studies.

## **Reproductive and Developmental Effects**

### Overview

As discussed in the Pharmacokinetics section (above), PFOA exposures in the developing human fetus are similar to those experienced by the mother, and neonatal exposure from breast-feeding or consuming formula prepared with contaminated drinking water is much higher than in the mother or other older individuals using the same drinking water source. As discussed in the Epidemiology section, prenatal exposure to PFOA is associated with decreased birth weight in human epidemiology studies (Johnson et al., 2014).

Reproductive or developmental effects of PFOA have not been studied in non-human primates. Prior to 2006, the reproductive and developmental effects of PFOA had been studied only in rats and rabbits. These species are not the most appropriate models for evaluation of the potential for human reproductive and developmental effects of PFOA because the half-life of PFOA in female rats and female (as well as male) rabbits is only a few hours (see Table 4 in Toxicokinetics section). Because of this rapid elimination, serum levels from a given dose of PFOA in females of these species are much lower than in other species with long half-lives, such as mice and humans, and PFOA does not reach steady state in females of these species with daily dosing by gavage.

Beginning in 2006, the reproductive and developmental toxicity of PFOA has been studied in mice. The mouse is a more appropriate species for evaluating the potential human effects of PFOA on reproduction and development, since the female mouse excretes PFOA slowly and steady state is achieved with continued dosing. As discussed in detail below, effects observed in mice include full litter resorptions, decreased postnatal survival and growth, delayed

development, and accelerated sexual maturation in males. More recent studies have found that delayed mammary gland development is caused by developmental exposure to doses as low as 0.01 mg/kg/day.

In this section, the mouse studies of developmental and reproductive effects from gestational dosing with PFOA are presented in Table 12 and discussed in the text. This is followed by a detailed discussion of effects of developmental exposures to PFOA on mammary gland development, the most sensitive developmental endpoint with serum data appropriate for dose-response modeling. Summaries of additional studies of effects of pre-pubertal exposure to PFOA on reproductive organs in female mice, and of reproductive effect in male mice, are then presented. Finally, the studies of developmental and reproductive effects in rats and rabbits that were conducted prior to the mouse studies are summarized.

### Mouse developmental studies

#### *Summary of study designs*

Table 12 summarizes data on reproductive and developmental effects from gestational and/or lactational exposure to PFOA in mice. Sixteen publications reporting such studies are included in the table. Several of these publications include multiple studies with different exposure protocols, in one case, different aspects of the same study are described in two publications (Wolf et al., 2007; White et al., 2009). In total, 18 separate studies, three of which used multiple strains of mice, are reported. Most but not all of the studies assessed both maternal/reproductive endpoints and developmental endpoints in the offspring. The table includes the six publications that evaluated developmental effects of PFOA on mammary gland development in the dam and/or the female offspring, as well as other endpoints. Effects on mammary gland development are further discussed in detail in a separate section below.

Several additional studies of effects of gestational dosing with PFOA are not included in the Table 12 because they did not evaluate standard reproductive or developmental endpoints and/or because they used non-standard exposure protocols. These papers are discussed elsewhere in this document. These include two studies of histopathological changes in the livers of mice in adulthood after low dose developmental exposures (Filgo et al., 2015; Quist et al., 2015), a study of intestinal tumorigenesis in wild type and genetically susceptible strains of mice (Ngo et al., 2014), and three studies of neurobehavioral effects at age 5-8 weeks in offspring of dams dosed during gestation (Johansson et al., 2008; Onishchenko et al., 2011; Sobolewski et al., 2014).

Most (11 of 16) of the studies in Table 12 used CD-1 mice, three used C57Bl/6 mice, one study (Tucker et al., 2015) used both strains, and one study used ICR mice (Yahia et al., 2010). Two studies included strains of mice with differing PPAR-alpha status (wild type, null, and/or humanized) and are discussed in more detail in the Mode of Action section.

In most of the studies (13), dosing was by oral gavage, while one study used drinking water exposure (Hu et al., 2010), one study used drinking water exposure with or without additional

gavage exposure in some, but not all, dose groups (White et al., 2011b), and one study used dietary exposure (van Esterik et al., 2016).

In most of the studies (10), the dams were dosed with PFOA throughout gestation, and postnatal development of the offspring was assessed. However, other protocols were used in some studies. In two studies (Hu et al., 2012; van Esterik et al., 2016), maternal dosing began before mating and continued until weaning. In other studies, the dosing period was shorter than the full period of gestation. Fenton et al. (2009) was primarily a pharmacokinetic study of a single dose of PFOA administered on GD 17 and is included because offspring body weight was assessed. In several other studies, PFOA was administered for only a portion of gestation (Hu et al., 2010, GD 6-17; Macon et al., 2011 “late gestation study”, GD 10-17; Suh et al., 2011, GD 11-16; White et al., 2007 “restricted exposure study”, GD 1-17, 8-17, and 12-17; Wolf et al., 2007, “restricted exposure study”, GD 7-17, 10-17, 13-17, and 15-17). Two studies, one with exposure on GD 8-17 (White et al., 2009) and one with exposure on GD 1-17 (Wolf et al., 2007; White et al., 2009) used a cross-fostering protocol in which offspring were exposed during gestation and/or lactation. In these studies, treated dams were dosed during gestation. The dams that were not dosed but fostered pups from dosed dams were exposed to PFOA via grooming of the pups and ingestion of excreted pup urine and feces. One study (White et al.; 2011b) was a multi-generation study in which effects on three generations were evaluated (P0, F1, and F2). Effects on P0 dams and F1 offspring are shown in Table 12; complete information on the study is presented in the section on mammary gland development (below).

Most of the studies (15) assessed both maternal/reproductive endpoints and endpoints of postnatal developmental in the offspring, although the specific endpoints that were evaluated differed among studies. Only two of the studies evaluated malformations at birth. These were Lau et al. (2006), which also evaluated postnatal development, and Yahia et al. (2010). Two studies (Macon et al., 2011, and Tucker et al., 2015) assessed effects in the offspring, but not maternal or reproductive effects. Several studies focused on specific effects in addition to standard developmental endpoints or mammary gland development. Hines et al. (2009) evaluated effects in female offspring of developmental exposures on body weight and hormones (insulin and leptin) in adulthood. Hu et al. (2010) and Hu et al. (2012) assessed effects of developmental exposures on immune endpoints at age 7 to 9 weeks and are also included in the summary table of immune effects (Table 13). Finally, Suh et al. (2011) focused on placental toxicity and other reproductive endpoints, and did not evaluate the offspring.

Two additional studies assessed the effects of pre-pubertal exposure to PFOA on the reproductive system in female mouse pups (Dixon et al., 2012; Yao et al., 2014). These are not included in the table and are discussed in the text later in this section.

#### Maternal and reproductive effects

PFOA caused reproductive effects in mice including increases in full litter resorptions (Abbott et al., 2007), increased litter loss, prenatal loss per live litter, or fetal resorptions/dead fetus (Abbott

et al., 2007; Lau et al., 2006; Suh et al., 2011; White et al., 2007; White et al., 2011b), and decreased number of live fetuses per litter or decreased litter size (Lau et al., 2006; Suh et al., 2010; van Esterik et al., 2016; Wolf et al., 2007; White et al., 2011b). In all of these studies, one or more of these reproductive effects occurred at doses below those which caused decreased maternal weight gain, an indicator of general maternal toxicity.

PFOA caused toxicity to the placenta, including decreased placental weight, decreased fetal/placental weight ratio, and decreased expression of genes for prolactin family hormones (hormones that support fetal growth and nutrition), after treatment on GD 11-16 at the lowest dose tested, 2 mg/kg/day (Suh et al., 2011). Higher doses caused placental necrosis and reduced numbers of placental trophoblast cells. These results suggest that placental toxicity may contribute to the increased number of dead fetuses/decreased number of live fetuses and decreased fetal growth observed in this and other developmental studies of PFOA.

Effects on maternal relative liver weight and maternal mammary gland development are discussed below.

#### *Effects in Offspring: Fetal through Weaning*

##### *Fetal teratology*

Two studies evaluated fetal teratology. In CD-1 mice, gestational exposure to PFOA caused reduced ossification of phalanges, limb and tail defects, and microcardia at doses below those which affected maternal weight gain (Lau et al., 2006). Reduced ossification of proximal phalanges of both the forelimb and the hindlimb was significantly increased at the lowest dose used (1 mg/kg/day) but was not significantly increased in some higher dosed groups. This effect represents a delay in timing of development rather than a permanent structural change, since the phalanges developed normally in mice treated with PFOA that were not sacrificed prior to delivery in this study (personal communication with C. Lau). Ossification at other sites (caudal vertebrae, metacarpals, and metatarsals) was delayed only at a much higher dose (20 mg/kg/day) which also caused maternal and reproductive toxicity.

In ICR mice, gestational exposure to PFOA caused increased incidence of cleft sternum, delayed ossification of phalanges, and delayed incisor eruption. The LOAEL and NOAEL for increased incidence of cleft sternum, delayed ossification of phalanges, delayed incisor eruption were 10 mg/kg/day and 5 mg/kg/day, respectively (Yahia et al., 2010). In this study, maternal weight gain, fetal weight, and pup survival until PND 4, were significantly decreased at dose(s) below the LOAEL for increased cleft sternum, delayed ossification, and delayed incisor eruption. No pups survived until PND 4 at 10 mg/kg/day, the LOAEL for delayed ossification.

##### *Birth weight, and growth and postnatal development until weaning*

PFOA caused decreased body weight at birth, postnatal mortality, reduced postnatal growth until weaning, and delayed development (as indicated by day of eye opening) in mice. As shown in Table 12, one or more of these effects was reported by Abbott et al. (2007), Hines et al. (2009),

Hu et al. (2010), Hu et al. (2012), Lau et al. (2006), Tucker et al. (2015), van Esterik et al. (2016), White et al. (2007), White et al. (2009), Wolf et al. (2007), White et al. (2011b), and Yahia et al. (2010). Exposure during only the latter part of gestation (GD 15-17) was sufficient to cause decreased body weight at birth, increased postnatal mortality, and decreased postnatal growth (Wolf et al., 2007). Furthermore, decreased postnatal growth resulted from either gestational or lactational exposure, as shown by the results of the cross-fostering study by White et al. (2009).

Effects on relative liver weight and mammary gland development during the period from birth until weaning are discussed below.

#### Effects in Offspring: Post-Weaning

In studies which continued to assess offspring during the post-weaning period, effects at these later time points were observed in some studies (summarized below) but not in others (e.g. no effect on body weight in Abbott et al., 2007, or on immune parameters in Hu et al., 2012).

In the restricted exposure component of Wolf et al. (2007), developmental exposure to 5 mg/kg/day PFOA beginning on GD 10 caused decreased body weight in male offspring until age 10-11 weeks. In the cross-fostering study component of the same publication, body weight remained decreased until PND 36 in male offspring after *in utero* and lactational exposure to 5 mg/kg/day PFOA, and until PND 85 in females exposed to the same dose *in utero* even without postnatal exposure from breastmilk.

Markers of sexual maturation (vaginal opening and first estrus) were delayed in female CD-1 mouse offspring with gestational exposure to PFOA. However, sexual maturation (preputial separation) in male offspring was accelerated by PFOA exposure, despite the fact that PFOA caused decreased body weight at 6.5 weeks of age (Lau et al., 2006). Notably, acceleration puberty in males did not follow a typical dose-response curve. The greatest acceleration occurred at the lowest dose (1 mg/kg/day) with a smaller effect at each increasing dose. At the highest dose (20 mg/kg/day), puberty was delayed rather than accelerated.

Body weight and hormone (insulin and leptin) levels were increased in early adulthood (20-29 weeks) in CD-1 mice with gestational exposure to low doses (0.01 to 0.1 mg/kg/day for obesity; 0.01 to 0.3 mg/kg/day for hormones) but not at higher doses. In this study, body weight at birth or early in life was not affected by the low doses that caused increased body weight later in life (Hines et al., 2009). In contrast, Ngo et al. (2014) found no effect on body weight at age 12-20 weeks in male or female wild type or Min/+ (a strain susceptible to intestinal tumorigenesis) C57Bl/6 mice offspring of dams dosed with 0.01, 0.1, and 3 mg/kg/day PFOA during gestation. These studies are of interest because several epidemiology studies evaluated associations of prenatal and/or early life PFOA exposure with parameters associated with increased body weight in childhood or adulthood. Three studies found associations of maternal serum PFOA levels during pregnancy with increased risk of overweight/obesity and changes in metabolic hormones

in young women (Halldorsson et al., 2012), more rapid weight gain in girls (Maisonet et al., 2012), or greater adiposity and more rapid increase in BMI in childhood (Braun et al., 2016), while two studies did not find such an association with prenatal or early childhood exposure to PFOA (Andersen et al., 2013; Barry et al., 2014).

Splenic T cells were decreased at age 6 weeks in C57Bl/6 mice exposed gestationally to 2 mg/kg/day PFOA, with no effect at 0.2 mg/kg/day (Hu et al., 2012). The NOAEL and LOAEL for this effect were the same as for decreased body weight gain on PND 1-21, suggesting that the immune system effect from developmental exposure was not a more sensitive endpoint than other developmental effects in this study.

Effects on relative liver weight and mammary gland development during the post-weaning period are discussed below.

### *Relative liver weight*

Increased relative liver weight is a sensitive toxicological endpoint for PFOA. This effect was evaluated in dams and/or offspring in 12 of the 18 mouse developmental studies summarized in Table 12. Maternal relative liver weight was assessed at delivery in three studies, between delivery and weaning in two studies, and at weaning in five studies. In offspring, relative liver weight was evaluated at birth in two studies, between birth and weaning in three studies, at weaning in five studies, and post-weaning in five studies.

The NOAELs and LOAELs for increased relative liver weight in dams and/or offspring and for other reproductive and developmental effects are presented in Table 12. (*Note:* Fenton et al., 2009, is not included in this table because no effects were seen at the single dose used.) In all studies except one (Hu et al., 2010), the LOAEL and/or NOAEL for increased relative liver weight in the dam and/or the offspring is the same or lower than the LOAEL and/or NOAEL for reproductive developmental effects (other than delayed mammary gland development). In Hu et al. (2010), offspring liver weight was evaluated on PND 48 and PND 63; these are post-weaning time points at which PFOA was almost totally eliminated from the body. These data indicate that increased relative liver weight (maternal and/or offspring) is an endpoint for PFOA toxicity in mice that is as sensitive or more sensitive than most of the other reproductive and developmental effects that were evaluated, with the exception of delayed mammary gland development which is discussed below.

## **Mammary gland development**

### Background information on mammary gland development as a toxicological endpoint

Because the developmental patterns of the mammary gland are similar in humans and rodents, rodents provide a good model for studying the effects of environmental contaminant on human mammary gland development (Rudel and Fenton, 2009; Fenton et al. 2012; Rudel et al., 2011; Fenton and Birnbaum, 2015; Osborne et al., 2015). The development of the mammary gland in

these species involves a complex series of events that is regulated by a balance of hormones, growth factors and stromal factors (Osborne et al., 2015; Gore et al., 2015). Agents that affect any of these steps can interfere with the normal process of mammary gland development, and this process is particularly sensitive to effects of endocrine disrupting chemicals, including environmental contaminants such as bisphenol A, atrazine, and dioxin (Gore et al., 2015; Fenton and Birnbaum, 2015; Fenton et al., 2012; IOM 2011; Rudel et al., 2011). As reviewed in detail below, adverse effects on mammary gland development are sensitive toxicological endpoints for PFOA, and a NOAEL for these effects has not been identified.

The mammary gland is most sensitive to the effects of toxic substances during critical periods when development occurs including fetal, neonatal, puberty, and pregnancy (Gore et al., 2015; Osborne et al., 2015), while the mammary glands of non-pregnant adult females may not be affected by the same exposures. Adverse effects on the mammary gland from early life exposures can include accelerated or delayed development (Macon and Fenton, 2013; Osborne et al., 2015), and effects of the same chemical on mammary gland development may vary depending on the life stage when exposure occurs (Osborne et al., 2015). These disruptions of mammary gland development may result in adverse effects later in life including impaired lactation and increased cancer risk (Fenton 2006; IOM, 2011; Rudel et al., 2011; Fenton et al., 2012).

The mammary gland is distinct from other tissues in that it undergoes a significant portion of its development postnatally; in addition to the fetal/neonatal period, puberty and pregnancy are critical periods of mammary gland development (Osborne et al., 2015). The mammary gland grows and differentiates slowly during embryonic and juvenile life and does not mature until after puberty. Mammary gland development in rodents has been reviewed by Sakakura (1987) and Daniel and Silberstein (1987) and in humans by Russo and Russo (1987) and Howard and Gusterson (2000).

The mammary gland is an organ that is unique to the class *Mammalia*, and its embryonic, postnatal, and adult development is highly conserved between species. Mammary gland development in humans and rodents takes place at a similar biological pace, although the absolute timeframes differ (Table 12 and Figure 10, both from Fenton, 2006). The description of mammary gland development below is taken from concise summaries provided by Osborne et al. (2015) and Fenton et al. (2012).

In rodents and humans, mammary gland development begins with the formation of the mammary, or milk line, during embryonic development. This structure separates into individual placodes (areas of thickening of the ectodermal layer), each of which develops into a ductal tree that embeds in a fat pad to form the mammary bud. Although there are slight timing differences in mammary gland development among species, ductal branching begins during the prenatal period in both rodents and humans. Subsequent to the fetal/neonatal period, there is little epithelial growth until puberty. During puberty, exponential growth of the female mammary

gland occurs for several weeks in rodents and several years in humans. During puberty, the fat pad rapidly fills with epithelial cells to become the adult form of the gland. The epithelium develops bundles of ducts, which then form club-like structures, called terminal end buds (TEB) in humans. Each TEB cleaves into alveolar buds and sprouts into ductules, forming a structure called the terminal ductal lobular unit. In rodents, TEBs are the sites of future ductal branching and disappear as the gland differentiates, and they are the structures considered to be most functionally equivalent to the terminal ductal lobular unit in humans. TEBs are particularly susceptible to the effects of carcinogens, and it has been suggested that “factors that lengthen the period when TEBs are present lengthen the period during which the mammary gland is susceptible to carcinogens (Osborne et al., 2015).” After puberty, the mammary gland remains in a resting state until pregnancy occurs. During pregnancy, the gland undergoes another period of rapid differentiation, involving branching and the development of lobulo-alveoli to prepare for lactation.

Developmental Event	Human	Rodent
Milk Streak Evident	EW4-6	GD10-11 (mice)
Mammary Epithelial Bud Forms	EW10-13	GD12-14 (mice), GD14-16 (rat)
Female Nipple and Areola Form	EW12-16	GD18 (mice), GD20 (rat)
Branching and Canalization of Epithelium	EW20-32	GD16 to birth (mice, GD18 to birth (rat)
Secretion is Possible	EW32-40 (ability lost Postnatally)	At birth, with hormonal stimuli
Isometric Development of Ducts	Birth to Puberty	Birth to Puberty
TEBs Present (Peripubertal)	8 to 13 yr old girls	23-60 d old (rat)
Formation of Lobular Units	EW32-40, or within 1-2 yr of first menstrual cycle	Puberty and into Adulthood

EW – embryonic week; GD – gestation day

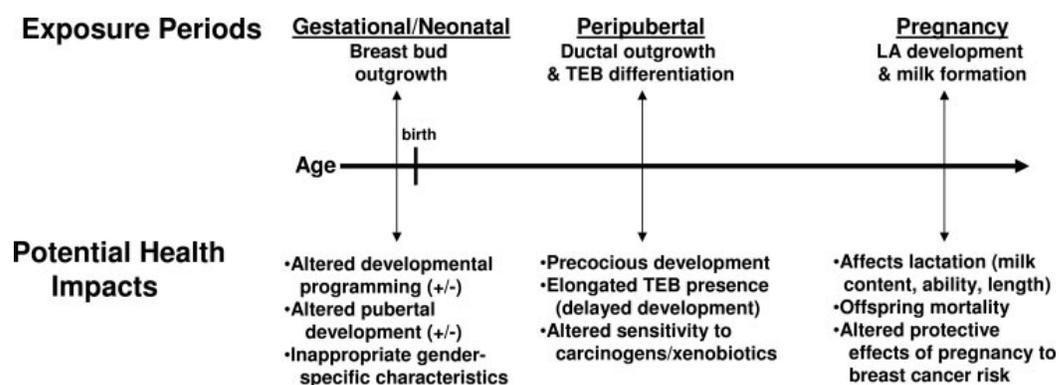


Figure 10. Timeline of critical periods of mammary gland development and potential effects of endocrine disrupting compounds on mammary gland development (Fenton, 2006).

Effects on mammary gland development in rodents are commonly assessed by evaluation of “whole mounts”. In this approach, the entire fourth and/or fifth abdominal mammary gland fat pad is mounted flat on a slide, fixed, stained, defatted, and permanently affixed to the slide.

Whole mounts are assessed microscopically for parameters such as numbers of mammary terminal ductal structures (i.e., TEBs, terminal ducts, alveolar buds, and lobules), extension of the epithelial cells through the fat pad, and branching patterns and density at different times during development branching (Rudel et al., 2011). Whole mounts were evaluated in the nine studies of the effects of PFOA on mammary gland development in mice described below, and tissue sections stained with hematoxylin and eosin (H&E) were also examined in some of these studies. Effects on mammary gland development are reported as overall age-adjusted developmental scores based on a number of parameters and/or as quantitative values for specific parameters.

#### Effects of PFOA on mammary gland development in mice

Delayed mammary gland development in mice is a sensitive toxicological endpoint that is considered relevant to humans because developmental patterns are similar in both species. Nine publications have reported the effects of PFOA on mammary gland development in mice, and some of these publications include multiple studies. These studies are summarized in Table 16, and details of each study are provided in an individual study table for each publication in Appendix 5. Information related to the mode of action of PFOA's effects on mammary gland development is reviewed in the Mode of Action section, below.

#### Studies of effects of maternal and fetal/neonatal exposure to PFOA

Six publications evaluated effects of PFOA exposure during gestation and/or lactation on mammary gland development. Studies presented in these publications are summarized in Table 16A, and details for each study are provided in Appendix 5. Some of these publications include multiple studies, and, in total, ten separate studies were reported. Nine of these studies reported delayed mammary gland development, and one reported no effect on mammary gland development. Five of these publications report studies in CD-1 mice (White et al., 2007, three studies; White et al., 2009, two studies; Macon et al., 2011, two studies; White et al., 2011b; Tucker et al., 2015), and one of these also includes C57Bl/6 mice (Tucker et al., 2015). The sixth publication evaluated wild type, PPAR-alpha null, and PPAR-alpha humanized mice of the Sv/129 genetic background (Albrecht et al., 2013). Nine of the ten studies evaluated female offspring, and four of the studies (White et al., 2007; White et al., 2009; White et al., 2011b) evaluated pregnant and/or lactating dams.

#### Effects on structure of the mammary gland development

PFOA exposure during critical developmental periods (fetal, neonatal, pregnancy, and lactation) caused delayed mammary gland development in both lactating dams (White et al., 2007; White et al., 2009; White et al., 2011b) and pups (White et al., 2007; White et al., 2009; Macon et al., 2011; White et al., 2011b; Tucker et al., 2015), while even a high dose (5 mg/kg/day) did not affect mammary gland development in non-pregnant adult female mice (White et al., 2007).

As shown in the comparison of LOAELs and NOAELs for mammary gland development and other endpoints in Table 12, mammary gland development was a more sensitive endpoint for PFOA than other effects found in dams and pups in mouse developmental studies including

reproductive endpoints (number of fetuses per litter, prenatal loss, prenatal survival), pubertal markers (day of vaginal opening or day of first estrus; Tucker et al., 2015), estrogen or progesterone levels (Tucker et al., 2015), body weight, or liver weight (Macon et al., 2011; Tucker et al., 2015).

#### Lactating dams

Delayed mammary gland development occurred in lactating dams after dosing with PFOA during gestation (White et al., 2007; 2009; 2011b) or from exposure via treated pups (White et al., 2009). At the end of pregnancy just prior to initiation of nursing (GD 18; White et al., 2007) and on PND 1 after one day of nursing (White et al., 2009), mammary glands of treated dams were not saturated with milk filled alveoli, as is normally seen, but rather exhibited stunted, immature development. On PND 10, normally the peak of lactation, mammary glands from treated mice were delayed in development and resembled those normally seen earlier in lactation (White et al., 2007; 2009). On PND 20-22, mammary glands from treated dams had milk-filled alveoli and resembled normal mammary glands at the peak of lactation on PND 10, instead of the normal involution that occurs at weaning, indicating delayed development of up to 10 days (White et al., 2007; 2011b).

#### Female offspring

Delayed mammary gland development occurred in female pups in both CD-1 and C57Bl/6 strains (Tucker et al., 2015). In CD-1 mice, the mammary gland developmental score was significantly decreased compared to controls at all doses including the lowest dose (0.01 mg/kg/day). In C57Bl/6 mice, the developmental score was decreased compared to controls at all doses at both timepoints assessed (PND 21 and PND 61). However, this difference was not statistically significant at the two lowest doses (0.01 and 0.1 mg/kg/day), but was significant at 0.3 and 1 mg/kg/day. Serum PFOA levels in C57Bl/6 pups were lower than in CD-1 pups at the same administered dose. Lack of statistical significance in C57Bl/6 offspring at the two lowest doses may be due to the lower serum PFOA levels and/or small number of animals in the two lower dose groups for this strain (n=2-5 per dose group) as compared to CD-1 mice (n=8-22 per dose group), rather than differences in intrinsic sensitivity to effects of PFOA on mammary gland development in the two strains mice (Tucker et al., 2015). It should be noted that the LOAELs in female offspring for other well-established developmental effects of PFOA (decreased body weight and increased relative liver weight, both on PND 21) were also higher in C57Bl/6 mice than in CD-1 mice in this study, possibly due to the factors mentioned above.

PFOA is found in breast milk after maternal exposure in both rodents and humans, resulting in lactational exposure to offspring (reviewed in Post et al., 2012; also Mogensen et al., 2015). Cross-fostering studies in which pups with no prenatal exposure were exposed via breast milk from exposed dams, and pups with prenatal exposure were fostered by untreated dams, show that delayed mammary gland development results from exposure during gestation, lactation, or both. Significant delays in mammary gland development occurred as early as PND 1 in non-gestationally exposed pups nursing from treated dams for only 12-24 hours, and as early as PND

3 in non-gestationally exposed dams nursing treated pups. The exposure in these dams was only through maternal behavior such as ingestion of treated pups' waste and grooming of treated pups (White et al., 2009).

In White et al. (2009), effects on the mammary gland persisted in exposed offspring until 18 months of age, long after PFOA had been eliminated from the body; these effects are considered to be permanent. Although the study was not designed to quantitatively evaluate mammary gland development in older animals, epithelial density appeared to be reduced in the mammary glands of exposed animals at 18 months. An increase in the number of unusual darkly staining foci per gland of approximately 5-fold was also seen at 18 months in the mammary glands of exposed mice. According to the authors, these foci appeared to result from hyperplasia of ductal epithelium, infiltration of inflammatory cells into ductal regions, increased stromal density surrounding the ducts, and/or inappropriate differentiation of the ductal epithelium. Gore et al. (2015) notes that abnormalities of this type can be associated with increased breast cancer risk.

Mammary gland development, as assessed on PND 21 by overall developmental score, number of terminal end buds, and other measures of mammary gland development, was delayed in a dose-related fashion in CD-1 mouse pups after late gestational exposure (GD 10-17) to doses lower than those used in earlier studies (Macon et al., 2011). The LOAEL was 0.01 mg/kg/day, with no NOAEL identified. These effects occurred at serum PFOA levels of 285 ng/ml or below, lower than the mean serum level (371 ng/ml) in a community exposed to highly contaminated drinking water (Emmett et al., 2006a).

In a multi-generation study of CD-1 mice exposed to 5000 ng/L (5 µg/L) PFOA in drinking water, mammary gland development was delayed in both F1 dams (PND 22) and F1 female pups (PND 22, 42, and 63) at serum levels relevant to human environmental exposures (White et al., 2011b). Pups were significantly affected at serum levels as low as 21.3 ng/ml on PND 22 (compared 0.6 ng/ml in controls at this time point). This serum level is below the mean serum level of 28 ng/ml in the six Ohio and West Virginia communities with contaminated drinking water that comprise the C8 Health Study population, and is within about 10-fold of the mean and 4-fold of the 95<sup>th</sup> percentile serum levels in the U.S. general population (CDC, 2015). This serum level would be expected in humans with ongoing exposure to drinking water concentrations of approximately 200 ng/L (0.2 µg/L), based on the serum:drinking water ratios discussed above.

In contrast to the delays in mammary gland development observed in CD-1 and C57Bl/6 mice in the five studies discussed above, no significant effects on mammary gland development were found in wild type, PPAR-alpha null, or humanized PPAR-alpha Sv/129 mouse pups exposed gestationally to 3 mg/kg/day PFOA (Albrecht et al., 2013). This is the only study of mammary gland development in these strains. Albrecht et al. (2013) report that postnatal lethality occurred in the wild type mice, but not in the PPAR-alpha null or humanized PPAR-alpha mice. They conclude that the study confirms the PPAR-alpha dependent postnatal lethality of PFOA

previously reported by Abbott et al. (2007). However, several problematic issues with this study limit the consideration of its results:

- Although postnatal lethality in wild type mice treated with 3 mg/kg/day (the only dose used in the study) on PND 20 was reported as statistically significant ( $p < 0.05$ ), this conclusion appears to be based on an inappropriate statistical comparison. In evaluating postnatal lethality, the number of pups per litter on PND 20 in the control and PFOA-treated groups of wild type mice were compared. However, this comparison does not appear to be valid because the control and PFOA treated litters initially had different numbers of pups on PND 0. The appropriate evaluation of this parameter is a comparison of the number of pups within the same litter on PND 0 and PND 20 (i.e. percent mortality within the litter between PND 0 and PND 20). In wild type pups, 96% of controls and 70% of PFOA-treated survived from PND 0 to PND 20. From the analysis presented, it is unclear whether postnatal lethality is actually significantly increased by PFOA in wild type pups. For this reason, the basis for the conclusion that wild type, but not humanized PPAR- $\alpha$ , mice are sensitive to developmental effects of PFOA is uncertain.
- An important concern is that Albrecht et al. (2013) state that elevated PFOA levels (up to > 1000 ng/ml) were found in liver and serum from some control fetuses, pups, and dams. However, no further information such as which groups of animals these samples came from, how many samples had elevated PFOA concentrations, or statistical data for serum levels in the control samples is provided. Importantly, data from control animals with elevated PFOA exposures do not appear to have been excluded in the comparisons of endpoints of toxicity in control and treated groups. Inclusion of these data from the control animals could have affected the results of these comparisons, especially since serum levels in some of the treated groups were only a few fold higher than those in some of the controls.
- Developmental effects observed in the same strain of mice (SV/129) in another study (Abbott et al., 2007) at lower doses (0.6 and 1 mg/kg/day) were not observed at the higher dose (3 mg/kg/day) used by Albrecht et al. (2013). Abbott et al. (2007) observed significantly increased postnatal lethality in wild type pups exposed gestationally to 0.6 and 1 mg/kg/day PFOA. Additionally, eye opening was significantly delayed in the 0.6 and 1 mg/kg/day wild type pups in Abbott et al. (2007), but not at 3 mg/kg/day in Albrecht et al. (2013).
- Although both studies used SV/129 mice, Albrecht et al. (2013) obtained them from NIH and Abbott et al. (2007) obtained them from Jackson Laboratories. Albrecht et al. (2013) suggest that pharmacokinetic differences in the wild type mice from the two different sources may explain the differences in effects of PFOA in these mice in the two studies. However, a close review of the data from the two studies (Table 3 of Abbott et al., 2007; Figure 10 of Albrecht et al., 2013) indicates that the serum levels in wild type pups in Albrecht et al. (2013) at which no developmental effects occurred were higher than the

serum levels in wild type pups at which delayed eye opening and postnatal mortality were reported by Abbott et al. (2007). Furthermore, the serum PFOA data for wild type dams on PND 20 appear to be inconsistent within the publication. Maternal serum levels in wild type dams on PND 20 are stated to range from 2066 – 6812 ng/ml, and no statistical parameters (e.g. median, mean, S.D.) are provided. However, the estimated serum level from the bar graph of maternal serum levels is 6700±3600 in the wild type dams (higher than what would be expected from the range provided in the text).

In summary, no effect of PFOA on mammary gland development was reported in pups from the three strains on PND 20; this is the only study that evaluated PFOA's effect on this endpoint in these strains. It is possible that these strains are less sensitive to this effect than are the other strains in which effects were reported. However, the general issues with this study create uncertainty about its conclusions related to mammary gland development. As discussed above, the inclusion of data from controls with elevated PFOA exposures may have affected the ability to observe effects in treated groups. Pertinent data on serum levels is not presented or appears to be presented inconsistently. The differences in developmental effects in wild type pups in Albrecht et al. (2013) versus Abbott et al. (2007) cannot be explained on the basis of pharmacokinetic differences in the two studies, since effects occurred at pup serum levels in Abbott et al. (2007) that are lower than the pup serum levels in Albrecht et al. (2013).

#### Effects on milk quality and quantity

In dams exposed to PFOA during pregnancy, the morphological delays observed in mammary glands during lactation suggest that milk production and/or composition may be impacted. Potentially relevant to this issue, maternal PFOA exposure was associated with shorter duration of breast feeding in both of the two human studies that evaluated this effect (Fei et al., 2010; Romano et al., 2016). However, the available toxicological information is not sufficient to make conclusions about the effects of developmental exposure to PFOA on lactational function, as only one study (White et al., 2007) evaluated effects on the composition of milk, and only one study (White et al., 2011b) evaluated the amount of milk produced. Evaluation of effects on growth of offspring is complicated by the fact that PFOA itself can cause decreased postnatal growth in mouse pups from only *in utero* exposure in a cross-fostering study (Wolf et al., 2007). Therefore, effects on postnatal growth could result from the intrinsic toxicity of PFOA and/or from decreased lactational function.

In the only toxicological study of effects on milk composition, expression of genes for four milk proteins (beta-casein, EGF, alpha-Lac, and LactoF) in mammary gland tissue were altered in dams exposed to 5 mg/kg/day PFOA during gestation. For example, peaks in LactoF expression normally seen early and late in lactation were delayed, consistent with the observed structural delays in mammary gland development at these time points (White et al., 2007).

The only toxicological data on effects of prenatal and early life exposure to PFOA on milk production in adulthood comes from the two-generation study in CD-1 mice (White et al.,

2011b). The design of this study is shown in Figure 11. As part of this study, a lactational challenge experiment was conducted in F1 dams and their F2 litters on PND 10, the time point at which lactation is at its peak. Except for the dose group receiving 5000 ng/L (5 µg/L) PFOA in drinking water throughout the experiment, the F1 dams that were evaluated for lactational function were exposed prenatally and in neonatal life until weaning through breast milk, but not after weaning or while pregnant and lactating. Milk production was evaluated by weighing a litter of 10 F2 pups before and after a nursing period of 30 minutes. There were no statistically significant effects on milk production, as measured by weight gain in the litter, or on time to initiate nursing behavior. However, high variability, the small number of animals assessed, and the lack of sensitivity of this assay may have limited the ability to detect effects on lactational function. Additionally, postnatal survival and body weight were not affected in the F2 pups, indicating that the ability of the F1 dams to provide nutritional support was not decreased. The authors note that it is not known whether deficits in lactational function were present, but were compensated for by increased frequency or longer duration of nursing events, since these parameters were not assessed.

Possibly relevant to this issue, the two available human studies both suggest that maternal exposure to PFOA may be related to shorter duration of breastfeeding. A study of 1400 Danish women from the general population found that serum PFOA concentration during early pregnancy was associated with shorter duration of breastfeeding among multiparous, but not nulliparous, women (Fei et al., 2010). Because women who breastfed previously were more likely to so again, and because longer duration of lactation would result in decreased serum PFOA due to excretion via breast milk, reverse causality could not be ruled out. A second study evaluated 336 U.S. women from a community with median serum PFOA levels about twice those in the U.S. general population, possibly due to past exposure to contaminated drinking water (Romano et al., 2016). In contrast to Fei et al. (2010), this study controlled for prior breast feeding history. Notably, serum PFOA levels during pregnancy were associated with shorter duration of breast feeding even after adjustment for previous breast feeding.

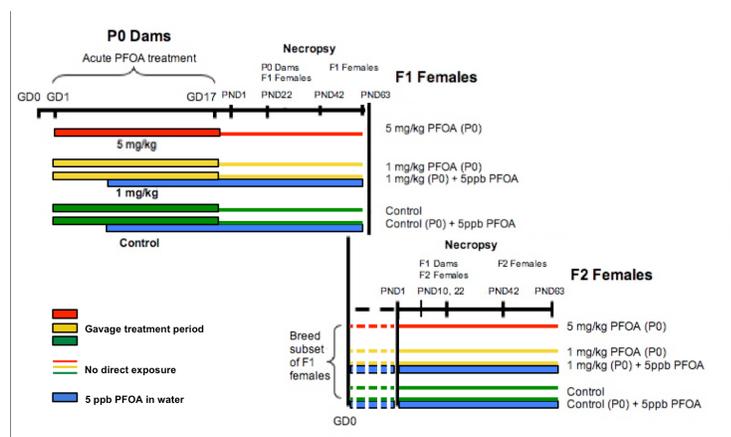


Figure 11. White et al. (2011b) study design and experimental timeline. Bar color denotes dose – green, 0 mg PFOA/kg body weight/day; yellow, 1 mg PFOA/kg body weight/day; red, 5 mg PFOA/kg body weight/day; blue, 5

ppb PFOA in drinking water – and bar thickness denotes timing of treatment – thick bars denote on-going direct treatment, thin bars denote only group identity subsequent to treatment.

### Studies of effects of peripubertal exposure to PFOA

Three studies reported effects of peripubertal (during periods between 3 and 7 weeks of age) exposure to PFOA on mammary gland development in female mice. Studies presented in these publications are summarized in Table 16B, and details for each study are provided in Appendix 5. Two studies used C57Bl/6 and Balb/C mice (Yang et al., 2009a; Zhao et al., 2012). One of these studies (Zhao et al., 2012) and an additional study (Zhao et al., 2010) evaluated C57Bl/6 PPAR-alpha null mice. Data on serum PFOA levels from all three of these studies are presented in Zhao et al. (2012). Interpretation of the combined results of these three studies is problematic because each PFOA dose level was used in each strain in only one of the three studies, and any dose-response interpretations must be made based on combining data from different studies. Because conditions (e.g. animals, housing conditions, time) may vary during different studies, dose-response curves based on combining data from different studies are difficult to interpret and conclusions based on such dose-response curves are highly uncertain.

Yang et al. (2009a) reported stimulation of mammary gland development in C57Bl/6 mice at 1 and 5 mg/kg/day PFOA, but complete inhibition at 10 mg/kg/day. In contrast, mammary gland development was inhibited at these three doses in a dose-related manner in Balb/c mice in this same study (Yang et al., 2009a).

A subsequent study (Zhao et al. 2012) attempted to further elucidate the dose response for mammary gland effects in these two strains by using a single dose in between those used in the first study in each strain (2.5 mg/kg/day in Balb/c; 7.5 mg/kg/day in C57Bl/6). Mammary gland development was inhibited at these doses in both strains. However, the doses used in the Yang et al. (2009) were not repeated in Zhao et al. (2012), and, importantly, the stimulatory effects reported at 1 and 5 mg/kg/day in C57Bl/6 mice by Yang et al. (2009a) were not replicated in Zhao et al. (2012).

It is important to note that the stimulation of mammary gland development in C57Bl/6 mice exposed to 5 mg/kg/day by Yang et al. (2009a) was not replicated in a second study. Additionally, this observation does not contradict the findings of delayed mammary development in this strain after gestational exposure (Tucker et al., 2015), since effects on mammary gland development may differ depending on life stage of exposure.

In C57Bl/6 PPAR-alpha null mice, mammary gland development was not affected by 7.5 mg/kg/day PFOA (Zhao et al., 2012), and was reported to be stimulated at 5 mg/kg/day, although quantitative data are not shown by Zhao et al. (2010). As was the case for the studies of C57Bl/6 wild type mice and Balb/C mice discussed above, each dose was used in PPAR-alpha null mice in only one of the studies (Zhao et al., 2010; Zhao et al., 2012). The effects on mammary gland development in the PPAR-alpha null mice therefore were similar to those in wild type mice of

the same strain at 5 mg/kg/day and differed at 7.5 mg/kg/day. As above, interpretation of these data is problematic because only one dose was used in each study.

### **Effects of pre-pubertal PFOA exposure on reproductive organs in female mice**

Two studies evaluated effects of pre-pubertal exposure to PFOA on reproductive organs in female mice. Groups of female CD-1 mouse pups (8 per group) to 0.01, 0.1, or 1 mg/kg/day were exposed to PFOA by gavage for three days starting on PND 18 (Dixon et al., 2012) and sacrificed one day after the last dose. Uterine weight (absolute and relative) was significantly increased (about 1.5-fold) only at 0.01 mg/kg/day, the lowest dose, while body weight was not affected by any dose of PFOA. As expected in this model, administration of 17-beta-estradiol greatly increased uterine weight by about 10-fold, and PFOA had no effect on uterine weight in the estradiol treated animals. In mice treated with PFOA alone, histopathological changes in the uteri in some, but not all, sections included minimal to mild endometrial and myometrial edema, and hyperplasia of the mucosal and endometrial glandular epithelia and smooth muscle layers. There was also focal minimal stromal edema of the cervix and focal areas of mucification of the vagina in some, but not all, sections from PFOA treated mice. The severity scores for these changes were statistically significant ( $p < 0.05$ ) only in the low dose (0.01 mg/kg/day) and may have contributed to the increased uterine weight in this dose group, indicating a non-monotonic dose response curve for this effect. In reproductive organs from mice treated with estradiol, histological changes that are known to result from estradiol were observed and these changes were more severe than those seen in PFOA treated mice. The authors note that PFOA did not appear to have anti-estrogenic effects in this study, since it did not decrease the uterine weight gain induced by estradiol, and that more research is needed regarding the mechanism for the observed histopathologic effects of PFOA.

In a second study of similar design, groups of 15 female CD-1 mouse pups were dosed with 0.005, 0.01, 0.02, 0.05, 0.1, or 1 mg/kg/day PFOA for 3 days beginning on GD-18 (Yao et al., 2014). Another group of 15 female mouse pups was dosed with 17-beta-estradiol (0.5 mg/kg/day) for the same 3-day time period. Mice were sacrificed one day after the last dose. In this study, PFOA did not affect relative uterine weight at any dose, while estradiol caused the expected increase in relative uterine weight. Body weight was not affected by PFOA or estradiol treatment. In histological examination of the uterus, cervix, and vagina from 5 mice from each treatment group, there were no differences in types or severity of observations between controls and PFOA treated groups, while estradiol treatment produced the expected histopathological changes. In uterine tissue from the other 10 mice per treatment group, expression of estrogen receptor target genes in the uterus was not changed in PFOA-treated mice. Additionally, the human estrogen receptor was not activated by PFOA *in vitro* studies. Based on these results, the authors concluded that PFOA does not activate the mouse or human estrogen receptor.

### **Reproductive effects in male mice**

Adverse reproductive effects were observed in four studies of male mice.

Li et al. (2011) studied the effects of 6 weeks of oral exposure to 0, 1, and 5 mg/kg/day APFO in wild type, PPAR-null, and humanized PPAR-alpha 129/sv mice. Serum testosterone was significantly decreased, and the percentage of abnormal sperm was significantly increased in a dose-dependent manner in wild type and humanized PPAR-alpha mice, but not in PPAR-alpha null mice. Histopathological examination of the testis found increased vacuolated cells in the seminiferous tubules of wild type and humanized PPAR-alpha at both doses, while no obvious effects occurred in the PPAR-alpha null mice.

Male Balb/C mice (10 per group) were dosed with PFOA by gavage for 28 days with 0, 0.31, 1.25, 5, and 20 mg/kg/day (Zhang et al., 2014). Sperm parameters were evaluated in five mice from the control and 5 mg/kg/day groups, histopathological studies were performed on testes from three mice from each dose group, and testes from the remaining animals were used in mode of action studies not described here. Relative testes weight was not affected by PFOA treatment, and absolute testes weight was decreased only at the highest dose (20 mg/kg/day). Sperm numbers, sperm motility, and sperm progression were significantly decreased, and percent teratosperm was markedly and significantly increased, by treatment with 5 mg/kg/day PFOA. Histopathological examination found no differences in testes in the two lower dose groups (0.31 and 1.25 mg/kg/day) as compared to controls, while the seminiferous tubules in the testes of the two higher dose groups (5 and 20 mg/kg/day) were severely damaged.

In a second study from this research group, fertility of male Balb/C mice (6-8 weeks old) was significantly reduced after 28 days of gavage dosing with 5 mg/kg/day PFOA (Lu et al., 2015). The number of mated females and the number of pregnant females per male mouse were significantly decreased by treatment of the males with PFOA. Further studies found that the blood-testis barrier was disrupted after 28 days of exposure to 1.25 or 5 mg/kg/day.

Finally, Liu et al. (2015), reported testicular toxicity in Kunming mice dosed with 2.5, 5, or 10 mg/kg/day PFOA for 14 days. Testicular damage including atrophy of seminiferous tubules, disrupted arrangement of spermatogenic cells, depletion of spermatogonial cells, detachment of germ cells from seminiferous epithelium, and decreased sperm production occurred in all treated groups, with severity increasing with dose. Epididymal sperm count was decreased in a dose-related fashion, with no NOAEL identified. Other parameters evaluated in this study are discussed in the Mode of Action section.

These adverse reproductive effects in male mice are in contrast to the results of the two-generation study in rats (Butenhoff et al., 2004b, see below). In this study, mating, fertility, and sperm parameters in F0 and F1 male rats were unaffected by PFOA at up to 30 mg/kg/day. Additionally, Cui et al. (2009) did not observe distinct histopathological changes in the testes of male Sprague-Dawley rats treated with 5 or 20 mg/kg/day of PFOA for 28 days and found that testes weight relative to body weight was significantly increased at both doses. As discussed below, PFOA increased the incidence of testicular Leydig cell tumors in two chronic studies of male rats (Biegel et al., 2001; Butenhoff et al., 2012). In the chronic study of male rats dosed

with 13.6 mg/kg/day PFOA in the diet for 24 months (Biegel et al., 2001), absolute testes weight was significantly increased by PFOA at 24 months but not at 21 months or earlier time points.

### **Reproductive and developmental studies in rats and rabbits**

As discussed above, the rat and the rabbit are not the most appropriate animal models for human reproductive and developmental effects of PFOA because of their rapid excretion of this chemical in female rats and both male and female rabbits. Studies of reproductive and developmental effects in these species are summarized below.

#### Rabbit developmental study

Gortner (1982, cited in USEPA, 2005a) studied developmental effects in New Zealand white rabbits (18/dose group) given 0, 1.5, 5, or 50 mg/kg/day PFOA on gestation days 6-18. Maternal body weight was decreased in treated groups on GD 6-9 but returned to control levels on GD 12-29. Parameters such as number of resorptions and implantations, and fetal viability, sex ratio, and weight, were not affected by treatment. A dose related increase in incidence of the skeletal variation, extra ribs or 13<sup>th</sup> rib, was observed, with incidence of 16, 20, 30, and 38% in the 0, 1.5, 5, and 50 mg/kg/groups. This increase was statistically significant in the highest dose group.

#### Rat studies

##### Rat developmental study

Gortner (1981, cited in USEPA, 2005a) gave Sprague-Dawley rats (22/dose group) 0, 0.05, 1.5, 5, and 150 mg/kg/day APFO on gestation days 6-15. The maternal NOAEL was 5 mg/kg/day and the LOAEL was 150 mg/kg/day, based on decreased body weight, ataxia, and mortality seen only in the high dose group. Parameters including number of resorptions and implantations, or fetal viability, sex ratio of offspring, and pup weight, were not affected by treatment. A significantly increased incidence of missing sternbrae occurred in the high dose group. Since this effect was also seen in the control and lower dose groups at lower frequency, the study authors did not believe that it was treatment related.

##### Two-generation rat reproductive study

A two-generation reproductive study in Sprague-Dawley rats using gavage doses of 0, 1, 3, 10, and 30 mg/kg/day APFO was conducted by York (2002) and was also reported by Butenhoff et al. (2004b). Various parameters related to reproduction and development, as well as general toxicology endpoints, were evaluated in each generation (F0, F1, F2). Because of the design of the study, some observed effects may have been due to developmental/reproductive toxicity and others due to adult toxicity (USEPA, 2005a).

The parental (F0) generation (30 per sex per group) were dosed for at least 70 days prior to cohabitation beginning at age 6 weeks and until after mating in males and through pregnancy and lactation until weaning of pups for females. Males of the F0 generation were sacrificed after mating, and females were sacrificed at weaning of offspring on PND 22.

Mean serum levels on the day of sacrifice in the F0 males in the control, 10, and 30 mg/kg/day groups were 34 ng/ml, 51,500 ng/ml and 45,300 ng/ml, with no increase in serum levels with increasing dose. In females, serum levels were much lower, < 5 ng/ml, 370 ng/ml, and 1020 ng/ml in the control, 10 and 30 mg/kg/day groups. Serum levels were not measured in the 1 and 3 mg/kg/day groups.

In the F0 males, there were no effects on mating, fertility, or sperm parameters. There were dose-related statistically significant decreases in body weight and body weight gain at 3, 10, and 30 mg/kg/day of 6, 11, and 25%, respectively, although relative food consumption was increased in these groups. Liver weights (absolute, relative to body weight, and relative to brain weight) were increased significantly in all dose groups (1 mg/kg/day and above). Kidney weights were also increased relative to brain and liver weights in all dose groups. Livers and kidneys were not examined histologically. Reproductive and endocrine organs were examined histologically, and hypertrophy and/or vacuolation of the zona glomerulosa of the adrenal glands were seen in 20% of the 10 mg/kg/day group and 70% of the 30 mg/kg/day group.

In F0 females, there were no effects on estrous cyclicity, mating, fertility, pup sex ratio, pup viability, pup birth weight, or other related parameters. Relative kidney weights were reduced at 30 mg/kg/day, and relative liver weight was reduced at 3 and 10 mg/kg/day. This decrease in relative liver weight is in contrast to findings in many other studies in which increased liver weight increases occurred. Histological examination was not performed on the kidneys or livers. Lau et al. (2006) concluded that the profile of maternal effects in the rat study differed from those seen in similar studies in mice because of the more rapid excretion in the adult female rat compared to the adult female mouse.

Dosing of F1 offspring with the same dose levels that their parents had received began at weaning (PND 22). Most of the F1 animals were sacrificed after sexual maturation (about 5 weeks in females and 7 weeks in males). From the F1 generation, 30 pairs per dose group were selected to be bred to produce the F2 generation. These F1 males were sacrificed at about 133 days of age, after 113 days of treatment, and F1 females were sacrificed at weaning of the pups, at 13 to 15 weeks of age.

The weight of the F1 pups (males and females combined) was reduced compared to controls through lactation on a per litter basis in the 30 mg/kg/day group. During the post-weaning period, signs of toxicity in the F1 males included increased incidence of annular constriction of the tail at all doses (significant at 1, 10, and 30 mg/kg/day), significant increases in the number of emaciated pups at 10 and 30 mg/kg/day, and significantly increased urine-stained fur, decreased motor activity, and abdominal distention at 30 mg/kg/day. Deaths were significantly increased in the 30 mg/kg/day males during the post-weaning period. Sexual maturation, as indicated by day of preputial separation, was significantly delayed in the high dose group (52.2 days) compared to controls (48.5 days).

No effects were observed on mating, fertility, or sperm parameters in the F1 males. There was a significant dose-related reduction in body weight gain at all doses (1 mg/kg/day and above) during the post-weaning period, although relative food consumption was significantly increased. At sacrifice (day 113 of dosing), body weights were reduced by 6, 6, 11, and 22% in the 1, 3, 10, and 30 mg/kg/day groups compared to controls. Absolute and relative liver weights were increased at all doses, and hepatocellular hypertrophy and necrosis were seen in livers of some animals in the 3, 10, and 30 mg/kg/day groups. Kidney weight relative to body and brain weight was increased in all treated groups, but histopathological examination was not performed. Hypertrophy and vacuolation of the adrenal cortex was seen in 70% of high dose animals, but not in other groups.

In F1 females, there was a significant increase (6/60) in mortality in the high dose group 2-8 days post-weaning. Sexual maturation, as indicated by day of vaginal opening, was significantly delayed in the 30 mg/kg/day group, from 34.9 days in controls to 36.6 days in treated females. Significant reductions in body weight were seen at several time points during post-weaning, gestation, and lactation, although relative food intake was not decreased, and body weight was not decreased at terminal sacrifice. No effects were seen in F1 females on mating or fertility parameters, or number of implantations, number of stillborn pups, or length of gestation.

The F2 pups were followed until weaning at PND 22. In the F2 generation, no treatment-related effects were seen on pup viability until weaning, percentage of male pups, litter size, average pup body weight on days 1, 5, 8, 15, or 22, or anogenital distance. Because these pups were sacrificed at weaning, post-weaning effects were not assessed.

Several LOAELs and NOAELs were identified in this study for males and females at different life stages, and the serum PFOA levels at these NOAELs and LOAELs were modeled (USEPA, 2005a). The LOAEL for adult males in this study was 1 mg/kg/day (modeled serum concentration – 42,000 ng/ml) with no NOAEL identified, based on increased liver weight in the F0 and F1 generations and decreased body weight in the F1 generation. For pregnant females, the LOAEL was 10 mg/kg/day, based on decreased body weight in F1 pups during post-weaning, and the NOAEL was 3 mg/kg/day (modeled serum concentration – 3500 ng/ml). For the male rat pups during post-weaning, the LOAEL was 10 mg/kg/day and the NOAEL was 3 mg/kg/day (modeled serum concentration - 8400 ng/ml at week 4), based on decreased body weight in the F1 generation. For the female rat pups during post-weaning, the LOAEL was 30 mg/kg/day and the NOAEL was 10 mg/kg/day (modeled serum concentration -13,000 ng/ml) at week 7, based on decreased body weight in the F1 generation.

### **Thyroid effects**

Effects of PFOA on the thyroid in animal toxicology studies are of interest because there have been many human epidemiological studies of PFOA and thyroid hormones and/or thyroid disease. However, only a few toxicology studies have evaluated effects of PFOA on thyroid function.

In the 4-week study of male cynomolgus monkeys (Thomford et al., 2001a), there were no effects on thyroid hormones (thyroid stimulating hormone, TSH; total and free triiodothyronine, T3; total and free thyroxin, T4) after 29 days of exposure to 0, 2, or 20 mg/kg/day APFO. It should be noted that the number of animals per dose group in this study was very small (controls, n=2; treated, n=3).

Changes in thyroid hormones were observed in PFOA-treated male cynomolgus monkeys in the 6-month study (Butenhoff et al., 2002). This study is described in detail above. At the beginning of the study, there were 6 monkeys in the control and high dose group, and 4 in the other two groups. Some animals in the treated groups did not complete the study because of mortality or overt toxicity (3 mg/kg/day, n=3; 30/20 mg/kg/day, n=2 at end of study.) Thyroid hormones (total and free triiodothyronine, T3; total and free thyroxin, T4) and TSH (thyroid stimulating hormone) were measured before dosing, and after 5, 10, 14, and 27 weeks of dosing with 0, 3, 10, 30/20 mg/kg/day APFO. Interpretation of the data is complicated by the fact that comparisons for these parameters are made both to the pretreatment values for the same animals and to the control animals at the concurrent time point. Statistically significant changes were reported for all hormones at one or more time points in one or more dosed groups.

TSH was significantly increased at week 35 compared to pretreatment values at 3 and 10 mg/kg/day. Total T4 was significantly decreased compared to concurrent controls in all dosed groups (3, 10, and 30/20 mg/kg/day) at week 35, at all time points during treatment (weeks 5 to 35) at 10 mg/kg/day, and at week 10 and week 35 at 30/20 mg/kg/day. Free T4 was significantly lower than in concurrent controls at 10 mg/kg/day at weeks 5, 10, and 27, and at 30/20 mg/kg/day at weeks 5 and 27. Total T3 was significantly increased compared to pretreatment values at two time points (weeks 10 and 14) at 3 mg/kg/day, and was decreased compared to concurrent controls in all dosed groups at 30/20 mg/kg/day; this change was not significant at 10 weeks. Finally, free T3 was significantly decreased compared to concurrent controls in the 30/20 mg/kg/day group at weeks 5, 10, and 35.

The authors reported that all thyroid hormone values were within normal range, and that no relevant histological observations were observed. They also state that there were no relevant changes in TSH or T4, despite the changes in levels of these hormones noted above. It is stated, in the 3 high dose monkeys that were removed from the study due to toxicity, the decreased T3 observed during treatment trended upward after treatment ceased. The authors state that the observed changes in thyroid hormone levels were likely due to stress or normal variation rather than a direct effect of APFO (PFOA).

Thyroid hormones (total T4, free T4, and total T3) were measured in male Sprague-Dawley rats one day after dosing for 1, 3, or 5 days with a high dose (20 mg/kg/day) of PFOA (Martin et al., 2007). Levels of free and total T4 in PFOA treated animals were significantly reduced to several fold below control levels at all three time points. Total T3 was also significantly decreased at all

three time points, although the magnitude of the decreases was smaller than for T4. The effects were similar or greater than those caused by PFOS at 10 mg/kg/day in the same study. Unfortunately, thyroid hormones have not been evaluated in longer term, lower dose rodent studies of PFOA.

### **Neurobehavioral and Central Nervous System Effects**

Several studies have found neurobehavioral effects, particularly increased activity, in rodents exposed to low doses of PFOA during development. Although human studies on these effects are not reviewed in this document, toxicological studies of neurobehavioral effects are of interest because some human studies have reported associations of PFOA and other PFCs with behavioral effects, particularly attention deficit hyperactivity disorder, in children (Hoffman et al., 2010; Ode et al., 2014).

Johansson et al. (2008) found significant behavioral effects in adult male NMRI mice given a single gavage dose of 0.58 or 8.7 mg/kg PFOA at 10 days of age. Behavioral tests were conducted at 2 and 4 months of age. These behavioral tests included spontaneous behavior (locomotion, rearing, total activity) over three consecutive 20-minute time periods (an indicator of habituation over time), nicotine-induced motor activity (locomotion, rearing, total activity), and behavior in an elevated plus-maze (a measure of anxiety). These single doses of PFOA did not affect weight gain or cause any overt signs of toxicity. At 2 and 4 months, some or all measures of spontaneous behavior were affected in both the low and high dose groups, with greater effects in the high dose group (8.7 mg/kg) and at the later time point (4 months). Habituation was greatly decreased at both 2 and 4 months in the high dose group compared to controls. This effect increased with age of the treated mice. Lack of effect in the elevated plus-maze test indicated that these effects were not likely to be caused by anxiety.

Responses over three 20-minute periods following an injection of 80 µg/kg nicotine, a measure of the susceptibility of the cholinergic system, was also significantly altered in high and low dose mice at 4 months. Control mice showed an increase in activity from the nicotine, followed by a decrease to baseline behavior. Low dose mice also showed increased activity after receiving nicotine, but less so than controls, and this increased activity was followed by greater activity than in controls during the last 20-minute time period. In contrast, high dose mice showed decreased activity during the first 20 minutes after receiving nicotine but were hyperactive during the later time period. The authors concluded that neonatal PFOA exposure caused deranged spontaneous behavior such as lack of habituation and hyperactivity that worsened with age in adult mice, and that PFOA exposure also affected the cholinergic system.

Significant effects were seen at both doses in this study. Therefore, the LOAEL was 0.58 mg/kg, and no NOAEL was identified. Serum levels were not measured in this study and have not been measured in neonatal mice administered a single dose of PFOA. A single oral dose of 1 or 10 mg/kg to adult CD-1 mice resulted in maximum serum concentrations of about 10,000 and 100,000 ng/ml (Lou et al., 2009). From these data, the serum concentrations from 0.58 and 8.7

mg/kg can be estimated as 5800 and 87,000 ng/ml, assuming that kinetics in these neonatal mice are similar to in adult mice.

The authors stated that the effects caused by PFOA in this study are similar to those seen with PCBs and PBDEs, known developmental neurotoxicants, and that PFOA should be classified as a developmental neurotoxicant along with these other persistent chemicals. It is noted that studies from this laboratory using the same dosing protocol (a single dose given to 10 day old mice) and the same behavioral tests are the basis for the chronic USEPA IRIS Reference Doses developed in 2008 for two PBDEs, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) (USEPA 2008b,c). Because the effects persisted into adulthood and were permanent, these effects were regarded by USEPA IRIS as chronic, not acute, even though only a single dose was given.

A second study in this laboratory (Johansson et al., 2009) examined the effects of a single gavage dose of 8.7 mg/kg PFOA to 10-day old mice on proteins important for neuronal growth and synaptogenesis in the developing brain. The mice were sacrificed 24 hours after dosing. Rapid brain development, the "brain growth spurt," occurs in mice during this time period. Levels of two proteins known to be involved with neuronal survival, growth, synaptogenesis, and other aspects of neuronal development were significantly increased in the hippocampus but not the cerebral cortex of treated mice, and two other proteins were increased in both areas of the brain. PBDEs, which are known to affect behavior after developmental exposures, had previously been found by these researchers to cause similar effects. The authors conclude that these changes may relate to some of the observations in their earlier behavioral study.

Onishchenko et al. (2010) found sex-related behavioral changes in offspring of C57Bl/6 mice exposed to 0.3 mg/kg/day PFOA throughout gestation. Liver weights were significantly increased in treated pups that were sacrificed at birth. PFOA exposure did not affect locomotor behavior in 5-8 week old mice tested individually, but significant sex-related effects were seen on circadian activity when these mice were housed in social groups. Initially, PFOA treated males were more active than controls, while treated females were less active than controls. After habituation, activity in both light and dark phases was increased during the remainder of the first 24 hours in exposed males, while no effects were seen in exposed females. During the second 24-hour period when the mice were more adapted to the test environment, the number of inactive periods during the light phase was decreased in both male and female PFOA-treated mice, and also in the dark phase in PFOA-treated males. No effects of PFOA were seen in other tests, including the elevated plus-maze test for anxiety-like behavior, the forced swimming test for depression-like behavior, or muscle strength in the hanging wire test. Serum PFOA levels in this study were not measured, but they are not expected to be below the serum levels at the LOAELs and NOAELs in some other studies which evaluated other effects. The NOAEL for these effects is not known.

Finally, Sobolewski et al. (2014) studied behavioral effects of developmental exposures to PFOA in C57Bl/6 mice. Three other unrelated environmental contaminants and a mixture of all four chemicals were also evaluated; only results for PFOA alone are reported here. PFOA (0.1 mg/kg/day) was administered to dams in puffed wheat cereal on GD7 through weaning. Behavioral effects were evaluated in male and female offspring (one or two per sex per litter) beginning at age 60 days.

PFOA treatment caused behavioral effects, which differed between males and females. Locomotor behavior was increased in males treated with PFOA, while no effect was seen in females. Horizontal movement and ambulatory movements were increased and resting time was decreased during one or more of three test sessions, while vertical activity was unaffected in males. Behaviors related to novel object exploration and recognition were altered in both PFOA-treated males and females to a highly significant degree, although the specific parameters affected differed between sexes. Fixed interval reinforcement schedule-controlled behavior was not affected by PFOA treatment in males or females.

### **Chronic toxicity and carcinogenicity**

As discussed in the Epidemiology section, PFOA has been associated with increased incidence of kidney and testicular cancer in humans exposed through drinking water, after adjustment for smoking and other relevant factors. The chronic toxicity and carcinogenicity of PFOA has been evaluated in two dietary studies in rats. One study (Sibinski et al., 1987; Butenhoff et al., 2012) included male and female rats, while Biegel et al. (2001) studied only males. As such, chronic toxicity and carcinogenicity have been studied only in the rat, a species in which PFOA is rapidly excreted by females. Chronic studies in another species in which PFOA is persistent in both sexes, such as the mouse, would provide important information specific to females. Furthermore, the chronic studies did not assess effects including carcinogenicity which might result from exposures during the critical developmental stages now known to be sensitive periods for PFOA toxicity.

### **Two year chronic/carcinogenicity study in male and female rats**

Sprague-Dawley rats (30 per sex per group) were dosed with APFO through diets containing 0, 30, or 300 ppm APFO for two years (Sibinski, 1987; Butenhoff et al., 2012). Additional animals (15 per sex) were included in the control and high dose groups for evaluation at sacrifice after one year. From food consumption data, mean APFO doses were determined to be 1.3 and 14.2 mg/kg/day in males, and 1.6 and 16.1 mg/kg/day in females, for the 30 and 300 ppm groups, respectively.

Body weight gains were decreased in both sexes in the high dose group, with maximum differences between the high dose group and controls of 21% in males at 6 weeks and 11% in females at 92 weeks. Smaller decreases were seen in low dose males, and decreases were not consistently seen in low dose females. Decreases in weight gain were treatment-related, since food consumption on a body weight basis was increased in treated males, by about 13% in the

high dose group. Mortality was not increased by APFO treatment, and 2-year survival rates were 70%, 72% and 88% in males and 50%, 48% and 58% in females in the 0, 30 ppm, and 300 ppm groups, respectively.

The incidence of ataxia was increased in female rats treated with PFOA and occurred most frequently in moribund animals. Ataxia occurred in 3%, 18% and 23% of rats in the control, low- and high-dose groups (including animals sacrificed at one year), respectively.

Clinical chemistry changes occurred in high and low dose males, but not in females. The liver enzymes alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were increased in both low dose and high dose males between 2 and 18 months, but only in the high dose group at 24 months. Albumin was significantly increased in the male low dose group at 3 and 6 months, and until 24 months in the high dose group. Butenhoff et al. (2012) concluded that the elevations in liver enzymes in both high and low dose males may represent “borderline chronic liver toxicity”.

Hematological parameters including red blood cell counts, hemoglobin concentrations and hematocrit were significantly decreased in high and low dose males and females at different time points. In high dose males, these erythrocyte-related hematological parameters were decreased from 3 to 18 months. Additionally, leukocytes were increased in both dose groups of males during the first year. Statistically significant changes included increases in lymphocytes and neutrophils at 3 and 12 months in both dose groups, and in lymphocytes and 6 and 18 months in the low dose group.

In females, hematological parameters were significantly changed with equal frequency in the high and low dose groups. At 3 months, red blood cells were decreased in the low dose group and hematocrit was decreased in the high dose group. At 6 months, erythrocytes, hemoglobin, and hematocrit were decreased only in the low dose group, while at 12 months, these three parameters were decreased only in the high dose group.

At the one-year interim sacrifice, statistically significant changes in organ weights in high dose males included increased liver weight and decreased pituitary weight (absolute and relative to body or brain weight), increased kidney weight (only relative to body weight), decreased adrenal and heart weight (absolute and relative to brain weight). No organ weight changes were observed in high dose females sacrificed at one year.

At the one-year interim sacrifice, histopathological changes in the livers of high dose males included diffuse hepatocellular hypertrophy in 12/15 (compared to 0/15 controls); portal mononuclear cell infiltration in 13/15 (compared to 7/15 controls); and focal hepatocellular necrosis in 6/15 (compared to 0/15 controls). In high dose females, hepatocellular vacuolation occurred in 11/15 (compared to 5/15 controls). Testicular tubular atrophy and marked aspermatogenesis was found in 2/15 high dose males but in none of the controls.

At the two-year sacrifice, slight non-significant increases in liver weight occurred in all treated groups of males and females. Relative kidney weight was slightly increased in male and female high dose rats, and this change was significant in females.

Several non-neoplastic liver lesions were also increased in treated animals at two years. Hepatocellular hypertrophy did not occur in controls but was found in 12% and 80% of the males, and 2% and 16% of the females, in the low and high dose groups, respectively. Hepatic cystoid degeneration occurred in 8%, 14% and 56% of the control, low, and high-dose males. However, hepatocellular necrosis or hepatocellular vacuolation was not increased in treated animals compared to controls, although an increase of focal hepatocellular necrosis in high dose males and hepatocellular vacuolation in high dose females was observed at the one-year sacrifice.

Hepatocellular carcinomas were found in the control (6%), low dose (10%), and high dose (2%) males, and occurred in females only in the high dose group (2%). The incidence of hepatic hyperplastic nodules was 6% in high-dose males, and they were not found in the other groups of males. In females, these nodules were found in 2% of the control and high dose animals, but not in the low dose group. Butenhoff et al. (2012) state that diagnostic criteria for these nodules, which represent a regenerative process, have changed since the histopathological evaluation for this study was performed. The USEPA SAB (2006) concluded that these nodules may have been part of the continuum of proliferative lesions in the liver carcinogenic process. Butenhoff et al. (2012) further conclude that the increased incidence of focal necrosis and vacuolation in treated animals, which was observed at the one year sacrifice but not at the two year sacrifice (because of the higher background incidence of these changes in older rats), suggests a progression of lesions “from hepatocellular hypertrophy to fatty degeneration to necrosis followed by regenerative hyperplasia.”

The incidence of neoplastic lesions of the testes differed significantly between control and treated rats. Leydig cell adenomas of the testes were found in 0, 4, and 14% of the control, low, and high dose males, respectively, with a significantly increased incidence in the high dose group. Additionally, vascular mineralization of the testes occurred in 0, 6, and 18% of control, low, and high dose males, respectively, and reached statistical significance in the high-dose group.

The incidence of mammary gland fibroadenomas was 22%, 42%, and 48% in the control, low dose, and high dose females, and the increase in the high dose group was significant. Mammary gland adenocarcinomas were present in 15, 31, and 11% of the control, low, and high dose females, respectively. A subsequent Pathology Working Group reevaluation of the mammary gland slides concluded that the incidence of mammary gland proliferative lesions was not increased by PFOA in this study (Hardisty et al., 2010).

A statistically significant, dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats at the two-year sacrifice, with incidence of 0%, 14%, and 32% in the

control, low, and high dose groups. The authors of the study report stated that the biological significance of this effect was unknown, as there was no evidence of progression to tumors. Slides of the ovaries were later re-evaluated (Mann and Frame, 2004) using more recent pathology criteria. In the reevaluations, no statistically significant increases in hyperplasia (total number), adenomas, or hyperplasia/adenoma combined were seen in treated groups compared to controls. Although the size of stromal lesions was increased in the high dose group, the incidence of ovarian adenomas was higher in the controls than in the treated groups.

USEPA (2005a) concluded that “based on these toxic effects, the high dose selected in this study appears to have reached the Maximum Tolerated Dose. Based on a decrease in body weight gain, increase in liver and kidney weights and toxicity in the hematological and hepatic systems, the LOAEL for male rats is 300 ppm and the NOAEL is 30 ppm. The LOAEL for female rats is 300 ppm based on a decrease in body weight gain and hematologic effects and the NOAEL is 30 ppm.”

However, as noted above, a significant increase in the incidence of ataxia, as well as hematological effects equivalent to those in the high dose group, occurred in low dose females in this study. Also, in males in this study, there were significant changes in several clinical chemistry parameters at multiple time points in the low dose group. Thus, statistically significant effects occurred at multiple time points in the low dose males and female in this study. It should be noted that the study authors concluded that the elevation of the three liver enzymes, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase, in both dosed groups of males, in conjunction with liver weight changes and histopathology observations, suggested that PFOA affected hepatocytes in both the low dose and the high dose groups of male rats.

The serum level in the low dose (1.6 mg/kg/day) females, considered to be the NOAEL by USEPA (2005a), was estimated at 1800 ng/ml, based on average AUC (USEPA, 2005a; Post et al., 2009a). The serum levels in the low dose (1.3 mg/kg/day) males were much higher, and can be estimated at 55,000 ng/ml from the kinetic data presented by USEPA (2005a).

#### Chronic mechanistic study in male rats

The second chronic study (Biegel et al., 2001) was designed to investigate the mode of action for testicular tumors observed in male rats in the first chronic study (Sibinski et al., 1987; Butenhoff et al., 2012). Male Sprague-Dawley rats were given 300 ppm PFOA in the diet (mean dose of 13.6 mg/kg/day) for up to two years. Serum levels at this dose were estimated as 572,000 ng/ml (USEPA, 2005a). Two control groups were used, an *ad libitum* fed (AL) group and a pair-fed (PF) group in which the food intake was controlled to match the food intake of the PFOA exposed group. Another group of rats was treated with Wyeth 14,643 (WY), a model peroxisome proliferator. There were 156 rats in each of the control and dosed groups.

At interim time points of 1, 3, 6, 9, 12, 15, 18, and 21 months, the liver and testes from six rats per treatment group were weighed and evaluated for cell proliferation. At each time point,

peroxisome proliferation (PCO) was evaluated in 6 additional rats per group, and serum hormone levels (estradiol, testosterone, LH, FSH, and prolactin) were measured in 10 rats from each group.

Liver-to-body weight ratios were increased in PFOA treated animals at all time points compared to controls. This increase was greatest at the earliest time point (1 month) and decreased over time. At 24 months, there was only a slight difference in liver-to-body weight ratio between the treated and control animals. Similarly, the increase in peroxisomal beta-oxidation (PCO), a measure of peroxisome proliferation, in the liver was greatest at 1 month, and the magnitude of the increase over controls decreased with time. WY also increased liver weight and peroxisomal beta-oxidation (PCO). Liver cell proliferation was increased by WY but not by PFOA.

In contrast to the liver, relative organ weight, PCO activity, and cell proliferation in testes were not affected in PFOA or WY treated rats except for increased relative testicular weight at 18 and 24 months in PFOA treated animals and at 24 months in WY treated animals. In the pancreas, PFOA increased acinar cell proliferation at 12, 16, 18, and 21 months, while WY did not have this effect.

Estradiol levels were significantly increased by PFOA at the 1, 3, 6, 9, and 12-month time points. WY also increased serum estradiol. Testosterone, prolactin, LH, and FSH were not changed compared to pair-fed controls.

In this study, there was a significant increase of three types of tumors (Leydig cell adenomas, hepatic adenomas, and pancreatic acinar cell adenomas) in the PFOA treated group at the 24-month sacrifice. The incidence of these tumors in the ad libitum control (AL), pair fed control (PF), and treated groups was: Leydig cell adenomas (AL – 0/80; PF – 2/78, treated – 8/76), hepatic adenomas (AL – 2/80, PF – 1/79, treated – 10/76), and pancreatic acinar cell adenomas (AL – 0/80, PF – 1/79, treated – 7/76). These same three types of tumors also occurred at a higher rate in the WY treated animals. As discussed above, the incidence of testicular Leydig cell adenomas was also increased by PFOA in a dose-related fashion in the first chronic study (Sibinski et al., 1987; Butenhoff et al., 2012).

#### Information relevant to carcinogenicity from developmental exposure

Carcinogenicity studies using prenatal or perinatal exposure protocols have not been reported for PFOA. Very limited information is available that is relevant to carcinogenicity after developmental exposure to PFOA.

As discussed in the section on hepatic effects above, Filgo et al. (2015) evaluated hepatic histopathological effects, including tumor incidence, at age 18 months in female offspring of dams dosed on GD 1-17. The study evaluated three strains of mice - CD-1, 129/Sv wild type, and 129/Sv PPAR-alpha knockout. CD-1 dams were dosed with 0, 0.01, 0.1, 0.3, 1, and 5 mg/kg/day, and 129/Sv wild type and PPAR-alpha null dams dosed with 0, 0.1, 0.3, 1, and 3 mg/kg/day.

The authors emphasize that the study was not designed or intended to be a carcinogenicity bioassay. Rather, the incidence of liver tumors was evaluated by Filgo et al. (2015) because of the unexpected finding of liver tumors in some treated animals that died before the scheduled end of the study. In CD-1 mice in this study, single or multiple hepatocellular adenomas were found in one or more animals in each of the treated groups (n=21 to 37 per group) except for at the lowest dose (0.01 mg/kg/day), but not in controls (n=29). In total, adenomas occurred in 4.9% (7 of 144) treated animals, compared to a historic control incidence of 0.4% in untreated female CD-1 mice. Hepatocellular carcinomas occurred in two treated mice (0.3 and 5 mg/kg/day) but not in controls.

In 129/Sv mice, hepatocellular adenomas did not occur in control or treated groups (n=6 to 10 per group). In PPAR-alpha null mice of this strain, in contrast, there were no adenomas in the controls (n=6), one adenoma in the 0.1, 0.3, and 1 mg/kg/day groups (n=9 or 10), and two adenomas at 3 mg/kg/day (n=9). These tumors occurred in 13.2% of all treated PPAR-alpha null mice.

Possibly because of the small numbers of animals per dose-group, tumor incidences were not significantly increased in PFOA-treated groups compared to control in this study, with the exception of the CD-1 mice dosed with 0.3 mg/kg/day in which adenomas were found in 4 of 26 animals. The results of this study are suggestive of the potential for developmental exposures to PFOA to cause tumors in mice later in life, and also suggest that these tumors may occur through a PPAR-alpha independent pathway. An additional study with larger numbers of animals and designed to detect increased tumor incidence is needed to further evaluate these questions.

Ngo et al. (2014) evaluated the incidence of intestinal tumors and other endpoints in wild type and Min/+ C57Bl/6 mice offspring of dams dosed with 0.01, 0.1, and 3 mg/kg/day PFOA during gestation. The Min/+ strain has a mutation of a tumor suppressor related to intestinal adenomatous polyps and is a sensitive model for chemical-induced intestinal tumorigenesis. PFOA did not cause increased intestinal tumorigenesis at sacrifice at age 11 weeks in either wild type or Min/+ mice.

## Discussion

Leydig cell testicular tumors were the only tumor type that were increased by PFOA in the chronic study of Butenhoff et al. (2012), and the incidence of these tumors was also increased by PFOA in Biegel et al. (2001). In Biegel et al. (2010), the incidence of hepatic and pancreatic tumors was also increased to a similar degree as testicular tumors in male rats treated with PFOA.

Although hepatic tumors were not significantly increased in treated animals in Butenhoff et al. (2012), both the USEPA SAB (2006) and Butenhoff et al. (2012) point out that the incidence of hepatic hyperplastic nodules was increased in the high dose males in comparison to the control and low dose groups in this study. Butenhoff et al. (2012) state that diagnostic criteria for these nodules, which represent a regenerative process, have changed since the histopathological

evaluation for this study was performed. The EPA SAB (2006) concluded that these nodules may have been part of the continuum of proliferative lesions in the liver carcinogenic process, and Butenhoff et al. (2012) further conclude that the increased incidence of focal necrosis and vacuolation found at the one year sacrifice but not at the two year sacrifice (because of the higher background incidence of these changes in older rats) suggests a progression of lesions “from hepatocellular hypertrophy to fatty degeneration to necrosis followed by regenerative hyperplasia.”

In regard to pancreatic tumors, Frame and McConnell (2003) reevaluated the slides of the pancreas from both studies and found that different diagnostic criteria and nomenclature were used by the pathologists in Sibinski et al. (1987) and Biegel et al. (2001). Frame and McConnell (2003) concluded that the incidence of pancreatic focal acinar lesions, which can progress to adenomas, was increased by 300 ppm PFOA in the diet in both studies and that these lesions were larger, more frequent, and had a greater tendency to progress to adenomas in Biegel et al. (2001).

PFOA has been shown to promote liver carcinogenesis in rats initiated with diethylnitrosamine as well as with a more complex initiating protocol (Abdellatif et al., 1991; Nilsson et al., 1991). PFOA also promoted hepatocarcinogenicity in rainbow trout, a model species for human liver cancer (discussed in Mode of Action section, below).

An increased incidence of liver tumors from prenatal exposure to PFOA in CD-1 and PPAR-alpha null mice is suggested by the results of Filgo et al. (2015). However, this study is not definitive, and additional research on carcinogenicity later in life after developmental exposures to PFOA is needed.

Information related to the mode of action for carcinogenicity is discussed in the Mode of Action section below.

### **Summary of Conclusions of Toxicology Studies**

The review of the toxicology data identified increased relative liver weight and delayed mammary gland development from developmental (perinatal) exposure as the most sensitive systemic toxicological endpoints with data appropriate for dose-response modeling.

Delayed mammary gland development in mice is the most sensitive systemic endpoint with data appropriate for dose-response modeling. Increased liver weight is a more sensitive endpoint than most other systemic effects, and it co-occurs with and/or progresses to more severe hepatic effects including increased serum liver enzymes, hepatocellular necrosis, fatty liver, and/or hyperplastic nodules. PFOA also causes other types of toxicity including reproductive effects in both males and females, delayed growth and development, immune system toxicity, and neurobehavioral effects. PFOA caused tumors in two chronic rat studies, and one of these studies provides data for testicular tumors that is appropriate for dose-response modeling. All of these toxicological effects are considered relevant to humans for the purposes of risk assessment, as discussed in the Mode of Action section.

Table 10. Summary of Increased Relative Liver Weight Data from Rodents Studies Using Doses  $\leq 1$  mg/kg/day, and 90 Day Non-Human Primate Study

**TABLE 10A: STUDIES PROVIDING SERUM PFOA DATA AT END OF DOSING PERIOD\***

<i>Citation</i>	<i>Species</i>	<i>Life-stage</i>	<i>Doses (mg/kg/day)</i>	<i>Duration</i>	<i>NOAEL (mg/kg/day)</i>	<i>Serum NOAEL (ng/ml)</i>	<i>LOAEL (mg/kg/day)</i>	<i>Serum LOAEL (ng/ml)</i>	<i>Comments</i>
Lau et al. (2006)	Female mouse	Pregnant GD 18	0, 1,3, 5, 10, 20, 40	GD 1-18	----	----	1	22,000	<ul style="list-style-type: none"> <li>Data for absolute liver weight are presented in bar graph in publication. Numerical data were obtained from the investigator.</li> <li>Data on relative liver weight are based on comparisons with body weight after gravid uterus was removed. These data are not shown in the publication and were obtained from the investigator.</li> </ul>
Loveless et al. (2006)	Male rat	Adult	0, 0.3, 1, 3, 10, 30	2 weeks	Linear/Branched 0.3	19,000	1	51,000	<ul style="list-style-type: none"> <li>Three formulations of PFOA of differing isomer compositions were tested.</li> <li>This study also provides data on peroxisomal beta-oxidation. In mice, increased relative liver weight does not correlate with increased peroxisomal beta oxidation among formulations with differing isomer compositions, indicating PPAR-alpha independent effects on liver weight.</li> </ul>
					Linear 0.3	20,000	1	65,000	
					Branched 0.3	16,000	1	48,000	
	Male mouse	Adult	0, 0.3, 1, 3, 10, 30	2 weeks	Linear/Branched 0.3	10,000	1	27,000	
					Linear ---	---	0.3	13,000	
					Branched ---	---	0.3	14,000	
Macon et al. (2011)	Female mouse	Female pup PND 1	0, 0.01, 0.1, 1	GD 10-17	0.1	2300	1	16,000	<ul style="list-style-type: none"> <li>LOAEL for increased relative liver weight is 100 fold higher than LOAEL for delayed mammary gland development.</li> <li>PFOA serum levels were measured on PND 1.</li> <li>With longer GD 1-17 exposure, LOAEL for increased relative liver weight was 0.3 mg/kg/day in male and female pups on PND 7. PFOA serum levels were not measured in this component of the study.</li> </ul>
Perkins et al. (2004)	Male rat	Adult	0, 1,10, 30, 100 ppm in diet	4, 7, 13 weeks	0.07 (1 ppm) at 4 weeks	6500	0.71 (10 ppm) at 4 weeks	55,000	Steady-state serum levels were reached by 4 weeks. Dose-related increases in relative liver weight were similar at 4, 7, and 13 wks (see individual study table in Appendix 5).
Thomford et al. (2001); Butenhoff et al. (2002)	Male cynomolgus monkey	Adult	0, 3, 10, 20/30	26 weeks	----	---	3	72,000	<ul style="list-style-type: none"> <li>PFOA serum levels did not significantly differ at 3 and 10 mg/kg/day.</li> <li>NOAEL was not identified.</li> </ul>

\*LOAELs are based on statistical significance at  $p < 0.05$ . Effects that were not statistically significant occurred at doses below the NOAELs in some studies.

**TABLE 10B: STUDIES NOT PROVIDING SERUM PFOA DATA AT END OF DOSING PERIOD\***

<i>Citation</i>	<i>Species</i>	<i>Life-stage</i>	<i>Doses (mg/kg/day)</i>	<i>Duration</i>	<i>NOAEL (mg/kg/day)</i>		<i>Serum NOAE L (µg/L)</i>	<i>LOAEL (mg/kg/day)</i>	<i>Serum NOAEL (µg/L)</i>	<i>Comments</i>
Abbott et al. (2007)	Mouse (PND 21)	WT** dam	0, 0.1, 0.3, 0.6, 1.0	GD 1-17	0.3		NA	1	NA	<ul style="list-style-type: none"> <li>• Serum data are not presented in this table because they are from PND 21 and do not reflect maximum exposure at end of dosing period.</li> <li>• Liver weight was assessed in offspring on PND 21 and later time points. No data are available from earlier time points closer to the end of dosing.</li> </ul>
		WT pup			--			0.1		
		PPAR-α null dam			1			3		
		PPAR-α null pup			1			3		
DeWitt et al. (2008)	Female mouse	Adult	0, 0.94, 1.88, 3.75, 7.5	15 days	----		NA	0.94	NA	Serum PFOA data are not available for the two lowest doses in this study.
Loveless et al. (2008)	Male mouse	Adult	0, 0.3, 1, 10, 30	29 days	0.3		NA	1	NA	<ul style="list-style-type: none"> <li>• Linear PFOA was used in this study.</li> <li>• Relative liver weight increased in a dose-related manner at all doses below the LOAELs, but changes were not statistically significant.</li> </ul>
	Male rat	Adult			1			10	NA	
Quist et al. (2015)	Female mouse (PND 21)	Pup	0, 0.01, 0.1, 0.3, 1  (On control or high fat diet from PND 35-77)	GD 1-17	0.1  (Note: No NOAEL was identified for other hepatic effects including chronic active inflammation on PND 21 and hepatocellular hypertrophy on PND 91)		NA	0.3  (Note: LOAEL was 0.01 mg/kg/day for chronic active inflammation on PND 21 and hepatocellular hypertrophy on PND 91)	NA	<ul style="list-style-type: none"> <li>• Relative liver weight was increased on PND 21, but not on PND 91.</li> <li>• Frequency and severity of hepatocellular hypertrophy increased on PND 91 in all dosed groups, in the absence of increased liver weight. Statistical analysis was not presented.</li> <li>• Increased severity of chronic active hepatic periportal inflammation on PND 21 was significantly increased in all dosed groups. This effect occurred at doses below those that caused increased liver weight on PND 21.</li> <li>• Increased severity of periportal inflammation also occurred on PND 91, but was less severe than on PND 21.</li> <li>• Hepatic mitochondrial abnormalities occurred at 1 mg/kg/day on PND 21 and PND 91; they were more severe on PND 91. This effect was not evaluated at other doses.</li> <li>• Hepatic peroxisome proliferation was not observed at 1 mg/kg/day on PND 91; other doses were not evaluated.</li> </ul>
Son et al. (2008)	Male mouse	Adult	0.049, 2.64, 17.63, 47.21	4 weeks	-----		NA	0.49	NA	<ul style="list-style-type: none"> <li>• PFOA serum data are not available from this study.</li> <li>• Significantly increased levels of one or both liver enzymes, ALT and AST, occurred at all doses (data shown in Table 8).</li> </ul>
Tucker et al. (2015)	Female mouse	Pup PND 21	0, 0.01, 0.1, 0.3, 1	GD 1-17	CD-1	0.3	NA	1	NA	<ul style="list-style-type: none"> <li>• Serum data are not presented here because they are from PND 21 and do not reflect maximum exposure at end of dosing period.</li> <li>• Liver weight was assessed in offspring on PND 21 and later time points. No data are available from earlier time points closer to the end of dosing. Increased liver weight was not observed at time points later than PND 21. At these time points, PFOA body burdens would be lower than on PND 21.</li> <li>• Delayed mammary gland development occurred at lower doses than liver effects in both strains.</li> </ul>
		C57/BI			1	NA	-----	NA		
		WT pup			--			0.1		
		PPAR-α null dam			1			3		
PPAR-α null pup	1			3						

\*LOAELs are based on statistical significance at p<0.05. Effects that were not statistically significant occurred at doses below the NOAELs in some studies. \*\*WT – Wild Type

<i>Citation</i>	<i>Species &amp; Strain</i>	<i>Lifestage for Dosing</i>	<i>Dose(s) (mg/kg/day)</i>	<i>Dosing Duration</i>	<i>Endpoint(s)*</i>	<i>NOAEL** (mg/kg/day)</i>	<i>LOAEL** (mg/kg/day)</i>	<i>PFOA Serum Concentration (ng/ml)</i>	<i>Comments</i>
<b>Botelho et al. (2015)</b>	Male C57BL/6 (H-2) <sup>b</sup> mice	Adult	0, 20, 50, 100, and 200 ppm in diet  (20 ppm estimated as 3 mg/kg/day from Qazi et al., 2013)	10 days	↓ complement activation (various parameters) at end of dosing period.	100 ppm  (estimated as 15 mg/kg/day based on data for lower dose in Quazi et al., 2013)	200 ppm  (estimated as 30 mg/kg/day based on data for lower dose in Quazi et al., 2013)	Estimated serum level at 200 ppm (LOAEL): 152,000 ng/ml (Qazi et al., 2009b)	Effects associated with PPAR-alpha occurred at doses below the LOAEL for ↓ complement activation; authors concluded that ↓ complement activation is PPAR-alpha independent.  LOAEL for ↑ liver weight was lower than for immune effect.
<b>DeWitt et al. (2008)</b>	Female C57/BL6N mice	Adult	0, 3.75, 7.5, 15, and 30 mg/kg/day in drinking water.	15 days	↓ IgM, ↑ IgG after immunization with sheep red blood cells	1.88 mg/kg/day	3.75 mg/kg/day	74,913 ng/ml at 3.75 mg/kg/day (LOAEL), one day post-dosing	NOAEL and LOAEL for ↓ relative spleen and thymus weight were higher than for other effects.  NOAEL and LOAEL for ↓ relative thymus weight were higher than for other effects.  LOAEL for ↑ liver weight was lower than for immune effect.
			0, 0.94, 1.88, 3.75, and 7.5 mg/kg/day in drinking water.		↓ IgM, ↑ IgG after immunization with sheep red blood cells	-----	3.75 mg/kg/day		
					↓ relative spleen weight				
<b>DeWitt et al. (2009a)</b>	Female C57/BL6N mice	Adult	0, 3.75, 7.5, and 15 mg/kg/day in drinking water	10 days	↓ IgM	7.5 mg/kg/day (sham operated)  3.75 mg/kg/day (adrenalectomized)	15 mg/kg/day (sham operated)  3.75 mg/kg/day (adrenalectomized)	Not assessed	Authors conclude that effects on immune system are not due to increased serum corticosterone.
<b>Hu et al. (2010)</b>	Female C57/BL6N mice	Pregnant	0, 0.5, and 1 mg/kg/day  Gavage	GD 6-17	IgM (PND 49), IgG (PND 63) after immunization with sheep red blood cells.  Absolute and relative spleen and thymus weight (PND 49 and 63) in offspring	1 mg/kg/day	-----	Not assessed at end of dosing	

<i>Citation</i>	<i>Species &amp; Strain</i>	<i>Lifestage for Dosing</i>	<i>Dose(s) (mg/kg/day)</i>	<i>Dosing Duration</i>	<i>Endpoint(s)*</i>	<i>NOAEL** (mg/kg/day)</i>	<i>LOAEL** (mg/kg/day)</i>	<i>PFOA Serum Concentration (ng/ml)</i>	<i>Comments</i>
<b>Hu et al. (2012)</b>	Female C57/BL6N mice	Pregnant and lactating	0, 0.02, 0.2, and 2 mg/kg/day Gavage	Pre-pregnancy (10.1-15.7 days) through weaning	↓ percentage of splenic T regulatory cells in offspring (age - at least 6 weeks old)	0.2 mg/kg/day/---- (see Comments)	2 mg/kg/day/0.02 mg/kg/day (see Comments)	Not assessed	<i>Ex vivo</i> IL-10 production by splenic CD4+ T cells was ↓ at ≥ 0.02 mg/kg/day in male offspring; it was ↑ at 0.02 mg/kg/day but not other doses in females.  No effect on two serum autoantibodies. For 3rd serum autoantibodies (anti-ssDNA), ↓ at 0.02 and 2, but not 0.2 mg/kg/day, in females only. Significance of changes at 0.02 mg/kg/day are not clear.
<b>Iwai and Yamashita (2006)</b>	Male Crj:CD (SD)IGS rats	Adult	0, 0.5, 5, and 50 mg/kg/day Gavage	14 days	Percent lymphocytes in peripheral blood  Percent or number of lymphocytes subsets in peripheral blood  Absolute or relative spleen weight	50 mg/kg/day	-----	Not assessed	Relative liver weight ↑ at NOAEL for immune effects (50 mg/kg/day).
<b>Loveless et al. (2008)</b>	Male Crj:CD (SD)IGS rats	Adult	0, 0.3, 1, 10, 30 mg/kg/day	28 days Gavage	Several immune parameters	30 mg/kg/day	-----	Not assessed	Other immune effects in mice occurred at doses above LOAEL for decreased relative spleen weight.  LOAEL in mice was also 1 mg/kg/day for ↑ relative weight and necrosis in liver.  Although authors concluded immune effects are secondary to ↑ corticosterone, corticosterone was not ↑ at LOAEL for ↓ relative spleen weight.
	Male Crj:CD (SD)IGS mice				↓ relative spleen weight	0.3 mg/kg/day	1 mg/kg/day		

<i>Citation</i>	<i>Species &amp; Strain</i>	<i>Lifestage for Dosing</i>	<i>Dose(s) (mg/kg/day)</i>	<i>Dosing Duration</i>	<i>Endpoint(s)*</i>	<i>NOAEL** (mg/kg/day)</i>	<i>LOAEL** (mg/kg/day)</i>	<i>PFOA Serum Concentration (ng/ml)</i>	<i>Comments</i>
<b>Qazi et al. (2009a)</b>	Male C57BL/6 (H-2) <sup>b</sup> mice	Adult	10 or 200 ppm in diet	10 days	Numerous parameters related to immune function	10 ppm (data not shown)  Estimated as 1.5 mg/kg/day from Qazi et al. (2013).	200 ppm  Estimated as <30 mg/kg/day from Qazi et al. (2013)	Estimated serum level at 200 ppm (LOAEL) was 152,000 ng/ml (Qazi et al., 2009b)	
<b>Qazi et al. (2009b)</b>	Male C57BL/6 (H-2) <sup>b</sup> mice	Adult	200 ppm in diet	10 days	↓ relative spleen and thymus weight.  ↓ spleen and thymus cellularity.  ↓ splenocyte and thymocyte cell populations.  Histopathological changes in thymus.	-----	200 ppm  Estimated as <30 mg/kg/day from Qazi et al. (2013)	152,000 µg/L	Relative liver weight also ↑ at LOAEL.
<b>Qazi et al. (2010)</b>	Male C57BL/6 (H-2) <sup>b</sup> mice	Adult	20 ppm in diet	10 days	Several parameters of hepatic immune status	-----	20 ppm  Estimated as 3 mg/kg/day from Qazi et al. (2013).	87,600 ng/ml	↑ liver weight and histopathological changes in the liver also occurred at this dose.
<b>Qazi et al. (2012)</b>	Male C57BL/6 (H-2) <sup>b</sup> mice	Adult	10, 20, or 200 ppm in diet	10 days	↓ numbers of beta-lymphoid cells and beta-lymphoid cell subpopulations in bone marrow.	10 ppm  Estimated as 1.5 mg/kg/day from Qazi et al. (2013).	20 ppm  Estimated as 3 mg/kg/day from Qazi et al. (2013).	Estimated serum level at 20 ppm (LOAEL) was 87,600 ng/ml (Qazi et al., 2010)	Effects at 200 ppm may be secondary to decreased food consumption. Food consumption not decreased at LOAEL of 20 ppm.  ↑ relative liver weight at NOAEL for immune effects of 10 ppm.

<i>Citation</i>	<i>Species &amp; Strain</i>	<i>Lifestage for Dosing</i>	<i>Dose(s) (mg/kg/day)</i>	<i>Dosing Duration</i>	<i>Endpoint(s)*</i>	<i>NOAEL** (mg/kg/day)</i>	<i>LOAEL** (mg/kg/day)</i>	<i>PFOA Serum Concentration (ng/ml)</i>	<i>Comments</i>
<b>Qazi et al. (2013)</b>	Male C57BL/6 (H-2) <sup>b</sup> mice	Adult	0.5 or 20 ppm in diet  0.07 or 3 mg/kg/day	10 days (high dose)  28 days (low dose)	↑ Concavalin A-induced liver damage.  Changes in cytokine levels (both with and without response to Con A).	0.07 mg/kg/day (0.5 ppm) for 28 days	3 mg/kg/day (20 ppm) for 10 days	Estimated serum level at 20 ppm (LOAEL) was 87,600 ng/ml (Qazi et al., 2010)	
<b>Son et al. (2009)</b>	Male ICR mice	Adult	0.49, 2.64, 17.63, 47.21 mg/kg/day	21 days	↓ numbers of splenic T cell subpopulations (CD4-CD8+, CD4+CD8+).	---	0.49 mg/kg/day		These T cell subpopulations were not evaluated in other studies of PFOA.  ↑ liver weight at LOAEL for immune effects in this study, as reported in accompanying paper (Son et al., 2008)
<b>Yang et al. (2000)</b>	Male C57BL/6 mice	Adult	200 ppm in diet	Up to 10 days	↓ absolute and relative thymus and spleen weight beginning at 5 days.  ↓ thymocyte and splenocyte numbers, selective ↓ in immature thymocyte subpopulations, ↓ T and B cells in spleen, ↓ numbers of splenic T cell subpopulations (CD4+, CD4+CD8+), inhibition of thymocyte proliferation at 7 days (other time points not evaluated).	-----	200 ppm in diet  Estimated as <30 mg/kg/day from Qazi et al. (2013)	Estimated serum level at 200 ppm (LOAEL) was 152,000 ng/ml (Qazi et al., 2009b)	Relative liver weight also ↑ at LOAEL.

<i>Citation</i>	<i>Species &amp; Strain</i>	<i>Lifestage for Dosing</i>	<i>Dose(s) (mg/kg/day)</i>	<i>Dosing Duration</i>	<i>Endpoint(s)*</i>	<i>NOAEL** (mg/kg/day)</i>	<i>LOAEL** (mg/kg/day)</i>	<i>PFOA Serum Concentration (ng/ml)</i>	<i>Comments</i>
Yang et al. (2002a)	Male C57BL/6 mice	Adult	200 ppm in diet	10 or 16 days	↓ IgM and IgG response to horse red blood cell (HRBC) immunization.	-----	200 ppm in diet  Estimated as <30 mg/kg/day from Qazi et al. (2013)	Estimated serum level at 200 ppm (LOAEL) was 152,000 ng/ml (Qazi et al., 2009b)	
Yang et al. (2002b)	Male C57BL/6 mice (wild type)	Adult	200 ppm in diet	7 days	<i>C57BL/6 wild type:</i> ↓ spleen and thymus weight. ↓ # of splenocytes and thymocytes. ↓ CD4+CD8+ thymocytes. ↓ T and B cells in spleen. ↓ % of proliferating thymocytes. ↓ proliferation of splenocytes ex vivo in response to proliferative stimulation.		200 ppm in diet  Estimated as <30 mg/kg/day from Qazi et al. (2013)		Comparison of wild type versus PPAR-alpha null mice was between two different strains. Strain differences unrelated to PPAR-alpha status may have affected the results.  Similar increase in relative liver weight occurred in both wild type C57BL/6 and Sv/129 PPAR-alpha null mice. Peroxisome proliferation only increased in wild type mice.  Effects of PFOA on thymus occurred in PPAR-alpha null mice, to a lesser degree than in wild type mice. WY also caused these effects in PPAR-alpha null mice.  Effects of PFOA on spleen seen in wild type mice did not occur in PPAR-alpha null mice.
	Male Sv/129 PPAR-alpha null mice				<i>Sv/129 PPAR-alpha null:</i> ↓ thymus weight and number of thymocytes (attenuated). ↓ CD4+CD8+ thymocytes (attenuated). ↓ % of proliferating thymocytes (attenuated).				

\* IgG and IgM evaluations were based response to injection with sheep or horse red blood cells (RBC). In some studies, additional endpoints that are not shown in the table were evaluated; data for the most sensitive immune-related endpoint(s) are shown.

\*\* NOAELs are defined as the highest dose that did not produce a statistically significant (p<0.05) effect, and LOAELs are defined as the lowest doses with statistically significant (p<0.05) effects.

Table 12: Summary of studies of effects of gestational/lactational exposure to PFOA in mice (most sensitive effect(s) in each study are shown in *red italics*)\*

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring: Fetal through Weaning			Effects in Offspring: Post-weaning			Comments
Citation	Strain	Administered dose(s) and routes (mg/kg/day)	Duration	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	
Abbott et al. (2007)	129S1/SvImJ wild type	0, 0.1, 0.3, 0.6, 1, 5, 10, or 20  Gavage	GD 1-17	<ul style="list-style-type: none"> <li>• Maternal weight gain</li> <li>• % FLR</li> <li>• Implants/litter</li> <li>• % litter loss (includes FLR)</li> <li>• Pups/litter (live + dead; excludes FLR)</li> <li>• <b>↑ relative liver weight on PND 22.</b></li> </ul>	10 1 20 0.3 1 0.6	-- 5 -- 0.6 -- 1	<ul style="list-style-type: none"> <li>• Body weight at birth</li> <li>• Eye opening delay</li> <li>• Postnatal mortality</li> <li>• Neonatal growth</li> <li>• <b>↑ relative liver weight on PND 22.</b></li> </ul>	1 0.6 0.3 0.6 --	-- 1 0.6 1 <b>0.1</b>	<ul style="list-style-type: none"> <li>• Body weight at week 28 (M) &amp; week 52 (F)</li> </ul>	1	--	<ul style="list-style-type: none"> <li>• No wild type live pups at ≥ 5 mg/kg/day.</li> <li>• FLR is PPAR-alpha independent.</li> <li>• ↑ pup liver weight is most sensitive endpoint in both strains.</li> </ul>
	129S1/SvImJ PPAR-α null	0, 0.1, 0.3, 1, 3, 5, 10, or 20  Gavage	GD 1-17	<ul style="list-style-type: none"> <li>• Maternal weight gain</li> <li>• % FLR</li> <li>• Implants/litter</li> <li>• % litter loss (includes FLR)</li> <li>• Pups/litter (live + dead; excludes FLR)</li> <li>• <b>↑ relative liver weight on PND 22.</b></li> </ul>	20 3 20 3 1 1	-- 5 -- 5 -- <b>3</b>	<ul style="list-style-type: none"> <li>• Body weight at birth</li> <li>• Eye opening</li> <li>• Postnatal mortality</li> <li>• Neonatal growth</li> <li>• <b>↑ relative liver weight on PND 22.</b></li> </ul>	20 3 3 3 1	-- -- -- -- <b>3</b>	<ul style="list-style-type: none"> <li>• Body weight at week 28 (M) &amp; week 52 (F)</li> </ul>	3	--	

Citation and Study Design				Maternal/Reproductive Effects			Effects in Offspring: Fetal through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	Strain	Administered dose(s) and routes (mg/kg/day)	Duration	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	
Albrecht et al. (2013)	Sv/129 wild- type	0 or 3  Gavage	GD 1-17	• Maternal weight gain	3	--	• Body weight and crown-to-rump length on GD 18	3	--	NA	NA	NA	<ul style="list-style-type: none"> <li>• Issues related to this study are discussed in text and individual study table in Appendix 5.</li> <li>• No significant effect for any developmental endpoint in any strain. Not consistent with effects reported in Abbott et al. (2007).</li> <li>• ↑ maternal and pup liver weight is the most sensitive endpoint in all strains.</li> </ul>
	• Day of parturition			3	--	• Postnatal mortality	3	--					
	• Implants/litter			3	--	• Postnatal growth	3	--					
• % litter loss	3	--	• Eye opening	3	--								
• Live fetuses/litter	3	--	• ↑ <b>relative liver weight on GD 18</b>	--	<b>3</b>								
• Male/female ratio	3	--	• ↑ <b>relative liver weight on PND 20</b>	--	<b>3</b>								
• ↑ <b>relative liver weight on GD 18</b>	--	<b>3</b>											
• ↑ <b>relative liver weight on PND 20</b>	--	<b>3</b>											
• <b>MG on PND 20</b>	3	--											
	Sv/129 PPAR- alpha null			• Maternal weight gain	3	--	• Body weight & crown-to-rump length on GD 18	3	--	NA	NA	NA	
				• Day of parturition	3	--	• Postnatal mortality	3	--				
				• Implants/litter	3	--	• Postnatal growth	3	--				
				• % litter loss	3	--	• Eye opening delay	3	--				
				• Live fetuses/litter	3	--	• ↑ <b>relative liver weight on GD 18</b>	3	--				
				• Male/female ratio	3	--	• ↑ <b>relative liver weight on PND 20</b>	3	--				
				• ↑ <b>relative liver weight on GD 18</b>	--	<b>3</b>	• <b>MG on PND 20</b>	3	--				
				• ↑ <b>relative liver weight on PND 20</b>	3	--							
	Sv/129 Human- ized PPAR- alpha			• Maternal weight gain	3	--	• Body weight & crown-to-rump length on GD 18	3	--	NA	NA	NA	
				• Day of parturition	3	--	• Postnatal mortality	3	--				
				• Implants/litter	3	--	• Postnatal growth	3	--				
				• % litter loss	3	--	• Eye opening	3	--				
				• Live fetuses/litter	3	--	• ↑ <b>relative liver weight on GD 18</b>	--	<b>3</b>				
				• Male/female ratio	--	<b>3</b>	• ↑ <b>relative liver weight on PND 20</b>	3	--				
				• ↑ <b>relative liver weight on GD 18</b>	--	<b>3</b>	• <b>MG on PND 20</b>	3	--				
				• ↑ <b>relative liver weight on PND 20</b>	3	--							

MG – delayed mammary gland development.

Citation and Study Design				Maternal/Reproductive Effects			Effects in Offspring: Fetal through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
<i>Citation</i>	<i>Strain</i>	<i>Administered dose(s) and routes (mg/kg/day)</i>	<i>Duration</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	
Fenton et al. (2009)	CD-1	0, 0.1, 1, or 5 Gavage	GD 17 Single dose	<ul style="list-style-type: none"> <li>• Body weight on GD 17 and 18, and PND 1-18</li> <li>• <b>↑ relative liver weight on GD 18, PND 1,4, 8, 18</b></li> </ul>	5 5	-- --	Offspring body weight on GD 18 and PND 1-18	5	--	NA	NA	NA	<ul style="list-style-type: none"> <li>• This was primarily a pharmacokinetic study and used a single dose.</li> </ul>
Hines et al. (2009)	CD-1	0, 0.01, 0.1, 0.3, 1, 3 or 5	GD 1-17	<ul style="list-style-type: none"> <li>• Live pups/litter</li> </ul>	NA 5	NA --	<ul style="list-style-type: none"> <li>• ↓ Body weight at PND 1</li> <li>• ↓ Body weight at PND 22</li> </ul>	1 0.3	5 1	<ul style="list-style-type: none"> <li>• ↑ Body weight at 20-29 wks***</li> <li>• ↓ Body weight at 20-29 wks</li> <li>• ↑ insulin &amp; leptin at 21-33 wks*** (Only females evaluated)</li> </ul>	--- 1 ---	<b>0.01</b> <b>0.01</b>	<ul style="list-style-type: none"> <li>• Effects at 20-29 weeks on body weight (0.01-0.3 mg/kg/day) and hormones (0.01-0.1 mg/kg/day) occurred at low doses but not higher doses.</li> <li>• Mortality occurred in all groups including controls after 36 weeks.</li> <li>• Effects at 18 months are not in this table.</li> </ul>
Hu et al. (2010)	C57BL/6N	0, 0.5, 1 Drinking water	GD 6-17	<ul style="list-style-type: none"> <li>• Maternal body weight</li> <li>• M/F ratio</li> </ul>	1 1	-- --	<ul style="list-style-type: none"> <li>• ↓ Body weight at PND 2</li> <li>• ↓ Body weight at PND 7 &amp; 14</li> <li>• <b>↑ relative liver weight on GD 18</b></li> </ul>	-- 0.5 1	<b>0.5</b> 1 --	<ul style="list-style-type: none"> <li>• IgM on PND 48</li> <li>• IgG on PND 63</li> <li>• Spleen, adrenal, thymus weight, PND 48 &amp; 63</li> <li>• <b>↑ relative liver weight on PND 48 &amp; 63</b></li> </ul>	1 1 1 1	-- -- -- --	See also summary table of studies of immune effects (Table 11).
Hu et al. (2012)	C57BL/6N	0.02, 0.2 or 2 Gavage	Pre-mating – PND 21; (mean 12.9 days pre-pregnancy)	<ul style="list-style-type: none"> <li>• Maternal body weight (through weaning)</li> <li>• Pups/litter</li> <li>• M/F ratio</li> </ul>	2 2 2	-- -- --	<ul style="list-style-type: none"> <li>• ↓ Body weight at PND 1 - 21</li> </ul>	0.2	<b>2</b>	<ul style="list-style-type: none"> <li>• Body weight at 6 weeks.</li> <li>• ↓ T cells in spleen at ~ 6 wks (Only females evaluated).</li> </ul>	2 0.2	-- <b>2</b>	<ul style="list-style-type: none"> <li>• <i>Ex vivo</i> and inconsistent <i>in vivo</i> immune effects observed at 0.02 mg/kg/day; see summary table for immune effects (Table 11).</li> </ul>

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight)			Effects in Offspring: Post-weaning			Comments
Citation	Strain	Administered dose(s) and routes (mg/kg/day)	Duration	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	
Lau et al. (2006)	CD-1	0, 1, 3, 5, 10, 20, or 40  Gavage	GD 1-17 (sacrificed GD 18)  GD 1-18 (allowed to deliver)	<ul style="list-style-type: none"> <li>• ↓ Maternal weight gain</li> <li>• FLR</li> <li>• # of implants</li> <li>• Live fetuses/litter</li> <li>• Prenatal loss/live litter</li> <li>• ↑ <b>relative liver weight on GD 18</b></li> </ul>	10 3 -- 10 10 --	20 5 40 20 20 <b>1</b>	<ul style="list-style-type: none"> <li>• ↓ fetal body weight</li> <li>• ↑ reduced ossification (several sites)</li> <li>• ↑ Limb &amp; tail defects</li> <li>• ↑ Micro-cardia</li> <li>• ↑ Postnatal mortality</li> <li>• ↓ Postnatal growth</li> <li>• Delayed eye opening</li> </ul>	10 -- 3 10 3 1 3	20 <b>1<sup>a</sup></b> 5 <sup>a</sup> 10 5 3 5	<ul style="list-style-type: none"> <li>• ↓ body weight in males at 6.5 weeks (p&lt;0.05 for treatment effect)</li> <li>• Delay in vaginal opening.</li> <li>• Delay in first estrus.</li> <li>• Accelerated preputial separation.</li> </ul>	Not started 20 3 -- .	Not started 10 5 <b>1<sup>a</sup></b>	<ul style="list-style-type: none"> <li>• Reduced ossification and increased limb defects did not increase as dose increased. These effects were not significant at one or more doses above the LOAEL.</li> <li>• Accelerated preputial separation was not observed at 20 mg/kg/day.</li> <li>• Body weight in treated animals tended to be higher than in controls as animals aged (until age 60 wks).</li> <li>• <b>Maternal serum levels on GD 18 shown graphically.</b></li> <li>• This study is also summarized in liver weight tables (Table 10).</li> </ul>
Macon et al. (2011)	CD-1	0, 0.3, 1, or 3  Gavage	GD 1-17	NA	NA	NA	<ul style="list-style-type: none"> <li>• Body weight PND 7-21</li> <li>• ↑ <b>relative liver weight on PND 7</b></li> <li>• <b>MG at PND 14 &amp; 21<sup>a</sup></b></li> </ul>	3 -- --	-- <b>0.3<sup>a</sup></b> <b>0.3</b>	<ul style="list-style-type: none"> <li>• Body weight on PND 42-84</li> <li>• <b>MG at PND 42 &amp; 84<sup>a</sup></b></li> </ul>	3 --	-- <b>0.3</b>	<ul style="list-style-type: none"> <li>• LOAELs for ↑ pup liver weight were higher at later pre-weaning and post-weaning time points.</li> <li>• LOAEL for MG was higher at PND 7.</li> <li>• MG was not significant at 3 mg/kg/day on PND 63 and 84, possibly due to small n (n=2)</li> </ul>
		0, 0.01, 0.1, or 1  Gavage	GD 10-17	NA	NA	NA	<ul style="list-style-type: none"> <li>• Body weight at birth (females)</li> <li>• Body weight on PND 1-21</li> <li>• ↑ <b>relative liver weight on PND 4, 7, 14<sup>a</sup></b></li> <li>• <b>MG at PND 21</b></li> </ul>	1 1 <b>0.1</b> --	-- -- <b>1</b> <b>0.01</b>	NA	NA	NA	<ul style="list-style-type: none"> <li>• <b>Pup serum levels measured on PND 1 (end of dosing).</b></li> <li>• Increased relative liver weight not significant on PND 1 and 21.</li> <li>• Delayed mammary gland development more sensitive endpoint than increased relative liver weight in offspring.</li> </ul>

<sup>a</sup> See comments.

Citation and Study Design				Maternal/Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
<i>Citation</i>	<i>Strain</i>	<i>Administered dose(s) and routes (mg/kg/day)</i>	<i>Duration</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	
Suh et al. (2011)	CD-1	0, 2, 10 or 25 Gavage	GD 11-16	↓ Maternal body weight on GD 13-16  <i>Endpoints assessed on GD 16.</i> • ↓ placenta weight • Necrotic changes in placenta <sup>a</sup> • ↓ levels of gene expression for three placental lactogens (PRL family) and two pituitary-specific positive transcription factor 1 isoforms. • ↓ # of placental trophoblast cells • ↓ fetal weight • ↓ fetal/ placental weight ratio • # of implantations • ↑ # of resorptions/dead fetus • ↓ # of live fetus	10 <sup>a</sup>  -- 2 --  10 2 -- 25 -- -- 2	25 <sup>a</sup>           2 10       2 10	NA	NA	NA	NA	NA	NA	<ul style="list-style-type: none"> <li>• Doses at which maternal body weight ↓ are not stated, although significance for trend is shown on various GDs. NOAEL &amp; LOAEL in this table is based on graph of data.</li> <li>• Effects on placenta and production of placental factors may contribute to decreased fetal growth.</li> <li>• Fetal/placental ratio indicates efficiency of placenta.</li> <li>• Levels of placental lactogens significantly correlate with fetal weight on individual basis.</li> <li>• Statistical evaluation for necrotic changes in placenta is not provided; doses where this effect occurred are discussed in text.</li> <li>• NOAELs for potentially important placental effects not identified.</li> </ul>

<sup>a</sup> See comments.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	Strain	Administered dose(s) and routes (mg/kg/day)	Duration	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	
Tucker et al. (2015)	CD-1	0, 0.01, 0.1, 0.3, 1  Gavage	GD 1-17	NA	NA	NA	<ul style="list-style-type: none"> <li>• ↓ body weight on PND 21</li> <li>• ↓ body weight minus liver weight on PND 21</li> <li>• Estrogen &amp; progesterone, PND 21</li> <li>• ↑ <b>relative liver weight, PND 21</b></li> <li>• <b>MG at PND 21</b></li> </ul>	1	--	<ul style="list-style-type: none"> <li>• Body weight &amp; body weight minus liver weight on PND 35 &amp; 56</li> <li>• Day of vaginal opening</li> <li>• Day of first estrus</li> <li>• Estrogen &amp; progesterone on PND 35 &amp; 56</li> <li>• ↑ <b>relative liver weight, PND 35 &amp; 56</b></li> <li>• <b>MG at PND 35 &amp; 56</b></li> </ul>	1	--	PFOA serum data provided from PND 21 and later time points.  Higher serum levels at same PFOA dose in CD-1 mice than C57Bl/6 mice may contribute to the greater sensitivity of CD-1.
	C57Bl/6						<ul style="list-style-type: none"> <li>• ↓ body weight on PND 21</li> <li>• ↓ body weight minus liver weight on PND 21</li> <li>• Estrogen &amp; progesterone on PND 21</li> <li>• ↑ <b>relative liver weight, PND 21</b></li> <li>• <b>MG at PND 21</b></li> </ul>	1	--	<ul style="list-style-type: none"> <li>• Body weight &amp; body weight minus liver weight on PND 61</li> <li>• Day of vaginal opening</li> <li>• Day of first estrus</li> <li>• Estrogen &amp; progesterone, PND 61</li> <li>• ↑ <b>relative liver weight on PND 61</b></li> <li>• <b>MG at PND 61</b></li> </ul>	1	--	

<sup>a</sup> See comments.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
<i>Citation</i>	<i>Strain</i>	<i>Administered dose(s) and routes (mg/kg/day)</i>	<i>Duration</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	<i>Effect</i> <sup>a</sup>	<i>NOAEL</i>	<i>LOAEL</i>	
van Esterik et al. (2016)	C57Bl/6J	0, 003, 0,01, 0.1, 0.3, 1, or 3 mg/kg/day  Dietary	2 weeks before mating through weaning	<ul style="list-style-type: none"> <li>• Maternal weight gain</li> <li>• ↓ litter size</li> <li>• Male/female ratio</li> </ul>	3 <sup>a</sup>  0.3 <sup>a</sup> 3 <sup>a</sup>	--  1 <sup>a</sup> --	<ul style="list-style-type: none"> <li>• ↓ Body weight on PND 4</li> <li>• Neonatal survival</li> </ul>	--  3 <sup>a</sup>	0.003 <sup>a</sup>  --				<ul style="list-style-type: none"> <li>• Exposure regimen differed from other studies. Exposure began before mating and continued through lactation.</li> <li>• Data presented as BMD and BMDL for each endpoint. NOAELs and LOAELs in table are based on interpretation of graphs and/or discussion in text; statistical significance is not presented. Personal communication with authors indicates that study was not intended to identify LOAELs and NOAELs. Data for some endpoints (e.g. relative liver weight) not presented in usable form.</li> <li>• The LOAEL for decreased neonatal body weight appears to be much lower than in other studies. As above, personal communication with authors indicates that study was not intended to identify LOAELs and NOAELs.</li> <li>• Text states that body weight decrements persisted through lactation and until wk. 21. Doses at which this occurred not stated.</li> <li>• Later in life studies relate to mode of action and effects with high fat versus control diet.</li> </ul>

<sup>a</sup> See comments.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
<i>Citation</i>	<i>Strain</i>	<i>Administered dose(s) and routes (mg/kg/day)</i>	<i>Duration</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	<i>Effect</i>	<i>NOAEL</i>	<i>LOAEL</i>	
White et al. (2007)	CD-1	0 or 5 Gavage	GD 1-17, GD 8-17, GD 12-17  <i>Restricted exposure study****</i>	<ul style="list-style-type: none"> <li>• ↓ Maternal weight gain, GD 18</li> <li>• # of implants</li> <li>• Live fetuses/litter</li> <li>• % prenatal loss/live litter</li> </ul>	GD 1-17 GD 1-17 GD 1-17 GD 8-17	-- -- -- GD 1-17	<ul style="list-style-type: none"> <li>• ↓ Body weight on PND 1, 5, 10, 20</li> <li>• <b>MG on PND 21</b></li> </ul>	-- --	<b>GD 12-17</b> <b>GD 12-17</b>	NA	NA	NA	
White et al. (2009)	CD-1	0 or 5 Gavage	GD 8-17  <i>Cross-foster study*****</i>	<ul style="list-style-type: none"> <li>• ↑ Maternal weight gain (GD 8-17)</li> <li>• Litter size</li> <li>• <b>↑ relative liver weight, PND 1, 3, 5, 10</b></li> </ul>	-- 5 0 dam, 5U pups	5 -- 5 dam, control pups	<ul style="list-style-type: none"> <li>• ↓ Body weight on PND 1, 3</li> <li>• ↓ Body weight on PND 10, 20</li> <li>• <b>↑ relative liver weight, PND 1, 3</b></li> <li>• <b>↑ relative liver weight, PND 5, 10</b></li> <li>• <b>MG, PND 1, 3, 5, 10</b></li> </ul>	5L, 5U -- 5L -- --	5U+L <b>5L, 5U</b> 5U <b>5L, 5U</b> <b>5L, 5U</b>	NA	NA	NA	
White et al. (2009); Wolf et al. (2007)	CD-1	0, 3, or 5 Gavage	GD 1-17  <i>Cross-foster study*****</i>	<ul style="list-style-type: none"> <li>• ↑ Maternal wt. gain, GD 17</li> <li>• FLR</li> <li>• # of implants</li> <li>• Live fetuses/litter</li> <li>• Prenatal loss/live litter</li> <li>• <b>↑ relative liver wt., PND 22</b></li> <li>• <b>MG at PND 22</b></li> </ul>	-- 3 5 5 5 5 -- 3L	3 5 -- -- -- 3 3U	<ul style="list-style-type: none"> <li>• ↓ Body weight at birth</li> <li>• ↓ Body weight on PND 22 (males)</li> <li>• ↓ Body weight on PND 22 (females)</li> <li>• Delayed eye opening</li> <li>• ↓ survival to PND 22</li> <li>• <b>↑ relative liver weight, PND 22</b></li> <li>• <b>MG at PND 22</b></li> </ul>	3 3U, 5L 3U, 3L 3U, 5L 3U+L, 5U, 5L -- 3L	5 3U+L, 5U 3U+L, 5U, 5L 3U+L, 5U 5U+L <b>3L, 3U</b> 3U	<ul style="list-style-type: none"> <li>• ↓ Body weight until PND 36 (M)</li> <li>• ↓ Body weight until PND 85 (F)</li> <li>• <b>MG at PND 42, 63</b></li> </ul>	3U+L, 5U, 5L 3U+L, 5L --	5U+L 5U <b>3L, 3U</b>	Body weight of male 3U group was increased from PND 85 (week 12) to week 35.

U = exposure *in utero* only; L = exposure through lactation only; U + L = exposure both *in utero* and through lactation.

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring: Fetal through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	Strain	Administered dose(s) and routes (mg/kg/day)	Duration	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	
Wolf et al. (2007)  <i>(See White et al., 2009 for additional study from this publication)</i>	CD-1	0  5; GD 15-17, 13-17, 10-17, or 7-17.  20; GD 15-17 <i>(Post-natal endpoints were not assessed at this dose due to decreased post-natal survival.)</i>  Gavage	GD 15-17, GD 13-17, GD 10-17, GD 7-17  <i>Restricted exposure study****</i>	<ul style="list-style-type: none"> <li>• ↑ Maternal weight gain</li> <li>• # of implants</li> <li>• Pups/litter</li> <li>• % prenatal loss</li> </ul>	5, GD 10-17  5, GD 7-17  5, GD 7-17  5, GD 7-17	5, GD 13-17  --  --  --	<ul style="list-style-type: none"> <li>• ↓ Body weight at birth (males)</li> <li>• ↓ Body weight at birth (females)</li> <li>• ↓ survival until PND 22</li> <li>• Delayed eye opening</li> <li>• ↓ Body weight at PND 22</li> </ul>	5, GD 13-17  20, GD 15-17; 5, GD 7-17  5, GD 7-17  5, GD 13-17  --	20, GD 15-17; 5, GD 10-17  --  20, GD 15-17  5, GD 10-17  <b>5, GD 15-17</b>	<ul style="list-style-type: none"> <li>• ↓ Body weight until age 10-11 weeks (males)</li> </ul>	5, GD 13-17	5, GD 10-17	Body weight of 5 mg/kg/day females exposed on GD 13-17 was increased above controls after PND 161 (week 23).
White, et al. (2011b)	CD-1	0, 1 or 5 (gavage)  0 or 1 (gavage) plus 5 ppb (5000 ng/L) in drinking water  .	GD 1-17 (P0) for gavage exposures.  GD 1 (P0) – PND 63 (F2) for drinking water exposures  <i>Multi-generation study. (See comments.)</i>	<ul style="list-style-type: none"> <li>• ↓ Maternal weight gain</li> <li>• Implants/litter</li> <li>• Live fetus/litter</li> <li>• Prenatal loss</li> </ul>	5  5  1  1	--  --  5  5	<ul style="list-style-type: none"> <li>• ↓ Body weight at PND 22</li> <li>• ↓ Survival until PND 22</li> <li>• ↑ <b>relative liver weight, PND 22</b></li> <li>• <b>MG at PND 22</b></li> </ul>	5  --  --  --	--  5  1 (not significant at 1 + 5 ppb in drinking water)  <b>5 ppb in drinking water; 1 mg/kg/day</b>	<ul style="list-style-type: none"> <li>• ↓ Body weight at PND 42</li> <li>• ↓ Body weight at PND 63</li> <li>• ↑ <b>relative liver weight, PND 42</b></li> <li>• <b>MG at PND 42, 63</b></li> </ul>	1  5  1  --	5  --  5  <b>5 ppb in drinking water; 1 mg/kg/Day</b>	<p>Only effects for P0 dams and F1 offspring presented here.</p> <p>See Table 14 and individual study table in Appendix 5 for additional information on this study.</p>

Citation and Study Design				Maternal/ Reproductive Effects			Effects in Offspring: Fetal through Weaning (including fetal body weight and malformations)			Effects in Offspring: Post-weaning			Comments
Citation	Strain	Administered dose(s) and routes (mg/kg/day)	Duration	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	Effect	NOAEL	LOAEL	
Yahia et al. (2010)	ICR	0, 1, 5, 10 Gavage	GD 0-17 (assessed on GD 18) GD 0-18 (postnatal assessment)	<ul style="list-style-type: none"> <li>• ↓ Maternal weight gain</li> <li>• ↑ Relative kidney weight</li> <li>• ↓ absolute brain weight</li> <li>• ↑ serum liver enzymes.</li> <li>• ↓ serum lipids</li> <li>• ↑ <b>relative liver weight, PND 22.</b></li> </ul>	1 -- 5 5 5 --	5 1 10 10 10 <b>I</b>	<ul style="list-style-type: none"> <li>• ↓ fetal GD 18 body weight &amp; birth weight</li> <li>• ↓ survival until PND 4</li> <li>• ↑ cleft sternum, delayed ossification of phalanges, delayed incisor eruption</li> </ul>	1 1 1 5	5 5 5 10	NA	NA	NA	NOAEL for delayed ossification is higher than LOAEL (1 mg/kg/day) for this effect in Lau et al. (2006). All pups died by PND 4 at LOAEL for delayed ossification (10 mg/kg/day).

\* General notes:

NOAEL is defined as the highest dose that did not produce a statistically significant effect ( $p \leq 0.05$ ) compared to control. LOAEL is defined as the lowest dose with statistically significant effects observed ( $p \leq 0.05$ ) compared to control. For some studies, there were dose-related trends that included non-statistically significant changes at lower doses.

- In some studies, not all dose groups were evaluated for some endpoints. For some studies, not all endpoints evaluated are listed.
- Several studies that focused on other specific endpoints also evaluated reproductive and/or developmental endpoints but did not present data for these endpoints in usable form, or did not provide statistical significance. These studies are mentioned in the text but are not included in this table.
- Serum data are useful for dose-response modeling only when measured at end of dosing period. Serum data in many studies were from later time points; these data provide useful pharmacokinetic information but are not appropriate for dose-response modeling.

\*\* See Table 14 and individual study tables in Appendix 5 for more details on studies in which mammary gland development was evaluated.

\*\*\* Effect occurred at low, but not high, doses.

\*\*\*\* In **restricted exposure studies**, gestational exposure was to the same dose for varying periods of time. In determining LOAELs and NOAELs, shorter time periods of dosing were considered to have been lower exposures than longer time periods of dosing.

\*\*\*\*\* In **cross foster studies**, pups were exposed *in utero* (U), through lactation (L), or both (U+L). In determining LOAELs and NOAELs, all groups to exposed lower doses (L, U, or U+L) were considered to have had lower exposure than those with higher doses. Within the same dosage level (mg/kg/day), L and U groups were considered to have had lower exposure than U+L groups.

FLR – full litter resorptions. MG – Delayed mammary gland development. NA – not assessed.

Table 13. Identification of most sensitive endpoints in mouse developmental studies of PFOA\*

Citation and Study Design				Most Sensitive Reproductive/Developmental Endpoint(s) (other than increased relative liver weight or delayed mammary gland development)	Delayed Mammary Gland Development	Increased Relative Liver Weight	
Citation	Strain	Administered dose(s) and routes (mg/kg/day)	Duration				
Abbott et al. (2007)	129S1/SvImJ wildtype	0, 0.1, 0.3, 0.6, 1, 5, 10, or 20 Gavage	GD 1-17	% litter loss (includes full litter resorption); postnatal mortality NOAEL – 0.3 LOAEL – 0.6	NA	Pup, PND 22  <b>NOAEL-ND</b> <b>LOAEL-0.1</b>	
	129S1/SvImJ PPAR-alpha null	0, 0.1, 0.3, 1, 3, 5, 10, or 20 Gavage	GD 1-17	% full litter resorptions; % litter loss (includes full litter resorption) NOAEL – 3 LOAEL – 5	NA	Maternal and pup, PND 22  <b>NOAEL - 1</b> <b>LOAEL - 3</b>	
Albrecht et al. (2013)	Sv/129 wild-type	0 or 3 Gavage	GD 1-17	All effects:  NOAEL – 3 LOAEL - ND	NOAEL – 3 LOAEL - ND	Maternal and offspring, GD 18 and PND 20  <b>NOAEL – ND</b> <b>LOAEL - 3</b>	
	Sv/129 PPAR-alpha null			All effects  NOAEL – 3 LOAEL - ND		NOAEL – 3 LOAEL - ND	Maternal, GD18, offspring GD 18 and PND 20  <b>NOAEL – ND</b> <b>LOAEL - 3</b>
	Sv/129 humanized PPAR-alpha			Male/female offspring ratio  <b>NOAEL – ND</b> <b>LOAEL - 3</b>		NOAEL – 3 LOAEL - ND	Maternal and offspring, GD 18  <b>NOAEL – ND</b> <b>LOAEL - 3</b>
Hines et al. (2009)	CD-1	0, 0.01, 0.1, 0.3, 1, 3 or 5 Gavage	GD 1-17	Increased body weight, insulin & leptin in female offspring at weeks 20-29  <b>NOAEL – ND</b> <b>LOAEL – 0.01</b>  (No effects at some higher doses)	NA	NA	
Hu et al. (2010)	C57BL/6N	0, 0.5, 1 Drinking water	GD 6-17	Decreased offspring body weight at PND 2  <b>NOAEL – ND</b> <b>LOAEL – 0.5</b>	NA	Offspring, PND 48 and 63 NOAEL – 1 LOAEL – ND (not evaluated at earlier times)	
Hu et al. (2012)	C57BL/6N	0.02, 0.2 or 2 Gavage	Pre-mating – PND 21	↓ Body weight at PND 1 – 21; ↓ T cells in spleen at ~ 6 wks  <b>NOAEL – 0.2</b> <b>LOAEL – 2</b>	NA	NA	

Citation and Study Design				Most Sensitive Reproductive/Developmental Endpoint(s) (other than increased relative liver weight or delayed mammary gland development)	Delayed Mammary Gland Development	Increased Relative Liver Weight
Citation	Strain	Administered dose(s) and routes (mg/kg/day)	Duration			
Lau et al. (2006)	CD-1	0, 1, 3, 5, 10, 20, or 40  Gavage	GD 1-17 or GD 1-18	Reduced ossification (not significant at some doses >LOAEL); accelerated preputial separation (did not occur at 20 mg/kg/day)  <b>NOAEL – ND</b> <b>LOAEL – 1</b>	NA	Maternal, GD 18  <b>NOAEL – ND</b> <b>LOAEL – 1</b>
Macon et al. (2011)	CD-1	0, 0.3, 1, or 3  Gavage	GD 1-17	Offspring body weight, PND 7-84  NOAEL – 3 LOAEL – ND	Offspring, PND 21  <b>NOAEL – ND</b> <b>LOAEL – 0.3</b>	Offspring, PND 7  <b>NOAEL – ND</b> <b>LOAEL – 0.3</b>
		0, 0.01, 0.1, or 1  Gavage	GD 10-17	Body weight, birth – PND 21  NOAEL – 1 LOAEL – ND		Offspring, PND 4,7, 14  NOAEL – 0.1 LOAEL – 1
Suh et al. (2011)	CD-1	0, 2, 10 or 25  Gavage	GD 11-16	Decreased placenta weight; decreased gene expression for placental factors; decreased fetal/ placental weight ratio; increased number of resorptions/dead fetuses  NOAEL – ND LOAEL – 2	NA	NA
Tucker et al. (2015)	CD-1	0, 0.01, 0.1, 0.3, 1  Gavage	GD 1-17	Decreased body weight minus liver weight, PND 21  NOAEL – 0.3 LOAEL – 1	Offspring, PND 35 and 56  <b>NOAEL – ND</b> <b>LOAEL – 0.01</b>	Offspring, PND 21  NOAEL – 0.3 LOAEL – 1
	C57Bl/6			All effects evaluated:  NOAEL – 1 LOAEL – ND		
van Esterik et al. (2016)	C57Bl/6 J	0, 0.03, 0.01, 0.1, 0.3, 1, or 3 mg/kg/day  Dietary	2 weeks before mating through weaning	Decreased body weight on PND 4  <b>NOAEL – ND</b> <b>LOAEL – 0.003</b>	NA	NA
White et al. (2007)	CD-1	0 or 5  Gavage	GD 1-17, GD 8-17, or GD 12-17  <i>Restricted exposure study**</i>	% prenatal loss per live litter  NOAEL – 5 mg/kg, GD 8-17 LOAEL – 5 mg/kg, GD 1 – 17	Offspring, PND 10, 20  <b>NOAEL – ND</b> <b>LOAEL - 5 mg/kg, GD 12-17</b>	NA

Citation and Study Design				Most Sensitive Reproductive/Developmental Endpoint(s) (other than increased relative liver weight or delayed mammary gland development)	Delayed Mammary Gland Development	Increased Relative Liver Weight
Citation	Strain	Administered dose(s) and routes (mg/kg/day)	Duration			
White et al. (2009)	CD-1	0 or 5  Gavage	GD 8-17  <i>Cross-foster Study***</i>	Decreased body weight on PND 10, 20  <b>NOAEL – ND</b> <b>LOAEL – 5L, 5U</b>	Offspring, PND 1-10  <b>NOAEL – ND</b> <b>LOAEL – 5L, 5U</b>	Offspring, PND 5-10  <b>NOAEL – ND</b> <b>LOAEL – 5L, 5U</b>
White et al. (2009); Wolf et al. (2007)	CD-1	0, 3, or 5  Gavage	GD 1-17  <i>Cross-foster Study***</i>	Decreased body weight on PND 22  NOAEL – 3U, 3L LOAEL – 3 U+L, 5L, 5U	Offspring, PND 42, 63  <b>NOAEL – ND</b> <b>LOAEL – 3L, 3U</b>	Offspring, PND 22  <b>NOAEL – ND</b> <b>LOAEL – 3L, 3U</b>
White, et al. (2011b)  <i>Multi-generation study – only P0 dam and F1 offspring data shown here</i>	CD-1	0, 1 or 5 (Gavage)  0 or 1 (gavage) with 5 ppb in drinking water	GD 1-17 (P0) for gavage exposures.  GD 1 (P0) – PND 63 (F2) for drinking water exposures	Increased prenatal loss; decreased live fetus/litter; decreased body weight on PND 42  NOAEL – 1 LOAEL - 5	NA	Offspring, PND 22  <b>NOAEL-ND</b> <b>LOAEL-1</b>  (Not significant at 1 mg/kg/day plus 5 ppb in drinking water)
Wolf et al. (2007)  <i>(See White et al., 2009 for other study from this publication)</i>	CD-1	0  5; GD 15-17, 13-17, 10-17, or 7-17.  20; GD 15-17  Gavage	GD 15-17, GD 13-17, GD 10-17, or GD 7-17  <i>Restricted exposure study**</i>	Increased maternal weight gain  NOAEL – 5 mg/kg, GD 13-17 LOAEL – 5 mg/kg, GD 15-17	Offspring, PND 10,20  <b>NOAEL – ND</b> <b>LOAEL – GD 15-17</b>	NA
Yahia et al. (2010)	ICR	0, 1, 5, 10  Gavage	GD 0-17 or GD 0-18	Increased maternal relative kidney weight, GD 18  <b>NOAEL – ND</b> <b>LOAEL - 1</b>	NA	Increased maternal relative liver weight, GD 18  <b>NOAEL – ND</b> <b>LOAEL - 1</b>

\* General notes:

- NOAEL is defined as the highest dose that did not produce a statistically significant effect ( $p \leq 0.05$ ) compared to control. LOAEL is defined as the lowest dose with statistically significant effects observed ( $p \leq 0.05$ ) compared to control. For some studies, there were dose-related trends that included non-statistically significant changes at lower doses.
- In some studies, not all dose groups were evaluated for some endpoints. For some studies, not all endpoints evaluated are listed.

- Several studies that focused on other specific endpoints also evaluated reproductive and/or developmental endpoints but did not present data for these endpoints in usable form, or did not provide statistical significance. These studies are mentioned in the text but are not included in this table.

\*\* In **restricted exposure studies**, gestational exposure is to the same dose for varying periods of time. In determining LOAELs and NOAELs, shorter time periods of dosing were considered to have been lower exposures than longer time periods of dosing.

\*\*\* In **cross foster studies**, pups were exposed *in utero* (U), through lactation (L), or both (U+L). In determining LOAELs and NOAELs, both all groups to exposed lower doses (L, U, U+L) were considered to have had lower exposure than those with higher doses. Within the same dosage level (mg/kg/day), L and U groups were considered to have had lower exposure than U+L groups.

FLR – full litter resorptions

NA – not assessed

ND – not determined

Table 14A. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Includes studies with exposure during pregnancy, gestation, and/or lactation (6 publications/10 studies). (Note: Other effects evaluated in these studies are discussed in other tables in main document and/or individual study tables in Appendix 5)

<b>Citation</b>	<b>Strain</b>	<b>Lifestage(s) for Dosing</b>	<b>Dose(s) (mg/kg/day)</b>	<b>Dosing Duration</b>	<b>Lifestage for MG Assessment</b>	<b>Timepoint for MG Assessment</b>	<b>Endpoint(s) Assessed</b>	<b>Effect</b>	<b>NOAEL* (mg/kg/day)</b>	<b>LOAEL* (mg/kg/day)</b>	<b>Comments/Other Mammary Gland Effects</b>
<b>Albrecht et al. (2013)</b>	Sv/129 Wild Type (KO), PPAR-alpha null (KO), and humanized PPAR-alpha (H)	Gestation	0, 3	GD 1-17	Female pups	PND 20	MG whole mounts. # terminal end buds/gland; ductal length	No effect	3	----	-This is the only study of MG gland development in this strain. -Other developmental effects seen at similar serum PFOA levels in this strain by Abbott et al. (2007) were not observed in this study. - Some control animals had elevated PFOA levels in serum and liver. - These issues create uncertainty about the conclusion that PFOA did not affect MG development in this study.
<b>Macon et al. (2011)</b>	CD-1	Gestation	0, 0.3, 1, 3	GD 1-17 "Late Gestation"	Female pups	PND 7, 14, 21, 28, 42, 63, 84	MG whole mounts, scored 1-4.	Delayed development	-----	0.3	- Delays occurred in absence of effects on body weight. - Delays persisted until end of study on PND 84 (12 weeks of age)
<b>Macon et al. (2011)</b>	CD-1	Gestation	0, 0.01, 0.1, 1	GD 10-17 "Full Gestation"	Female pups	PND 14, 21	MG whole mounts. PND 14- longitudinal growth, change in long. Growth PND 22 – Score 1-4, longitudinal and later growth, lateral growth, change in long. and lateral growth, # terminal endbuds and terminal ends.	Delayed development	----	0.01 (Developmental Score, p<0.05)	- Serum levels in offspring on PND 1 are provided and can be used for dose-response modeling. Serum levels are highest on PND 1 (end of dosing period) and decline thereafter. - The serum level at the LOAEL (0.01 mg/kg/day) in PND offspring was 285 ng/ml. - Liver weight and body weight in the pups were not affected at 0.01 mg/kg/day and higher doses, indicating that MG development is a more sensitive endpoint than decreased body weight or increased liver weight

\* NOAELs are defined as the highest dose that did not produce a statistically significant (p<0.05) effect, and LOAELs are defined as the lowest doses with statistically significant (p<0.05) effects.

Table 14A. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Includes studies with exposure during pregnancy, gestation, and/or lactation (6 publications/10 studies). (Note: Other effects evaluated in these studies are discussed in other tables in main document and/or individual study tables in Appendix 5)

<b>Citation</b>	<b>Strain</b>	<b>Lifestage(s) for Dosing</b>	<b>Dose(s) (mg/kg/day)</b>	<b>Dosing Duration</b>	<b>Lifestage for MG Assessment</b>	<b>Timepoint for MG Assessment</b>	<b>Endpoint(s) Assessed</b>	<b>Effect</b>	<b>NOAEL* (mg/kg/day)</b>	<b>LOAEL* (mg/kg/day)</b>	<b>Comments/Other Mammary Gland Effects</b>
<b>Tucker et al. (2015)</b>	CD-1 C57Bl/6	Gestation	0, 0.01, 0.1, 0.3, 1	GD 1-17	Female pups	CD-1: PND 21, 35, 56 C57Bl/6: PND 21, 61	MG whole mounts. Scored 1-4.	Delayed development	--- (CD-1) 0.1 (C57Bl/6)	0.01 (CD-1) 0.3 (C57Bl/6)	- Delayed MG development occurred in the absence of effects on body wt, liver wt, day of vaginal opening, day of first estrus, or serum estradiol and progesterone in both strains. - Serum PFOA levels were first measured on PND 21 (3 weeks after dosing ended) and therefore cannot be used for dose-response modeling. - Higher serum levels from same dose in CD-1 mice than C57Bl/6 mice may contribute to the greater sensitivity of CD-1 to effects on MG development.
<b>White et al. (2007)</b>	CD-1	Gestation	0, 5	GD 1-17	Dams	GD 18	MG whole mounts, scored 1-4	Delayed development	-----	5, GD 1-17	
<b>White et al. (2007)</b>	CD-1	Gestation	0, 5	GD 1-17, 8-17, or 12-17 "Restricted Exposure"	Dams	PND 10 and 20	MG whole mounts, scored 1-4; H&E slides also evaluated.	Delayed development	5 GD 8-17 exposure (PND 10)	5 GD 12-17 exposure (PND 10). All treated groups on PND 20.	- No effect in non-pregnant adult females dosed for 17 days. - Expression of milk proteins changed on PND 10 and 20, consistent with delays on PND 20.
					Female Pups		MG whole mounts, scored 1-4	Delayed development	-----	5, GD 8-17 exposure	

\* NOAELs are defined as the highest dose that did not produce a statistically significant (p<0.05) effect, and LOAELs are defined as the lowest doses with statistically significant (p<0.05) effects.

Table 14A. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Includes studies with exposure during pregnancy, gestation, and/or lactation (6 publications/10 studies). (Note: Other effects evaluated in these studies are discussed in other tables in main document and/or individual study tables in Appendix 5)

<i>Citation</i>	<i>Strain</i>	<i>Lifestage(s) for Dosing</i>	<i>Dose(s) (mg/kg/day)</i>	<i>Dosing Duration</i>	<i>Lifestage for MG Assessment</i>	<i>Timepoint for MG Assessment</i>	<i>Endpoint(s) Assessed</i>	<i>Effect</i>	<i>NOAEL* (mg/kg/day)</i>	<i>LOAEL* (mg/kg/day)</i>	<i>Comments/Other Mammary Gland Effects</i>
<b>White et al. (2009)</b>	CD-1	Gestation	0, 5	GD 8-17 (Cross-fostering: exposure during gestation, lactation, or both)	Dams	PND 1,3,5, 10	MG whole mounts, numerical data not shown.	Delayed development	-----	5	
					Female pups		MG whole mounts, scored 1-4	Delayed development	-----	5	
<b>White et al. (2009)</b>	CD-1	Gestation	0, 3, 5	GD 1-17 (Cross-fostering: exposure during gestation and/or lactation)	Female pups	PND 22, 42, 63, and 18 months	MG whole mounts, scored 1-4	Delayed development	-----	3	At 18 months, changes in MG persisted in treated groups (whole mounts and H&E slides) including increased number of darkly staining foci per gland and reduced epithelial density. These effects could not be scored using the criteria that were used at earlier time points.
<b>White et al. (2009)</b>	CD-1	Gestation	0, 5	GD 15-17, 13-17, 10-17, 7-17 "Restricted Exposure"	Female pups	PND 29, 32 and 18 months	MG whole mounts, scored 1-4	Delayed development	-----	5, GD 15-17 exposure.	At 18 months, changes in MG persisted in treated groups (whole mounts and H&E slides) including increased numbers of darkly staining foci per gland in all treated groups.

\* NOAELs are defined as the highest dose that did not produce a statistically significant (p<0.05) effect, and LOAELs are defined as the lowest doses with statistically significant (p<0.05) effects.

Table 14A. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Includes studies with exposure during pregnancy, gestation, and/or lactation (6 publications/10 studies). (Note: Other effects evaluated in these studies are discussed in other tables in main document and/or individual study tables in Appendix 5)

<b>Citation</b>	<b>Strain</b>	<b>Lifestage(s) for Dosing</b>	<b>Dose(s) (mg/kg/day)</b>	<b>Dosing Duration</b>	<b>Lifestage for MG Assessment</b>	<b>Timepoint for MG Assessment</b>	<b>Endpoint(s) Assessed</b>	<b>Effect</b>	<b>NOAEL* (mg/kg/day)</b>	<b>LOAEL* (mg/kg/day)</b>	<b>Comments/Other Mammary Gland Effects</b>
											These effects could not be scored using the criteria used at earlier time points
<b>White et al. (2011b)</b>	CD-1	Gestation and/or postnatal (multi-generation study)	GD 1-17 (0, 1, 5 mg/kg/day) and/or 5 ppb in drinking water (through PND 22 in P0 and F1 dams; through PND 63 in F1 and F2 pups)	P0 Dams	PND 22	MG whole mounts, scored 1-4. H&E slides for some groups.	Delayed development	----	(P0 dams)	1 mg/kg/day (exposure P0 GD 1-17) or 5 ppb in drinking water (exposure P0 GD 7- F1 PND 22)	In lactational challenge test in F1 dams and F2 litters on PND 10, milk volume was decreased and time to initiate was increased in all treated groups. However, these changes were not statistically significant. High variability limited power to detect statistically significant differences.
				F1 Dams	PND 10, 22			----	(F1 dams on PND 10).	On PND 10: 1 mg/kg/day (exposure P0 GD 1-17) or 5 ppb in drinking water (exposure P0 GD 7- F2 PND 22)	
				F1 Female pups	PND 22,42, 63			-----	(F1 pups)	1 mg/kg/day (exposure P0 GD 1-17) or 5 ppb in drinking water (exposure P0 GD 7- F1 PND 63). Serum PFOA level in 5 ppb group was 21.3 ng/ml	
				F2 Female pups	PND 10, 22, 42, 63			No significant effects at most doses and time points. Significant delays at 3 of 16 data points.		(F2 pups)	

\* NOAELs are defined as the highest dose that did not produce a statistically significant (p<0.05) effect, and LOAELs are defined as the lowest doses with statistically significant (p<0.05) effects.

Table 14B. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Studies with peripubertal exposure (3 publications)

<b>Citation</b>	<b>Strain</b>	<b>Lifestage(s) for Dosing</b>	<b>Dose(s) (mg/kg/day)</b>	<b>Dosing Duration</b>	<b>Lifestage for MG Assessment</b>	<b>Timepoint for MG Assessment</b>	<b>Endpoint(s) Assessed</b>	<b>Effect</b>	<b>NOAEL* (mg/kg/day)</b>	<b>LOAEL* (mg/kg/day)</b>	<b>Comments/Other Mammary Gland Effects</b>
<b>Yang et al. (2009a)</b>	Balb/C C57Bl/6	Peripubertal	0,1,5,10	4 weeks, starting at 3 wks of age.	Peripubertal females	7 weeks of age	MG whole mounts. Ductal length, # terminal end buds, # terminal ducts	Balb/C - Delayed development  C57Bl/6 - Stimulated development & totally inhibited development	Balb/C - 1  C57Bl/6 - 1	Balb/C - 5  C57Bl/6 - Stimulation at 5  Total inhibition at 10	MG cell proliferation measured by BrDu incorporation: - In Balb/C, proliferation of MG cells was inhibited at 1 and 5 mg/kg/day. - In C57Bl/6, proliferation of MG cells was stimulated at 1 and 5 mg/kg/day, and totally stopped at 10 mg/kg/day  Serum PFOA data provided in Zhao et al. (2012)
<b>Zhao et al. (2010)</b>	C57Bl/6 Wild Type Ovariectomized (OVX)	Peripubertal. OVX at 3 weeks (before PFOA treatment)	0, 5	4 weeks starting at 4 weeks of age	Peripubertal OVX females	8 weeks of age	MG whole mounts scored based on longitudinal growth, # of terminal end buds, stimulated/enlarged terminal ducts.	Total inhibition of development in both control and PFOA-treated OVX mice	NA	NA	Groups of non-OVX mice were not included in these studies.
	C57Bl/6 PPAR-alpha null (KO)	Peripubertal. OVX at 7 weeks (after PFOA treatment)		4 weeks starting at 3 weeks of age	Peripubertal OVX Females, treated with saline, or progesterone and/or estradiol after OVX	9 weeks of age	Numerical data not shown.	In mice treated with hormones, stimulation of development by PFOA compared to controls (no PFOA).	NA	NA	
		Peripubertal		4 weeks, starting at 3 wks of age.	Peripubertal females	7 weeks of age	Stimulated development	----	5	C57Bl/6 WT were not included in this study. Serum PFOA data provided in Zhao et al. (2012)	

\* NOAELs are defined as the highest dose that did not produce a statistically significant (p<0.05) effect, and LOAELs are defined as the lowest doses with statistically significant (p<0.05) effects.

Table 14B. Summary of publications/studies evaluating effects of PFOA on mammary gland (MG) development in mice – Studies with peripubertal exposure (3 publications))

<b>Citation</b>	<b>Strain</b>	<b>Lifestage(s) for Dosing</b>	<b>Dose(s) (mg/kg/day)</b>	<b>Dosing Duration</b>	<b>Lifestage for MG Assessment</b>	<b>Timepoint for MG Assessment</b>	<b>Endpoint(s) Assessed</b>	<b>Effect</b>	<b>NOAEL* (mg/kg/day)</b>	<b>LOAEL* (mg/kg/day)</b>	<b>Comments/Other Mammary Gland Effects</b>
<b>Zhao et al. (2012)</b>	Balb/C  C57Bl/6 (WT)  C57Bl/6 PPAR-alpha null (KO)	Peripubertal	Balb/C: 0, 2.5  C57Bl/6 WT and KO: 0, 7.5	4 weeks, starting at 3 wks of age.	Peripubertal females	7 weeks of age	MG whole mounts. Ductal length, # terminal end buds, # terminal ducts	Balb/C – Delayed development  C57Bl/6 WT – Delayed development  KO – no effect	Balb/C & C57Bl/6 WT – -----  C57Bl/6 KO - 7.5	Balb/C – 2.5  C57Bl/6 WT – 7.5  C57Bl/6 KO – -----	- PFOA doses in all 3 strains of mice in this study are different than those used in earlier studies of these strains (Yang et al., 2009a; Zhao et al., 2010). Doses from the earlier study in each strain were not repeated in this study. Data from different studies may not be directly comparable. - Lack of inhibition of MG development in KO mice may be due to lower serum PFOA levels rather than PPAR status. - Serum PFOA levels for this study and the 2 earlier studies are presented in this study.

\* NOAELs are defined as the highest dose that did not produce a statistically significant (p<0.05) effect, and LOAELs are defined as the lowest doses with statistically significant (p<0.05) effects.

## **MODE OF ACTION**

### **Overview**

The mode(s) of action of PFOA are not fully characterized. PFOA structurally resembles a free fatty acid, and it thus may act similarly to a free fatty acid in activating nuclear receptors, binding to transporters and carrier proteins, and interacting with membranes (Butenhoff, 2009). However, it is non-reactive and therefore is not a substrate for biochemical reactions involving fatty acids. It also has been shown to have estrogenic activity and to act through other mechanisms. The mode of action for some of the effects of PFOA is unknown. A summary of information on PFOA's mode of action, with emphasis on potential human relevance, is presented below.

### **Genotoxicity**

Since PFOA is not chemically reactive, it is not metabolized to reactive intermediates and does not covalently bind to nucleic acids and proteins. Therefore, it is considered unlikely to be genotoxic. Information on the genotoxicity of PFOA was reviewed by Butenhoff et al., (2014). PFOA was not mutagenic or genotoxic in most of a series of *in vitro* assays in bacterial and mammalian cells, with or without metabolic activation. It did not cause chromosomal aberrations in Chinese hamster ovary cells at non-cytotoxic concentrations, although some positive results were reported at cytotoxic concentrations. It did not cause chromosomal aberrations in human lymphocytes in whole blood with or without metabolic activation, and it did not transform cells in culture. Although PFOA caused DNA strand breaks and increased the incidence of micronuclei in cultured human hepatoma cells in a dose-related manner (Yao and Zhong, 2005), high doses of PFOA did not induce micronuclei in mice *in vivo*. In male rats, PFOA increased the levels of 8-hydroxydeoxyguanosine in liver DNA, but not in kidney DNA (Takagi et al., 1991). These effects were accompanied by a significant increase in reactive oxygen species, which the investigators suggested caused the DNA damage. In contrast, PFOA increased the formation of reactive oxygen species but was not genotoxic in *in vitro* studies by other investigators.

### **PPAR-alpha and Other Nuclear Receptors**

PFOA activates the nuclear receptor, peroxisome proliferator-activated receptor-alpha (PPAR-alpha) as well as other nuclear receptors including PPAR-gamma, CAR (constitutive activated receptor), PXR (pregnane X receptor) and estrogen receptor-alpha (reviewed by Peters and Gonzalez, 2011). When activated, nuclear receptors bind to DNA and alter the expression of genes that control many biological processes involved in development, homeostasis, and metabolism. PPAR-alpha and other nuclear receptors are found at varying levels in many tissues in rodents, humans, and other species. PPARs affect many biological processes beyond stimulation of peroxisome proliferation in rodents, the effect for which they were originally named. Levels of PPARs may vary during different stages of development, and their role in development is discussed further below.

Much attention has been focused on the role of PPAR-alpha activation in the toxicity of PFOA and on the potential human relevance of effects that occur through activation of this receptor. The

major role of PPAR-alpha is regulation of energy homeostasis. Levels of PPAR-alpha are highest in tissues with high rates of catabolism of fatty acids and peroxisomal activity, including liver, brown adipose tissue, heart, kidney, and intestine. Hepatic effects of PPAR-alpha include increased fatty acid oxidation, increased degradation of cholesterol, increased gluconeogenesis, synthesis of ketone bodies, and control of lipoprotein assembly. By increasing oxidation of fatty acids, activation of PPAR-alpha decreases serum triglycerides and serum cholesterol and prevents the accumulation of fat in the liver (Michalik et al., 2006). Consistent with PPAR-alpha's inhibition of hepatic lipid accumulation, PPAR-alpha null mice developed fatty livers, which were more severe with a high fat diet, while fatty liver did not occur in wild type mice on either a regular or high fat diet (Abdelmegeed et al., 2011). In addition to its effects on metabolism, activation of PPAR-alpha also decreases inflammatory response. PPAR-alpha activators such as fibrate drugs are used in humans to decrease serum lipids. The anti-inflammatory effects of these drugs may contribute to their actions in preventing atherosclerosis and reducing the incidence of cardiovascular events (Michalik et al., 2006).

*In vitro* activation of human and rodent PPAR-alpha by PFOA has been evaluated in several studies of cultured cells transfected with plasmids containing PPAR-alpha from these species. As noted by Vanden Heuvel et al. (2006), these *in vitro* assays measure only the first step in the series of complex steps involved in regulation of gene expression by PPAR-alpha. Therefore, interspecies comparisons based solely on these *in vitro* data may not necessarily be valid.

Relative sensitivities of human and rodent PPAR-alpha to PFOA differed among these studies, and the results do not clearly indicate that human PPAR-alpha is less sensitive than rodent PPAR-alpha in *in vitro* systems. Maloney and Waxman (1999) reported that somewhat higher concentrations of PFOA were needed to cause maximal activation of human PPAR-alpha as compared to mouse PPAR-alpha. In contrast, Vanden Heuvel et al. (2006) found that mouse and human PPAR-alpha were similarly responsive to PFOA, while rat PPAR-alpha was less responsive than mouse or human PPAR-alpha. Two studies from the same laboratory reported differing results on the responsiveness of mouse and human PPAR-alpha to PFOA. In the first study (Wolf et al., 2008), human PPAR-alpha was somewhat less responsive to PFOA than mouse PPAR-alpha. In the second study (Wolf et al., 2012), the dose-response curves for activation by PFOA were identical for mouse and human PPAR-alpha.

Many PPAR-alpha activators (e.g. phthalates, trichloroethylene, and perchloroethylene) cause liver tumors in rodents. The human relevance of these tumors is subject to debate because of the lower levels and/or differences in intrinsic activity of hepatic PPAR-alpha in humans as compared to rodents (NRC, 2006; Corton, 2010). However, the uncertainty about human relevance does not necessarily apply to PPAR-alpha mediated effects other than liver tumors, as illustrated by the use of fibrate drugs to decrease cholesterol and lipids in humans by activation of PPAR-alpha.

Furthermore, as discussed in detail below, effects of PFOA clearly occur through both PPAR-alpha independent and PPAR-alpha dependent processes. Therefore, conclusions from studies with pure PPAR-alpha activators such as Wyeth 14,643 (WY) cannot necessarily be extrapolated to PFOA.

## **Hepatic Toxicity**

As discussed in the Epidemiology section, PFOA is associated with increased serum liver enzymes in human. The studies in non-human primates, standard strains of rats and mice, PPAR-alpha null mice, and humanized PPAR-alpha mice that are summarized below support the conclusion that hepatic effects of PFOA in experimental animals are relevant to humans for the purposes of risk assessment.

### **Data from non-human primates**

In the 13-week study in male cynomolgus monkeys (Butenhoff et al., 2002), PFOA increased relative liver weight in a dose-related fashion. Several monkeys that did not complete the study due to overt toxicity exhibited notable increases in liver weight, highly elevated serum liver enzymes, and/or severe hepatic toxicity.

Subcellular biochemical markers were evaluated in the livers of the monkeys that completed the study; animals that had been removed due to toxicity were not included. Hepatic DNA content was decreased by PFOA (statistically significant at highest dose); a marker of peroxisome proliferation (palmitoyl CoA oxidation; PCO) was increased in a dose-related manner (2.6-fold and statistically significant at highest dose; Figure 12); and the mitochondrial enzyme, succinate dehydrogenase, was increased although not in a dose related manner (statistically significant at highest dose). Hepatic alkaline phosphatase (a lysosomal marker) and glucose-6-dehydrogenase (a marker for endoplasmic reticulum) were not affected by PFOA treatment.

Although no histopathological changes were reported in the livers of the treated monkeys that completed the study, the authors state that the dose-related decrease in hepatic DNA content is indicative of hepatocellular hypertrophy. The dose-related increase in peroxisome proliferating activity (PCO) was similar in magnitude to the increases reported in PFOA treated rats (below), demonstrating that substantial hepatic PPAR-alpha activity occurs in response to PFOA in non-human primates. The authors also state that the increased succinate dehydrogenase activity suggests mitochondrial proliferation, although this possibility was not evaluated by microscopic studies.

As discussed below, data from rats (Perkins et al., 2004) indicate that the increase in PCO activity in response to PFOA becomes smaller in magnitude with longer exposure duration, while the increase in relative liver weight remains stable over time. Since PCO data are available only from a single time point (13 weeks) in the monkeys, it is unknown whether they represent the maximum PCO activity in response to PFOA. The USEPA SAB (2006) stated: "Because the available data for PFOA in rats and monkeys indicate similar responses in the livers of rodents and primates (increased liver weight and induction of hepatic peroxisomal enzyme activity), about three quarters of the Panel members shared the view that human relevance for liver effects induced by PFOA by a PPAR $\alpha$  agonism mode of action cannot be discounted."

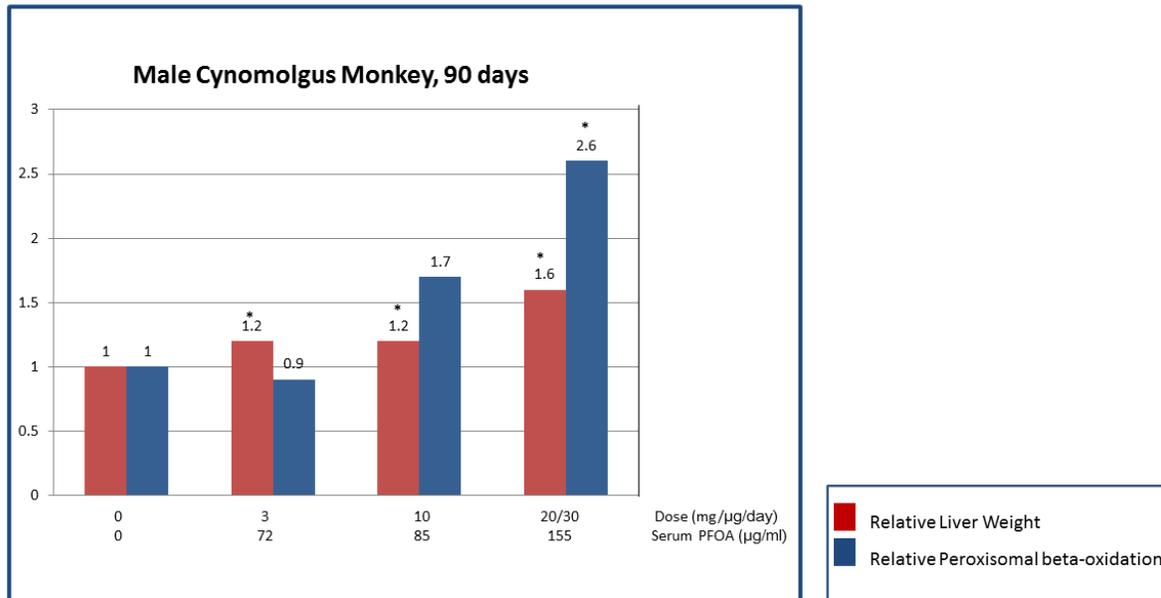


Figure 12. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male cynomolgus monkeys dosed with PFOA for 6 months (Butenhoff et al., 2002).

Data from a 90-day study of rhesus monkeys (Goldenthal, 1978) also suggest that PFOA caused increased liver weight, although this effect was not statistically significant likely due to the small number of animals.

#### Data from standard strains of laboratory rodents

In a standard strain of laboratory rats, PFOA activated PPAR-alpha, as indicated by increased PCO activity, and also activated other nuclear receptors, CAR and PXR, as indicated by induction of specific cytochrome P450 proteins associated with these receptors (Elcombe et al., 2010).

Similarly, PFOA treatment increased gene expression for cytochrome P450 proteins associated with each of these three receptors in wild type mice (Cheng and Klaasen, 2008).

Loveless et al. (2006) evaluated the effects of three isomeric forms of PFOA (linear isomers, branched isomers, and mixed linear/branched isomers) on relative liver weight and hepatic PCO activity in standard strains of male rats and mice. As can be seen in Figure 13, increased relative liver weight did not correlate with hepatic peroxisome proliferation, as indicated by PCO activity. In mice, liver weight increased with administered dose and serum PFOA level, but PCO activity was lower at the highest administered doses and serum levels than at lower doses and serum levels. Additionally, in rats (Figure 14), branched isomers of PFOA were more potent in increasing relative liver weight than linear isomers, but were less potent in increasing PCO activity. These results illustrate the involvement of PPAR-alpha independent processes in the increased relative liver weight caused by PFOA even in standard strains of rodents with normal PPAR-alpha function.

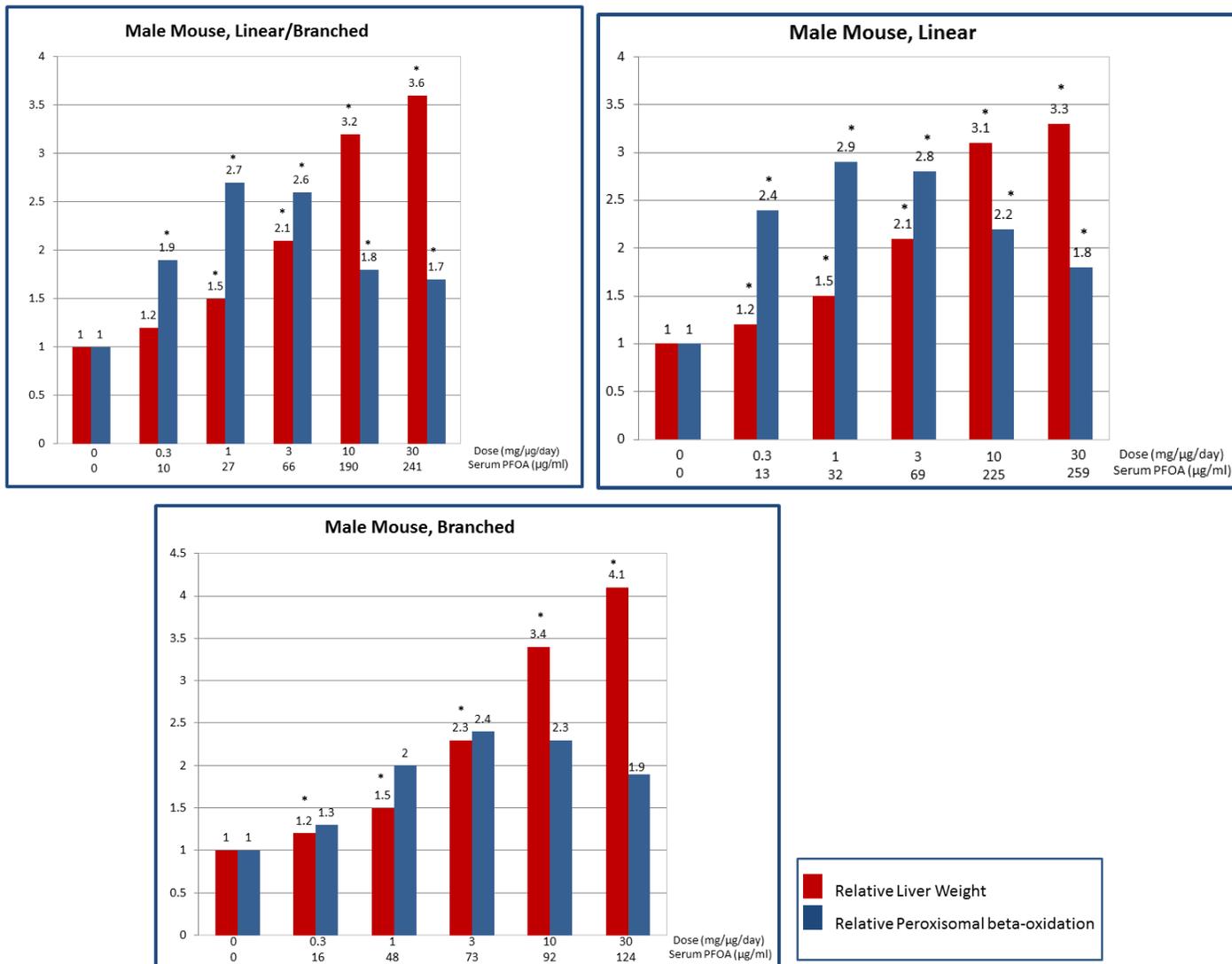


Figure 13. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male mice dosed with linear/branched, linear, or branched PFOA for 14 days (Loveless et al., 2006).  
 \*  $p < 0.05$ .

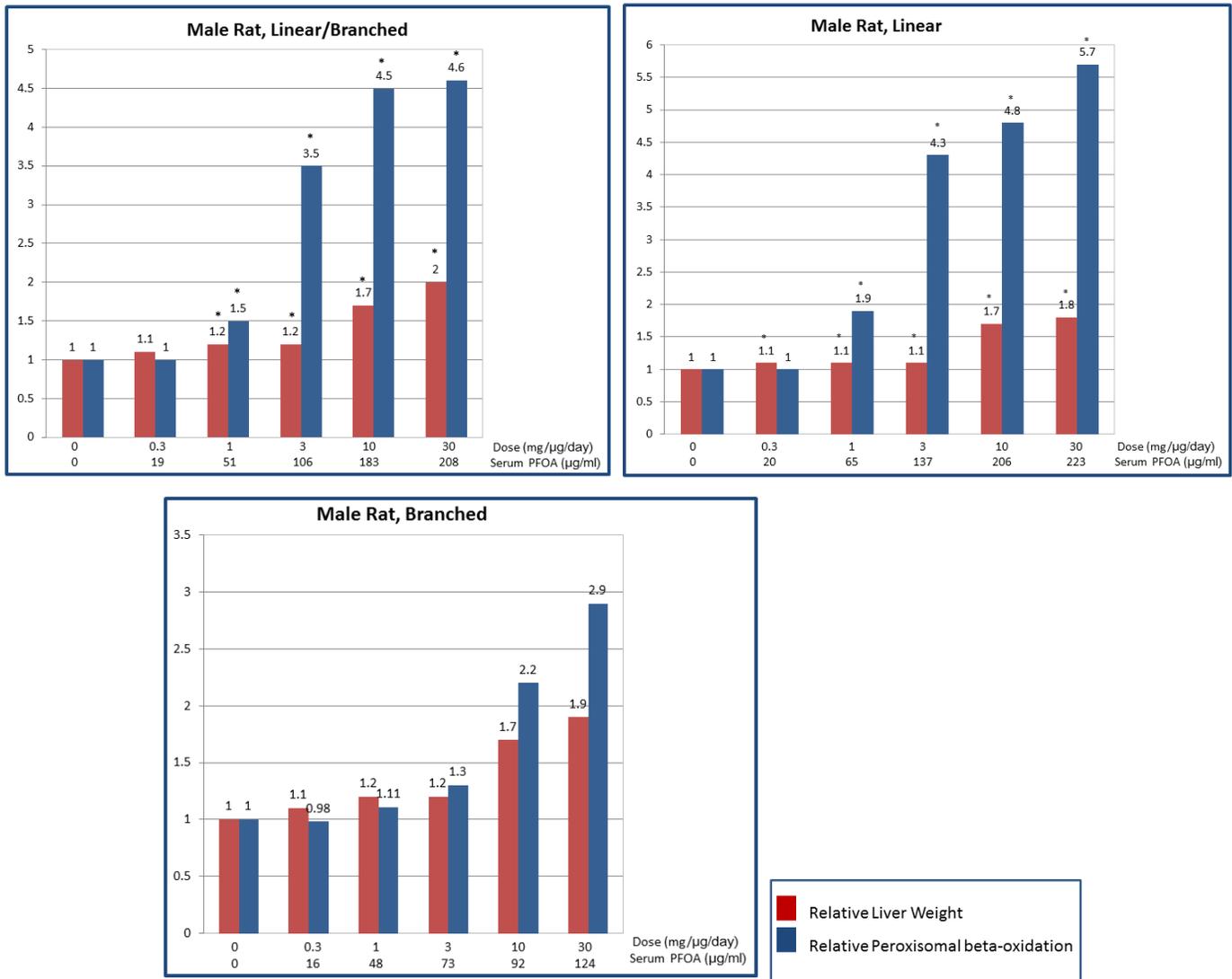


Figure 14. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male rats dosed with linear/branched, linear, or branched PFOA for 14 days (Loveless et al., 2006).  
 \*  $p < 0.05$ .

Data from a standard strain of male rats exposed for 4, 7, or 13 weeks (Figure 15) also indicate that PCO activity does not necessarily correlate with the increased liver weight caused by PFOA (Perkins et al., 2004). Although relative PCO activity was much greater at 7 weeks than 4 weeks, and was lower at 13 weeks than at the two earlier time points, the dose-response curve for increased relative liver weight was similar at all three time points.

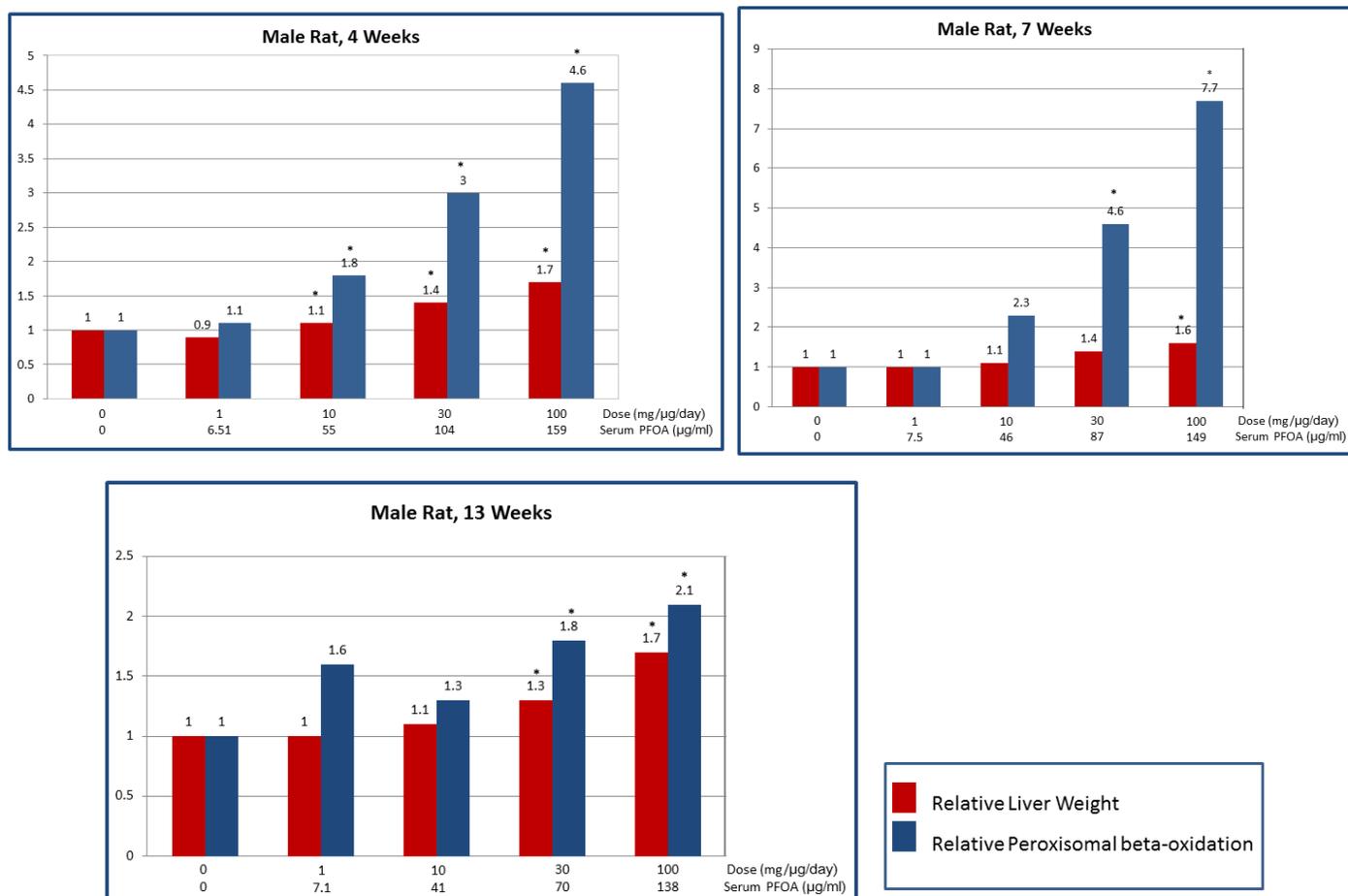


Figure 15. Relative liver weight and relative PCO activity (compared to controls) versus serum PFOA concentrations in male rats and mice dosed with PFOA for 4, 7, or 13 weeks (Perkins et al., 2004).

A recent study of histopathological change in livers of female CD-1 mouse offspring with developmental exposure to PFOA does not support the involvement of peroxisome proliferation in the observed hepatic toxicity (Quist et al, 2015; described in the Animal Toxicology section). Transmission electron microscope studies of the livers found no evidence that PFOA caused peroxisome proliferation, but rather indicated that it caused mitochondrial proliferation and abnormal mitochondrial morphology.

Activation of PPAR-alpha inhibits accumulation of fat in the liver. In contrast, PFOA increased the accumulation of hepatic triglycerides, cholesterol, and phospholipids in a standard strain of laboratory rats, and the authors noted that these effects are not consistent with the actions of other peroxisome proliferators such as clofibric acid and di(2-ethylhexyl) phthalate (Kawashima et al., 1995). Additionally, PFOA caused a dose-dependent increase in hepatic triglycerides in mice exposed to perilla oil and soybean oil, although there was no effect in mice exposed to fish oil (Kudo and Kawashima, 1997). In more recent studies of standard strains of mice given either a regular or high fat diet, PFOA caused increased triglyceride levels in the liver and decreased relative adipose tissue weight in animals on both diets (Tan et al., 2013; Wang et al., 2013).

#### Data from wild type mice, PPAR-alpha null mice, and humanized PPAR-alpha mice

Studies comparing wild type and PPAR-alpha null mice clearly demonstrate that PFOA causes PPAR-alpha independent and PPAR-alpha dependent hepatic effects. Yang et al. (2002) reported that liver weight was increased in both wild type and PPAR-alpha null mice treated with PFOA (0.02% in the diet) for 7 days, while peroxisomal acyl CoA oxidase (a marker of peroxisome proliferation) was increased only in wild type mice. In contrast, the model PPAR-alpha activator, WY, increased liver weight and peroxisome proliferating activity in wild type mice but had no effect in PPAR-alpha null mice. Based on this study, which was the only study of PFOA in wild type and PPAR-alpha null mice available at the time, the USEPA Science Advisory Board (2006) concluded that PPAR-alpha independent modes of action may be important in hepatic effects of PFOA in rodents. Numerous studies that have been published subsequent to the USEPA SAB (2006) review confirm this conclusion and provide additional relevant information.

Consistent with Yang et al. (2002), Wolf et al. (2008) also reported that PFOA (0, 1, 3, or 10 mg/kg/day for 7 days) increased liver weight similarly in both PPAR-alpha null mice and wild type mice, while WY increased liver weight only in wild type mice. Cell proliferation and hepatocyte hypertrophy were also increased by PFOA in a dose-related fashion in both wild type and PPAR-alpha null mice. However, the livers of wild type and PPAR-alpha null mice treated with PFOA differed histologically. Increased numbers of peroxisomes were seen in the livers of treated wild type mice, while the livers of PPAR-alpha null mice had no peroxisomes but had numerous vacuoles.

Gene profiling studies in wild type and PPAR-alpha null mice treated with PFOA and WY found that PFOA, but not WY, also activates CAR (constitutive activated/androstane receptor), a receptor that is activated by phenobarbital and other compounds, and possibly PXR (pregnane X receptor). Both compounds caused a similar profile of gene changes in wild type mice, including up-regulation of genes involved with PPAR-alpha activation. However, while there were few gene changes in WY treated PPAR-alpha null mice, gene expression was altered in PFOA-treated PPAR-alpha null mice, including some of the same genes affected by PFOA in the wild type mice (Rosen et al., 2008a). Further study (Rosen et al., 2008b) showed that 85% of the genes altered by PFOA were PPAR-alpha dependent. The PPAR-alpha independent genes included genes involved with lipid homeostasis and xenobiotic metabolism, and many were consistent with activation of CAR.

Additional studies suggest that PPAR-alpha may be protective against some types of hepatic toxicity caused by PFOA. Minata et al. (2010) showed that hepatic or biliary damage occurred in both wild type and PPAR-alpha null mice treated with PFOA, but the profile of toxicity differed greatly between the strains. They investigated the effects of PFOA in wild type and PPAR-alpha null mice given 4.4, 10.8, or 21.6 mg/kg/day for four weeks. Concentrations of PFOA in serum and liver were similar in wild type and PPAR-alpha null mice, but were much lower in bile of PPAR-alpha null mice than wild type mice, indicating a much lower capacity for transport of PFOA to bile in the null strain. Relative liver weights were similarly increased in both wild type

and PPAR-alpha null mice at all doses of PFOA, to about three times the control value. Hepatocyte and bile duct injury was assessed by light and electron microscopy, by levels of serum enzymes, bile acids, and bilirubin, and by biochemical markers. Hepatocyte hypertrophy and elevated liver enzymes occurred in both wild type and PPAR-alpha null mice, but bile duct injury was much more severe in PPAR-alpha null mice. PFOA increased the levels of two inflammatory cytokines associated with liver injury in PPAR-alpha null, but not wild type, mice. The authors concluded that PPAR-alpha protects against cholestasis and bile duct injury caused by PFOA in mice. They suggested that rodents may, in fact, be less sensitive to PFOA than humans at some doses.

Finally, the hepatic effects of developmental PFOA exposure in 18-month old offspring of gestationally exposed dams were studied in CD-1, wild type, and PPAR-alpha null mice (Filgo et al., 2015). At this time point long after PFOA has been eliminated from the body, the incidence and/or severity of hepatocellular hypertrophy was increased by prenatal and early life exposure to PFOA in all three strains of mice, and this effect was more pronounced in PPAR-alpha null than wild type mice. Additionally, consistent with Minata et al. (2010), PFOA caused a dose-dependent increase in incidence and severity of bile duct toxicity in PPAR-alpha null, but not wild type, mice. Additional findings from this study related to carcinogenicity of PFOA are discussed below.

Three additional studies evaluated hepatic effects of PFOA in wild type, PPAR-alpha null, and humanized PPAR-alpha (having hepatic expression of human PPAR-alpha) mice of a Sv/129 genetic background (Nakamura et al., 2010; Nagakawa et al., 2011; Albrecht et al., 2013). PFOA caused hepatic toxicity in all three of these mouse strains, including in fetal liver. It should be noted that differences in response in humanized mice are not necessarily indicative of a different response of human PPAR-alpha to PFOA, for reasons that may include species differences in binding of PFOA to recognition sites on mouse DNA (cognate DNA). PFOA concentrations in liver and serum were measured in only one of these three studies (Albrecht et al., 2013).

Hepatic effects were studied in male wild type, PPAR-alpha null, and humanized PPAR-alpha mice treated with 0, 0.1, or 0.3 mg/kg/day PFOA for two weeks (Nakamura et al., 2009). Relative liver weight was not affected at 0.1 mg/kg/day in any strain and was increased at 0.3 mg/kg/day only in wild type mice. Expression (mRNA and protein levels) of five genes that are targets of PPAR-alpha was measured in the three strains. Expression of four of these genes was significantly increased only in the wild type mice, while expression of the fifth gene was significantly increased only in the humanized PPAR-alpha mice. None of the PPAR-alpha target genes were affected by PFOA in the PPAR-alpha null mice. Histopathological examination showed mild hepatocellular hypertrophy in both wild type and humanized PPAR-alpha mice. Cytoplasmic vacuoles indicating lipid accumulation were observed in PPAR-alpha null mice, consistent with those described in this strain by Wolf et al. (2008).

Interpretation of these results is complicated by the fact that PFOA levels in serum and liver were not measured in this study and may vary between strains. Relative liver weight was increased at 0.3 mg/kg/day in wild type mice, but was not increased at this dose in the other two strains. However, there was no effect in wild type mice at 0.1 mg/kg/day, indicating that 0.3 mg/kg/day is

relatively close to the NOAEL in this strain. In the absence of serum and liver PFOA data, it is not known whether the increased liver weight observed at 0.3 mg/kg/day in wild type, but not PPAR-alpha null or humanized PPAR-alpha, strains is due to an intrinsic difference in sensitivity or to toxicokinetic differences.

Hepatic effects were also evaluated in male wild type, PPAR-alpha null and humanized PPAR-alpha mice treated with 0, 1, or 5 mg/kg/day for six weeks (Nagakawa et al., 2011). The results of this study do not support the conclusion that rodent PPAR-alpha is required for the hepatic toxicity of PFOA. Hepatocyte hypertrophy occurred in both strains with PPAR-alpha activity (wild type and humanized), and activity of the liver enzyme ALT was increased in all three strains including PPAR-alpha null. Single cell hepatocyte necrosis occurred in all three strains, but hydropic degeneration of hepatocytes occurred only in PPAR-alpha null and humanized mice.

Macrovesicular steatosis and inflammatory cells were found only in the PPAR-alpha null mice, while microvesicular steatosis occurred in the PPAR-alpha null and humanized strains but not the wild type strain. Liver triglyceride and cholesterol levels were increased several fold in the PPAR-alpha null mice, but not in the other strains, while plasma triglycerides were decreased only in the wild type mice. The authors concluded that PPAR-alpha may be important in protecting from hepatic damage caused by PFOA at the doses used in the study. They also concluded that, since hepatic PPAR-alpha may be weaker in its function and present in lower amounts in humans than in mice, humans may be susceptible to hepatic damage from similar doses of PFOA.

Finally, hepatic effects of PFOA were evaluated in pregnant wild type, PPAR-alpha null and humanized PPAR-alpha mice and their offspring after exposure to 3 mg/kg/day on GD 1-17 (Albrecht et al., 2013). Serum and liver PFOA levels were measured in dams and fetuses on GD 18, and in dams and offspring on PND 20. There were large differences in serum and/or liver PFOA concentrations among strains, and these differences were not consistent at the different life stages and time points. Therefore, differences in responses to PFOA among the strains may be due to toxicokinetic differences in addition to, or instead of, intrinsic strain differences in sensitivity to PFOA's effects.

In dams on GD 18, relative liver weight was significantly increased to a similar degree in all three strains. Expression of two target genes for PPAR-alpha was increased by PFOA in both wild type and humanized PPAR-alpha dams, but not PPAR-alpha null dams, although the increase in expression of one of the genes was not significant in the humanized mice. Expression of target genes for CAR and PXR was increased by PFOA in all three strains, with the biggest increase in the humanized PPAR-alpha dams. Minimal to mild hepatocellular hypertrophy was observed by histopathological examination in all three strains, although the morphological features differed among the strains.

On PND 20, relative liver weight remained increased in the wild type dams treated with PFOA, but not in PPAR-alpha null or humanized PPAR-alpha strains. Expression of a gene associated with PPAR-alpha was also increased by PFOA only in wild type dams. Expression of a gene associated with CAR was not affected in any strain, while expression of a gene associated with PXR

remained significantly increased in all three strains. Histopathological changes were observed in livers from PFOA-treated dams of all three strains, but were decreased in incidence and severity compared to on GD 18 in wild type and humanized PPAR-alpha dams.

In fetuses on GD 18, relative liver weight was significantly increased in wild type and humanized PPAR-alpha, but not PPAR-alpha null, mice. Expression of two genes associated with PPAR-alpha was significantly increased in wild type and humanized fetal livers, but not in PPAR-null. The increased expression compared to controls was greater for both genes in the humanized PPAR-alpha fetuses than in the wild type fetuses, and the increase for one of them was about 5-fold greater in humanized than wild type (humanized PPAR-alpha significantly different from wild type). Fetal expression of a gene associated with CAR was not affected by PFOA in any of the three strains, and expression of a gene associated with PXR was increased only in the humanized PPAR-alpha fetal liver. Histopathological changes consistent with peroxisome proliferation were seen in some wild type mice, while no definitive changes were seen in the other strains.

In offspring on GD 20, relative liver weight and expression of genes associated with PPAR-alpha were increased only in wild type mice. Expression of a gene associated with CAR was increased in all three strains, with the largest increase in the PPAR-null offspring, while expression of a gene associated with PXR was increased in wild type and humanized, with the larger increase in wild type. At this time point, histopathological changes in PFOA-treated wild type offspring were similar to those on GD 19, while changes were not clearly evident in livers from the other two strains.

These results indicate that hepatic effects of PFOA in adult, fetal, and neonatal mice are mediated by human PPAR-alpha and through PPAR-alpha independent pathways, as well as by mouse PPAR-alpha. As noted above, interpretation of these results is complicated by the fact that PFOA levels in serum and liver differed among the strains in dams, fetuses, and offspring. For example, serum and liver PFOA levels were lower in humanized dams and offspring than in wild type dams and offspring on PND 20. Therefore, it is unknown whether the greater hepatic effects in wild type than humanized PPAR-alpha mice at this time point are due to an intrinsic difference in sensitivity or to toxicokinetic factors.

### **Serum cholesterol and lipids**

As discussed above, activation of PPAR-alpha causes decreased serum cholesterol and triglycerides in both humans and experimental animals, and this is the basis for the use of fibrates as cholesterol-reducing agents in humans. Consistent with other PPAR-alpha activators, PFOA decreased serum lipids levels in rodents in a number of studies (e.g. Loveless et al., 2006, and other studies summarized in Rebholz et al., 2016). In contrast, as discussed in the Epidemiology section, serum PFOA has been consistently associated with increased serum cholesterol, suggesting a human versus rodent difference in the effect of PFOA on this endpoint.

Two recent studies suggest the possibility that dietary factors, specifically differences in dietary fat content, may contribute to the observed differences in effects of PFOA on serum lipids in rodents

versus humans. Male Balb/C mice on a regular diet or a high fat diet were dosed with 0, 5, 10, or 20 mg/kg/day PFOA for 14 days (Tan et al., 2013). Consistent with earlier mouse studies, both serum cholesterol and serum triglycerides decreased in a dose-related fashion in mice on the regular diet. In contrast, serum cholesterol and triglycerides were unaffected by PFOA in mice on the high fat diet. Additionally, hepatic toxicity from PFOA was more severe in mice on the high fat diet (discussed in Animal Toxicity section).

In another study, male and female C57BL/6 and BALB/c mice were fed a high fat diet containing 32% of calories from fat (similar to the mean of 33% calories from fat in the U.S. diet; CDC, 2016) and 0.25% cholesterol for 6 weeks (Rebholz et al., 2016). In both sexes of C57BL/6 mice and male Balb/c mice, plasma cholesterol was significantly increased by PFOA treatment (3.5 ppm in the diet, resulting in a dose of about 0.5 mg/kg/day), while PFOA had no effect on plasma cholesterol in female Balb/C mice. These results contrast with the decreased serum cholesterol and triglycerides caused by PFOA reported in other studies in which rodents were fed a standard laboratory diet (e.g. containing 13% of calories from fat and 0.02 % cholesterol; LabDiet, 2016). Interestingly, Minata et al. (2010) also observed that cholesterol was increased at the highest PFOA dose, and triglycerides were increased at all doses, in PPAR-alpha null mice.

### **Immune System Toxicity**

Effects of PFOA on the immune system in humans and experimental animals are discussed in the Epidemiology and Toxicology sections above. Additionally, immunotoxicity of PFOA and other PFCs, including mode of action studies, was reviewed by DeWitt et al. (2012). PFOA suppressed the immune system in studies of both non-human primates and mice. Effects include decreased absolute and relative spleen and thymus weights, decreased thymocyte and splenocyte counts, decreased immunoglobulin response, and changes in total numbers and/or specific populations of lymphocytes in the spleen, thymus, peripheral blood, and bone marrow. Based on the available data, rats are less sensitive than mice to immunotoxic effects of PFOA. Immune system effects were not observed in the two studies of rats; these studies included doses higher than those which generally caused immune effects in mice. In humans, exposures to PFOA within the general population range have been associated with decreased antibody levels in response to vaccination. Fletcher et al. (2009) also reported several statistically significant associations between several markers of immune function (decreased IgA, decreased IgE in females only, increased anti-nuclear antibody, decreased C-reactive protein) and serum PFOA levels in communities with drinking water exposure to PFOA in an unpublished C8 Science Panel report.

### **PPAR-alpha dependence of immune effects in rodents and humans**

Two studies in wild type and PPAR-alpha null mice indicate that PFOA's effects on the immune system in mice occur through both PPAR-alpha dependent and PPAR-alpha independent mechanisms. Yang et al. (2002b) studied PFOA's effects on immune response in wild type and PPAR-alpha null mice given 0.02% PFOA in the diet (resulting in a dose of 30 mg/kg/day) for 7 days. Although food intake was similar in the treated wild type and PPAR-alpha null mice, body weight was significantly reduced by PFOA in the wild type mice, but not the null mice, suggesting that weight loss is related to activation of PPAR-alpha, possibly through PFOA's effects on

metabolism, rather than to non-specific toxicity. Consistent with studies discussed in the section on hepatic effects (above), liver weights were increased by PFOA similarly in wild type and null mice, but peroxisome proliferation (as measured by PCO oxidation) occurred only in wild type mice, indicating that increased liver weight was not PPAR-alpha dependent. PFOA caused some immune system effects in wild type but not PPAR-alpha null mice, including decreased spleen weight and splenocyte numbers, and reduced splenocyte proliferation in response to mitogens. However, PFOA also caused other effects (reduced thymus weight and thymocyte numbers, and alterations in the distribution of thymocyte subpopulations) in the null mice, although to a lesser degree than in the wild type mice. These findings led the authors to conclude that PFOA's effects on the immune system are due to mechanisms both dependent and independent of PPAR-alpha.

DeWitt et al. (2009a) studied C57 and Sv/129 wild type and PPAR-alpha null mice exposed to 30 mg/kg/day PFOA in drinking water for 15 days to determine the involvement of PPAR-alpha in the immunotoxic effects of PFOA. Sensitivity to PFOA toxicity appeared to differ in the C57 and the Sv/129 mice, regardless of their PPAR-alpha status, as PFOA treatment decreased body weight, spleen weight, thymus weight, or antibody titer in the C57 wild type mice but did not have these effects in the Sv/129 wild type mice. IgM titer was decreased in both C57 and Sv/129 PPAR-alpha null mice, although this effect did not occur in the wild type Sv/129 mice, suggesting that this effect is not PPAR-alpha dependent. Relative liver weight was similarly increased in all four types of mice given 30 mg/kg/day PFOA in drinking water for 15 days, indicating that this response was not dependent on strain or PPAR-alpha status.

A more recent study suggests that the T-cell dependent antibody response (TDAR) in mice is independent of PPAR-alpha. DeWitt et al. (2016) evaluated the IgM response to immunization with a T-cell dependent antigen (sheep red blood cells: SRBC) in wild type and PPAR-alpha C57Bl/6 null mice exposed to 0, 7.5, or 30 mg/kg/day PFOA for 15 days. PFOA at 7.5 mg/kg/day did not affect TDAR, and production of sheep red blood cell-specific antigens was inhibited equally in wild type and PPAR-alpha null mice at 30 mg/kg/day.

In the C8 Health Study population, serum PFOA was significantly associated with a strong downward trend with C-reactive protein, a liver protein that is a marker for inflammation (Fletcher et al., 2009). Genser et al. (2015) found consistent and significant associations of serum PFOA with decreased C-reactive protein, both within each of the six water districts included in the study and on an aggregated basis. They concluded that these within- and between-district associations strengthen the evidence of causality for this effect. Fibrate drugs act through activation of PPAR-alpha to decrease C-reactive protein in humans (Kleemann et al., 2003; Wagner et al., 2011). These drugs also cause decreases in other markers of inflammatory response *in vivo* in humans and animals, and *in vitro* through PPAR-alpha activation (Kleemann et al., 2003; Budd et al., 2007; Wagner et al., 2011). As it is well established that PFOA also activates PPAR-alpha, the decrease in C-reactive protein in populations exposed to PFOA through drinking water may have similarly occurred through PPAR-alpha activation.

Additionally, statins are a group of drugs that inhibit HMG-CoA reductase, a hepatic enzyme involved with cholesterol synthesis. Statins also activate PPARs including PPAR-alpha (Balakumar and Mahadevan, 2012). Two recent studies have reported that these drugs decrease the effectiveness of influenza vaccines in humans (Black et al., 2016; Omer et al., 2016). As above, it is well established that PFOA is an activator of PPAR-alpha, and HMG-CoA reductase activity was reduced by 50% in rats treated with 0.02% PFOA in the diet (22.7 mg/kg/day) for 7 days (Haughom and Spydevold, 1992). Taken together, these observations suggest that the decreased immune response associated with PFOA in humans may also be related to these effects.

Finally, an *in vitro* study in the human promyelocytic cell line THP-1 showed that PPAR-alpha plays a role in the immunotoxicity of PFOA in human cells (Corsini et al., 2011). Incubation of these cells with 100,000 ng/ml PFOA inhibited the lipopolysaccharide-stimulated release of two pro-inflammatory cytokines (TNF-alpha and IL-8) and matrix metalloproteinase-9, a molecule which plays a role in mobilization of inflammatory cells. This inhibition was reversed when PPAR-alpha was silenced by the addition of small interference RNA specific for PPAR-alpha.

#### Role of corticosterone production secondary to stress in immune effects in mice

Loveless et al. (2008) studied immune system and other effects in male CD rats and CD-1 mice given linear APFO by gavage at 0, 0.3, 1, 10, and 30 mg/kg/day for 29 days. This study evaluated the role of corticosterone in the immunotoxicity of PFOA. Hepatic effects from this study were described above.

Immunotoxicity caused by PFOA in the mice included decreased relative spleen weight at 1 mg/kg/day and above, and decreased numbers of spleen and thymus cells, decreased thymus weight, thymic depletion/atrophy, granulocytic bone marrow hyperplasia, and decreased anti-SRBC IgM titer at 10 and 30 mg/kg/day. The authors stated that the LOAEL for immunotoxic effects was 10 mg/kg/day, based on decreased IgM titers. However, the significant decrease in relative spleen weight at 1 mg/kg/day suggests that the LOAEL may actually have been 1 mg/kg/day.

Corticosterone levels were above the normal value of 400 ng/ml in 7 of 10 mice given 10 mg/kg/day PFOA and in 6 of 10 mice given 30 mg/kg/day PFOA, and decreased IgM correlated with increased corticosterone for individual mice ( $p < 0.002$ ). Based on this observation, the authors suggested that the IgM decreases from PFOA were secondary to stress-related increases in corticosterone.

In this study, rats were less sensitive to immunotoxic effects of PFOA than mice, and there were no effects of PFOA on spleen or thymus weight or on antibody production in the rats. Corticosterone levels were increased above the level considered normal (300 ng/ml) in 2 of 10 rats in the 10 mg/kg/day group and 4 of 10 rats in the 30 mg/kg/day group.

DeWitt et al. (2009b) further investigated the hypothesis that immune effects of PFOA are secondary to corticosterone production in response to stress by studying adrenalectomized and

sham-operated mice. Female C57BL/6N female mice (adrenalectomized or sham-operated) were dosed with 0, 7.5, or 15 mg/kg/day PFOA in drinking water for 10 days. Corticosterone levels were much higher in untreated sham-operated than adrenalectomized mice, and PFOA increased the levels of this hormone in sham-operated, but not adrenalectomized, animals. However, the immunotoxic effects of PFOA were not reduced by adrenalectomy, as SRBC-specific IgM levels were significantly reduced by 7.5 mg/kg/day PFOA in the adrenalectomized, but not sham-operated, mice, and in both groups of mice by 15 mg/kg/day PFOA. These authors concluded that the suppression of IgM synthesis by PFOA was not secondary to stress-related corticosterone production.

### **Developmental toxicity**

As discussed in the Epidemiology section, a systematic review of relevant epidemiological evidence concluded that there is “sufficient” human evidence (the highest strength of evidence in the evaluation scheme) that developmental exposure to PFOA reduces fetal growth in humans (Johnson et al., 2014). The developmental effects of PFOA in mice have PPAR-alpha dependent and independent components (Dewitt et al., 2009; Abbott et al., 2007) and may involve toxicity to the placenta. Based on the information presented below, developmental effects of PFOA in laboratory animals are considered relevant to humans for the purposes of risk assessment.

The USEPA Science Advisory Board (2006) concluded that PPAR-alpha mediated effects of PFOA in human fetuses, neonates, and children are of potential concern because there was minimal information about human levels of PPAR-alpha during these life stages. Subsequent to the USEPA SAB (2006) review, additional relevant information on the role of PPARs in human development became available. It is now known that PPAR-alpha, -beta, and -gamma are expressed in many fetal and adult tissues in rodents and humans. Abbott et al. (2010, 2012) found that PPARs are present in nine human fetal tissues examined (liver, heart, lung, kidney, intestine, stomach, adrenal, spleen, and thymus) from embryonic days 54 to 125. They found that the levels may increase or decrease with age of the fetus, or between the fetus and the adult. In some fetal tissues, PPARs were expressed at levels equivalent to or higher than in adults. Although the role of PPAR-alpha and other PPARs in human and animal development is not well characterized, based on their physiological actions, they are expected to have important roles in reproduction and development (Abbott et al., 2010). For these reasons, it can be assumed that PPAR-alpha mediated effects on development are relevant to humans.

In 129S1/SvImJ wild type and PPAR-alpha null mice dosed with PFOA on GD 1-17, full litter resorptions and increased pup liver weight caused by PFOA were independent of PPAR-alpha, while PFOA caused postnatal mortality, delayed eye opening, and decreased weight gain in wild type, but not PPAR-alpha null mice (Abbott et al., 2007). More recently, Albrecht et al. (2013) studied effects of gestational exposure of PFOA in wild-type, PPAR-alpha null, and humanized PPAR-alpha (expressing human PPAR-alpha) mice. They stated that PFOA decreased postnatal survival in wild type mice, but not PPAR-alpha null or humanized PPAR-alpha mice, suggesting that the humanized PPAR-alpha strain is less sensitive to this effect. However, a detailed review

of this study, presented in the Animal Toxicology section, suggests that a firm conclusion on this point cannot be made from the data presented.

In the lungs and the liver of fetal (GD 18) CD-1 mice exposed during gestation, PFOA primarily affected the expression of genes related to intermediary metabolism and inflammation, including genes both associated and not associated with PPAR-alpha activation (Rosen et al., 2007). The authors suggested that PFOA tends to shift metabolism in the direction of a fasted animal, consistent with the metabolic changes and obesity in adult mice exposed to PFOA during gestation (Hines et al., 2009).

Developmental exposure to PFOA was found to alter the expression pattern of PPAR-alpha, -beta, and -gamma in many tissues in fetal and neonatal CD-1 mice (Abbott et al., 2012). The expression of genes regulated by PPARs and other nuclear receptors (CAR and PXR), including genes involved with homeostatic control of lipid and glucose metabolism, was also altered as early as GD14. The authors suggested that these effects on metabolism could contribute to the neonatal mortality and decreased rate of growth caused by gestational exposure to PFOA.

Wolf et al. (2010) discussed the potential human relevance of developmental effects of PFNA that are mediated by PPAR-alpha in mice, and these comments are applicable to PFOA as well. They state: “Relevance of the PPAR-alpha mechanism to humans has been criticized primarily based on the lower number of these receptors in the liver of human versus mouse. However, PPAR-alpha is implicated here in the developmental effects of PFNA as well, and the etiology of PPAR-alpha in other tissues of the embryo, fetus and neonate of the human and the mouse that are involved in gross development has not been fully determined. Therefore, the possibility of relevance of PPAR-alpha to a human response to PFNA cannot be dismissed.”

Although many of the developmental effects of PFOA appear to be PPAR-alpha dependent, PFOA's developmental effects are not shared by other potent PPAR-alpha activators. Palkar et al. (2010) evaluated the developmental effects of two well-studied PPAR-alpha activating compounds, WY and clofibrate, in wild type and PPAR-alpha null mice. Pregnant mice were given doses of WY and clofibrate known to cause the same increase in maternal liver weight that is produced by doses of PFOA that also cause developmental toxicity. As expected, both WY and clofibrate caused increased maternal relative liver weight on GD 18 in wild type, but not PPAR-alpha null, mice. However, WY did not increase fetal liver weight on GD 18, although PFOA caused this effect on GD 18 and/or at early postnatal time points (GD 1, GD 4) in other studies (Albrecht et al., 2013; Macon et al., 2011; White et al., 2009). Although WY did not increase fetal liver weight, both WY and clofibrate increased expression of two genes associated with PPAR-alpha in fetal and maternal liver of wild type mice on GD 18. Furthermore, unlike PFOA, which is considered a low affinity PPAR-alpha agonist, the two higher affinity PPAR-alpha activators, WY and clofibrate, had no effect on reproductive and developmental parameters such as litter loss, number of live pups, fetal growth, day of eye opening, or post-natal mortality in either wild type or PPAR-alpha null mice. Although the reasons for these differences between PFOA and the two higher affinity PPAR-alpha activators is not known, these results suggest that PFOA may cause

increased fetal liver weight, and reproductive and developmental effects, through a mechanism unrelated to PPAR-alpha activation.

Toxicity to the placenta may play a role in developmental effects of PFOA such as fetal growth retardation (Suh et al., 2011). In pregnant CD-1 mice treated with PFOA (2, 10, or 25 mg/kg/day) on GD 11-16, fetal and placental weights were decreased in a dose-dependent manner. PFOA also increased resorptions and dead fetuses, and decreased the number of live fetuses, resulting in significant increases in post-implantation loss. Placental efficiency (ratio of fetal weight to placental weight) showed a dose-dependent decrease. Necrotic changes occurred in the placenta at the two higher doses, and decreases in several types of placental trophoblast cells occurred at all doses. These placental trophoblast cells produce prolactin family hormones that are vital to placental and fetal growth, adaptation to physiological stressors, and maintenance of pregnancy. Placental mRNA for these hormones was significantly decreased in all dose groups. Additionally, mRNA for the pituitary-specific transcription factor (Pit-1) that activates the prolactin family genes was also decreased. The authors concluded that reduced placental efficiency due to effects on placental trophoblast cells and placental hormones may play a role in PFOA's reproductive and developmental effects.

### **Male reproductive effects**

As discussed above, PFOA caused toxicity to the male reproductive system in several mouse studies. Additionally, PFCs have also been associated with adverse effects on sperm parameters and/or effects on male reproductive hormones in humans, although these associations are not necessarily specific to PFOA (Joensen et al., 2009; Toft et al., 2012). Additionally, prenatal exposure to PFOA was associated with decreased sperm count and concentration and increased LH and FSH in young men (Vested et al., 2013).

Li et al. (2011) suggest that PPAR-alpha plays a role in male reproductive effects of PFOA and that these effects occur through activation of either mouse or humanized PPAR-alpha. Decreased serum testosterone and an increased percentage of abnormal sperm were statistically significant effects in wild type and humanized PPAR-alpha mice, but statistically significant changes in these parameters did not occur in PPAR-alpha null mice. Additionally, abnormal seminiferous tubules with vacuoles or lack of germ cells were observed in the wild type and humanized PPAR-alpha mice, while no obvious structural changes occurred in the PPAR-alpha null mice.

Other studies suggest that PFOA may decrease levels of enzymes involved with testosterone synthesis (Zhang et al., 2014), disrupt the blood-testis barrier (Lu et al., 2015), and/or affect cellular pathways in the testes leading to increased oxidative stress (Liu et al, 2015). In regard to the latter effect, it was noted by Liu et al. (2015) that the testes are very sensitive to oxidative damage produced by reactive oxygen species, and that oxidative stress is known to be an important cause of male infertility in humans.

### **Effects on mammary gland development**

Because the developmental patterns of the mammary gland are similar in humans and rodents, rodents are considered to be a good model for studying the effects of environmental contaminants on human mammary gland development (Rudel and Fenton, 2009; Fenton et al. 2012; Rudel et al., 2011; Fenton and Birnbaum, 2015; Osborne et al., 2015).

PPAR-alpha null mice are viable, healthy, and fertile (Lee et al., 1995), suggesting that PPAR-alpha is not required for mammary gland development (Yang et al., 2006). However, activation of PPAR-alpha was shown to disrupt maternal mammary gland development in mice in two different mouse models (Yang et al., 2006). First, mammary gland development during pregnancy was impaired in transgenic mice with constitutively activated PPAR-alpha. Additionally, mammary gland development was suppressed in wild type mice administered the PPAR-alpha activator WY during pregnancy, and this effect was decreased when dosing with WY was started later in pregnancy. In contrast, no significant effects on mammary gland development were observed in PPAR-alpha null mice similarly treated with WY during pregnancy.

The role of PPAR-alpha in PFOA's effects on mammary gland development in mice is not known. As discussed in detail above, prenatal and/or neonatal exposures to very low doses of PFOA cause persistent delays in mammary gland development in mice. However, the effects of gestational and/or lactational exposure to PFOA on mammary gland development have not been studied in wild type versus PPAR-alpha null mice. Limited data from two studies of effects of peripubertal exposure to PFOA on mammary gland in wild type and PPAR-alpha null mice (Zhang et al., 2010; Zhang et al., 2012) are insufficient to support a conclusion on this question.

In Zhao et al. (2010), PPAR-alpha null mice were treated with a single dose level of 5 mg/kg/day PFOA, 5 days per week for 4 weeks, starting at age 3 weeks. It was reported that PFOA caused stimulation of mammary gland development in the PPAR-alpha null mice, and, based on this single data point, the authors concluded that PFOA causes PPAR-alpha independent stimulation of mammary gland development. However, numerical data for this effect were not provided, and a similarly treated group of wild type mice was not included for comparison to the PPAR-alpha null mice in the study. A subsequent study (Zhao et al., 2012) reported no effect on mammary gland development in PPAR-alpha null mice from 7.5 mg/kg/day PFOA given 5 days per week for 4 weeks starting at age 3 weeks, while mammary gland development was inhibited in similarly treated wild type mice. However, the differences seen in the two strains may not have resulted from PPAR-alpha dependence of the effect, but rather from toxicokinetic differences, since the serum levels in the PPAR-alpha null mice (38,000 ng/ml) were much lower than in the wild type mice (93,000 ng/L). Additionally, the serum levels in PPAR-alpha null mice dosed with 5 mg/kg/day where stimulation was reported (28,000 ng/ml; Zhao et al., 2010) were close to the serum levels (38,000 ng/ml) in the same strain given 7.5 mg/kg/day at which no effects were reported. In summary, the stimulation of mammary gland development in PPAR-alpha null mice has not been clearly demonstrated. It was reported only in one study using a single dose level without a comparison group of wild type mice, and was not replicated in a second study of PPAR-alpha null mice with similar serum PFOA levels.

### **Estrogenic effects**

Estrogenic activity and/or increased estrogen levels may be involved in the mode of action of PFOA. As discussed in the Toxicology section, serum estradiol was increased in male Sprague-Dawley rats exposed to 13.6 mg/kg/day APFO in the diet for 1 month to 12 months in a two-year dietary study (Biegel et al., 2001). PFOA was also shown to bind to and/or activate human estrogen receptor-alpha in several *in vitro* studies (Benninghoff et al., 2011; Kjeldsen and Bonefeld-Jorgensen, 2013; Buhrke et al., 2015).

Exposure to PFOA increased levels of the sensitive estrogen-dependent biomarker protein, vitellogenin, and the expression of estrogen receptor-beta in the livers of mature male and female rare minnows (Wei et al., 2007). Male fish exposed to PFOA developed oocytes in the testes, and the ovaries of exposed females underwent degeneration. PFOA (as well as PFNA and perfluorodecanoic acid [PFDA, C10]), induced vitellogenin in young rainbow trout, and these PFCs also activated rainbow trout estrogen receptor *in vitro* (Benninghoff et al., 2011).

Studies in rainbow trout, a species used as a model for human liver carcinogenesis because it is insensitive to peroxisome proliferation, suggest that PFOA promotes liver tumor development through an estrogenic mechanism (Tilton et al., 2008; Benninghoff et al., 2012). PFOA and two other peroxisome proliferating compounds (clofibrate and dehydroepiandrosterone, DHEA) were tested for tumor promoting activity in rainbow trout which had been initiated with aflatoxin. PFOA and DHEA (Tilton et al., 2008), as well as PFNA and PFDA (Benninghoff et al., 2012), increased the incidence and number of liver tumors and also induced a genomic signature similar to that induced by 17-beta-estradiol, while clofibrate did not promote liver tumors and did not regulate genes in common with 17-beta-estradiol (Tilton et al., 2008).

### **Carcinogenicity**

As discussed in the Epidemiology section, PFOA has been associated with testicular and kidney tumors in communities with drinking water exposure after adjustment for smoking and other relevant factors. As discussed in the Toxicology section, PFOA caused hepatic, testicular Leydig cell, and pancreatic acinar cell tumors in chronically exposed male rats (Biegel et al., 2001; Butenhoff et al., 2012). These tumor types are also caused by several other PPAR-alpha activating compounds in male rats (Klaunig et al., 2003).

### **Hepatic tumors**

Many PPAR-alpha activators cause liver tumors in rodents through a mode of action involving PPAR-alpha activation. These chemicals are not directly genotoxic, and thus cause tumors through a non-genotoxic mechanism. Activation of PPAR-alpha causes a number of effects in the liver, but all of these effects are not necessarily causal for carcinogenesis. As summarized by USEPA (2009c), peroxisome proliferation and increases in biochemical markers for peroxisome proliferation such as PCO are considered indicative of PPAR-alpha activation, but they are not

considered causal events for carcinogenicity since their correlation with carcinogenic potency is poor. As discussed by the USEPA SAB (2006), the key causal events for PPAR-alpha induced liver carcinogenesis are believed to be PPAR-alpha activation, followed by increased cell proliferation and decreased apoptosis mediated by gene expression changes, leading to clonal expansion of preneoplastic foci and tumor formation (Klaunig et al., 2003; NRC, 2006). However, aside from the initial event (PPAR-alpha activation), the other causal events are not specific to a PPAR-alpha mode of action but rather are also common to other modes of action for hepatic carcinogenesis (USEPA, 2006; Klaunig et al., 2012).

Much attention has been focused on the potential human relevance of rodent liver tumors induced by PPAR-alpha activators. There is wide variation among species in the ability of PPAR-alpha activators to cause hepatic peroxisome proliferation and liver tumors, with rats and mice the most sensitive, hamsters intermediate in sensitivity, and humans, monkeys, and guinea pigs least sensitive to these effects. These species differences may be due to lower levels of PPAR-alpha expression in humans than in rodents (NRC, 2006) or to differences in PPAR-alpha structure and function among species (Corton, 2010). Studies of humans exposed to peroxisome proliferating fibrate drugs have not shown increased risk of liver cancer (NRC, 2006), although these studies have limitations that are discussed by Guyton et al. (2009).

The majority of the USEPA SAB (2006) panel believed that the human relevance of the PPAR-alpha mode of action for liver tumors caused by PFOA could not be dismissed. This conclusion was based on data indicating similar responses to PFOA in the livers of rodents and primates (increased liver weight and induction of hepatic peroxisomal enzyme activity, discussed above). Although increased cell proliferation was not found in the monkeys (Butenhoff et al., 2002), the USEPA SAB (2006) noted that this endpoint was only measured after 6 months of exposure in the monkeys but not at an earlier time point during the first 1-2 weeks of exposure when it would have been more likely to occur, and apoptosis was not evaluated at any time point. The lack of data on cell proliferation at an appropriate time point and apoptosis at any time point in the monkeys treated with PFOA precluded analysis of dose-response concordance between these key events and tumor induction for PFOA as compared to other PPAR-alpha agonists. Subsequent to the USEPA SAB (2006) evaluation, hepatic effects of PFOA were observed in three studies of mice with humanized PPAR-alpha, although chronic studies to evaluate tumor incidence in humanized PPAR-alpha mice have not been conducted (Nakamura et al., 2009; Nagakawa et al., 2011; Albrecht et al., 2013; discussed above).

The USEPA SAB (2006) also noted that the role of hepatic PPAR-alpha in human fetuses, neonates, and children is not known, and that a PPAR-alpha mode of action for hepatic effects could not be ruled out in these age groups. As discussed above, it was subsequently demonstrated that PPARs are present in many human fetal tissues, including liver, and that levels of PPARs increase and decrease with age of the fetus, and between the fetus and the adult (Abbott et al., 2010, 2012). Additionally, PFOA caused increased liver weight and expression of genes associated with PPAR-alpha in fetal livers from mice with humanized PPAR-alpha (Albrecht et al., 2013).

The USEPA SAB (2006) further concluded that PPAR-alpha activation may not be the sole mode of action for liver tumors caused by PFOA. This conclusion was based on the single study of PFOA in wild type and PPAR-alpha null mice that was available at the time (Yang et al., 2002b). In this study, PFOA increased liver weight in both wild type and PPAR-alpha null mice, while a model PPAR-alpha activator (WY) increased liver weight in wild type mice but had no effect in PPAR-alpha null mice. Numerous studies published subsequent to the USEPA SAB (2006) review confirm this conclusion and provide additional relevant information. These include several studies demonstrating PPAR-alpha independent hepatic effects of PFOA in standard strains of rodents and PPAR-alpha null mice, as well as the PPAR-alpha independent hepatic tumors, possibly related to estrogenic effects, in rainbow trout exposed to PFOA. The USEPA SAB (2006) also noted that some of the causal events in the PPAR-alpha mode of action for liver tumors had not been demonstrated for PFOA, including increased cell proliferation in the liver at early time points after dosing and/or decreased apoptosis in liver cells.

Newer information relevant to this issue shows that the hepatic effects of PFOA and WY differ, and that increased hepatic cell proliferation caused by PFOA is not totally dependent on PPAR-alpha activation. PFOA caused liver tumors in a two-year study of male rats (Biegel et al., 2001), but it did not increase hepatic cell proliferation at any of eight time points between one and 21 months after dosing began. In contrast, the PPAR-activating compound WY, which also caused liver tumors in this study, significantly increased hepatic cell proliferation at most of these time points.

Elcombe et al. (2010) suggested that the earliest time point evaluated by Biegel et al. (2001), one month after dosing began, may have been too late to observe hepatic cell proliferation in response to PFOA since cell proliferation occurs early in the sequence of events leading to liver tumors. They studied effects of PFOA and WY on hepatic endpoints including nuclear receptor activation, cell proliferation, and apoptosis in male rats at earlier time points (one day after the end of dosing for 1, 7, or 28 days). While PFOA acted as an activator of PPAR-alpha, CAR, and PXR, WY was a specific activator of PPAR-alpha only. WY increased hepatic cell proliferation and decreased apoptosis, and these effects were accompanied by increased hepatic DNA content. PFOA also increased hepatocellular proliferation, but, unlike WY, it did not cause decreased apoptosis or increased liver DNA content.

Results from wild type and PPAR-alpha null mice demonstrate that PFOA causes hepatic cell proliferation, one of the key causal events for PPAR-alpha dependent liver carcinogenesis, through PPAR-alpha independent pathway(s). PFOA caused a similar or greater dose-dependent increase in hepatic cell proliferation in mice lacking PPAR-alpha as compared to wild type mice, and relative liver weight was also increased to a similar degree in both strains (Wolf et al., 2008). In contrast to PFOA, WY increased cell proliferation and relative liver weight only in the wild type mice in this study.

A carcinogenicity bioassay of PFOA has not been conducted in a standard strain of mice, or in wild type and PPAR-alpha null mice. However, the incidence of liver tumors at age 18 months was evaluated in female CD-1, 129/Sv wild type, and 129/Sv PPAR-alpha knockout offspring after developmental exposures to PFOA (Filgo et al., 2015). The authors emphasize that the study was not designed or intended to be a carcinogenicity bioassay, but that tumor incidence was assessed because of the unexpected finding of liver tumors in some animals that died before the scheduled end of the study.

In CD-1 mice, single or multiple hepatocellular adenomas occurred in one or more animals in each of the treated groups (n=21 to 37 per group; 0, 0.01, 0.1, 0.3, 1, and 5 mg/kg/day) except for at the lowest dose (0.01 mg/kg/day), but were not found in controls (n=29). In total, adenomas occurred in 4.9% (7 of 144) treated animals, compared to a historic control incidence of 0.4% in untreated female CD- mice. Additionally, hepatocellular carcinomas occurred in two treated mice (0.3 and 5 mg/kg/day) but not in controls.

In 129/Sv wild type mice, hepatocellular adenomas did not occur in control or treated groups (n=6 to 10 per group; 0, 0.1, 0.3, 1, and 3 mg/kg/day). In contrast, in PPAR-alpha null mice of this strain, there were no adenomas in the controls (n=6), one adenoma in each of the 0.1, 0.3, and 1 mg/kg/day PFOA-treated groups (n=9 or 10), and two adenomas in the 3 mg/kg/day group (n=9). These tumors occurred in 13.2% of all PFOA-treated PPAR-alpha null mice. The results of this study suggest the possibility that developmental exposure to low doses PFOA may cause hepatic tumors later in life through a PPAR-alpha independent mode of action, although more research is needed before any firm conclusions can be made.

#### Testicular Leydig cell and pancreatic acinar cell tumors

Modes of action that have been suggested for the non-hepatic tumors caused by PFOA are reviewed in USEPA (2005a) and Klaunig et al. (2012). Several other PPAR-alpha agonists have been found to induce the same three tumor types (hepatic adenomas, pancreatic acinar cell tumors, and testicular Leydig cell tumors) as PFOA in Sprague-Dawley rats. However, the modes of action for the latter two types of tumors have not been fully characterized, and it has not been shown that they occur through a PPAR-alpha mediated mode of action.

The mode of action for testicular Leydig cell tumor induction by PFOA is unknown. Proposed modes of action include 1) increased serum estradiol through PPAR-alpha mediated induction of hepatic aromatase activity, leading to estradiol-dependent increased production of growth factors inducing the tumors, and 2) inhibition of testosterone biosynthesis leading to an increase in luteinizing hormone (LH), which promotes Leydig cell tumor development (Klaunig et al., 2012). However, for each of these proposed modes of action, data from the chronic mechanistic rat study (Biegel et al., 2001) do not support one of the key events and the mode of action (Klaunig et al., 2012).

The key events in the first proposed mode of action above are:

Activation of PPAR-alpha in the liver → increased hepatic aromatase → increased serum and testicular interstitial fluid estradiol levels → increased transforming growth factor-alpha → increased testicular Leydig cell proliferation → testicular Leydig cell tumors (Klaunig et al., 2012).

As discussed by Klaunig et al. (2012), testicular Leydig cell proliferation was not increased by PFOA at numerous time points during the chronic rat study of Biegel et al. (2001).

The key events in the second proposed mode of action above are:

Decreased testosterone levels → increased LH levels → testicular Leydig cell tumors.

As discussed by Klaunig et al. (2012), LH was not increased by PFOA at numerous time points during the chronic rat study (Biegel et al., 2001).

The mode of action for pancreatic acinar cell tumor induction by PFOA is also unknown. One possible hypothesis that has been suggested is that these tumors occur as a result of an increase in cholecystokinin secondary to hepatic effects of PFOA, but this has not been demonstrated experimentally (Klaunig et al., 2012).

The USEPA SAB (2006) concluded that insufficient data were available to determine the mode of action for the testicular Leydig cell tumors and the pancreatic acinar cell tumors found in PFOA treated male rats (Biegel et al., 2001). More recently, Klaunig et al. (2012) concluded that, based on information presented above, the available data do not definitively establish a mode of action for these tumors. In the absence of a defined mode of action for these tumor types, the USEPA SAB (2006) concluded that “they must be presumed to be relevant to humans, as suggested by EPA’s Cancer Guidelines,” and this conclusion remains valid at this time.

### **Other possible modes of action**

A number of other modes of action for PFOA have been suggested. Although a complete review of this topic is beyond the scope of this document, information on several potential modes of action is summarized below.

PFOA and other perfluorinated carboxylates of carbon chain lengths C7-C10 inhibited intercellular gap junction communication *in vitro* in cultured rat liver cells, while non-fluorinated fatty acids and PFCs of chain length C2-C5 or C16 and C18 did not (Upham et al., 1998). In further *in vivo* studies, male F-344 rats fed diets containing 0.02% PFOA (resulting in a dose of 37.9 mg/kg/day) for 7 days had significantly increased liver weight and significantly decreased hepatic gap junction intercellular communication, as measured by the distribution of a fluorescent dye that travels through gap junction channels (Upham et al., 2009). Perfluoropentanoic acid (C5) did not cause either of these effects. The authors suggested that disruption of gap junction intercellular communication can lead to tumorigenesis and can be important in tumor promotion. However, Lau et al. (2007) stated that inhibition of gap junction intercellular communication is a widespread

phenomenon, and that these effects of PFOA and other PFCs have not been shown to be species or tissue specific.

The mode of action for PFOA may also involve effects on mitochondria. As discussed above, developmental exposure to PFOA in female mice caused hepatic mitochondrial proliferation and abnormal mitochondrial morphology later in life (Quist et al., 2015). Furthermore, hepatic activity of the mitochondrial enzyme, succinate dehydrogenase, was increased in cynomolgus monkeys treated with PFOA (Butenhoff et al., 2002). Hepatic mitochondrial DNA copy number was also significantly increased in male rats 3 days after a single dose of 100 mg/kg PFOA and after 28 days of exposure to 30 mg/kg/day (Berthiaume and Wallace, 2002; Walters et al., 2009).

Starkov and Wallace (2002) investigated effects of PFOA and other PFCs in isolated mitochondria. The involvement of mitochondria in the mode of action of PFCs was suggested by earlier reports of proliferation of hepatic mitochondrial membranes after PFC exposure and uncoupling of mitochondrial respiration in isolated mitochondria exposed to high concentrations of PFCs (reviewed in Starkov and Wallace, 2002). They found that PFOA and PFOS caused slight effects only at high concentrations (e.g. 100  $\mu$ M for PFOA) which may have resulted from changes in membrane fluidity due to the surfactant properties of PFCs. In contrast, other PFCs were potent uncouplers of oxidative phosphorylation, such as perfluorooctane sulfonamide which had an  $IC_{50}$  of 1  $\mu$ M for this effect.

MicroRNAs (miRNAs) are small non-coding RNAs which affect gene expression by binding to complementary mRNA to block translation or trigger degradation. Changes in miRNA may play a role in various diseases by affecting the expression of relevant genes (Wang et al., 2012; Yan et al., 2014). Recent studies suggest that PFOA may alter miRNA levels in both humans and rodents. Elevated levels of specific miRNAs in serum were associated with serum PFOA concentrations in a study of Chinese residents and workers with PFOA exposures from a fluoropolymer manufacturing facility (Wang et al., 2012). In this study, 63 of the 754 miRNAs that were evaluated were detected in all serum samples. Levels of 9 miRNAs were significantly higher in workers compared to less highly exposed residents. In a further analysis, levels of two of these (miR-26b and miR-199a-3p) were significantly increased in an exposure-related fashion within the worker group. A subsequent study found that levels of circulating miRNAs were increased in male BALB/cJ mice dosed with PFOA for 28 days, and that more miRNAs were affected as PFOA dose increased. Interestingly, the two miRNAs that were associated with PFOA in workers in a dose-related fashion were also increased by PFOA exposure in mice (Yan et al., 2014).

In addition, the mode of action of PFOA may involve effects related to organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and other multispecific transporter proteins such as multidrug resistance-associated proteins (MRPs/Mrps). These highly conserved transporters are present in the membranes of many tissues throughout the body in humans, other mammalian species, and lower vertebrates. They are responsible for the transport of numerous endogenous and xenobiotic compounds both into and out of cells. Substrates for transporter

proteins include fatty acids, hormones, bile acids, drugs, environmental contaminants, and other exogenous and endogenous substances that are critical in homeostatic pathways (Roth et al., 2011).

It is well established that renal tubular secretion and reabsorption of PFOA occur through transport by various OATs and OATPs, and that the slow rate of excretion of PFOA in male (in contrast to female) rats and both genders of humans is due to reabsorption of PFOA by specific renal OATs and/or OATPs (Han et al., 2012). Recent studies have shown that effects of PFOA in organs other than the kidney may involve these transporter proteins as well. PFOA can act as an inhibitor of specific OATs and OATPs (Yang et al., 2009b; 2010), and it induced MRP in cultured human cells (Rusiecka and Skladanowski, 2008) and Mrp in mouse liver (Maher et al., 2008), potentially affecting transport of endogenous substances, drugs, and other transporter substrates. The role of OAT4 in placental transfer of PFOA in humans was recently studied (Kummu et al., 2015). OAT4 is found primarily in human kidney and placenta and has been identified as one of the specific transporters responsible for renal reabsorption of PFOA in humans (Han et al., 2012). The level of placental OAT4 was inversely correlated with the rate of transfer of PFOA from the maternal circulation to fetal circulation in studies of perfused human placenta, suggesting that higher levels of placental OAT4 may protect the fetus from PFOA after maternal exposure (Kummu et al., 2015).

### **Summary of conclusions about human relevance of toxicological effects of PFOA**

Based on the information presented above, the toxicological effects of PFOA are generally considered relevant to humans for the purposes of risk assessment.

#### Hepatic effects

Increased serum levels of liver enzymes are associated with exposure to PFOA in humans. Data from non-human primates, standard strains of rats and mice, PPAR-alpha null mice, and humanized PPAR-alpha mice support the conclusion that hepatic effects of PFOA in experimental animals are relevant to humans for the purposes of risk assessment.

PFOA caused increased relative liver weight in non-human primates, species for which the human relevance of hepatic effects is not in question. In a subchronic study in cynomolgus monkeys, several animals that did not complete the study due to overt toxicity exhibited notably increased liver weight, highly elevated serum liver enzymes and/or severe hepatic toxicity. In this study, hepatic peroxisome proliferating activity was increased in a dose-related fashion. As was noted by the USEPA SAB (2006), the effects of PFOA on both liver weight and peroxisome proliferation were similar in cynomolgus monkeys and rats. In contrast to rodents, the human relevance of hepatic effects mediated by PPAR-alpha in non-human primates is not subject to debate. Potential PPAR-alpha independent pathways for hepatic effects of PFOA have not been thoroughly investigated in non-human primates, although increased activity of a mitochondrial enzyme (succinate dehydrogenase) was reported in the subchronic cynomolgus monkey study.

Data from standard strains of mice and rats clearly show that PPAR-alpha independent mechanisms contribute to the hepatic effects of PFOA. Studies which evaluated both liver weight

and hepatic peroxisome proliferating activity provide data relevant to this question. At the doses and time points evaluated in these studies, the dose-response curves for increased liver weight and hepatic peroxisome proliferating activity did not directly correspond. These data indicate that hepatic effects of PFOA involve PPAR-alpha independent mechanisms even in standard strains of rodents with normal PPAR-alpha status. Furthermore, PFOA caused fatty liver in standard strains of mice and rats, although PPAR-alpha activation causes decreased hepatic lipid accumulation. Additionally, recent studies suggest that the differences in the effects of PFOA on serum lipids in rodents (decreased cholesterol and triglycerides) versus humans (increased cholesterol and triglycerides) may be related to the low-fat diet generally used in laboratory rodent studies versus the higher fat content of a typical Westernized human diet, rather than solely to interspecies differences. Finally, developmental exposure to PFOA caused abnormal mitochondria in livers of a standard strain of laboratory mice, with no evidence of peroxisome proliferation.

Data from studies comparing mice with normal PPAR-alpha status (wild type) and mice lacking PPAR-alpha (PPAR-alpha null) provide further evidence that hepatic effects occur through both PPAR-alpha dependent and PPAR-alpha independent pathways. PFOA causes similar increases in liver weight in wild type and PPAR-alpha null mice. PFOA also causes histopathological changes and increased liver enzymes in PPAR-alpha null mice; the histopathological changes differ from those seen in wild type mice, particularly as related to damage to the bile duct. Additionally, developmental exposures to PPAR-alpha null mice caused persistent histopathological changes in the liver.

Data from mice expressing human PPAR-alpha in the liver indicate that activation of human PPAR-alpha by PFOA causes hepatic effects. PFOA caused similar increases in liver weight in humanized PPAR-alpha mice and wild type mice. Other hepatic effects of PFOA in humanized PPAR-alpha mice include activation of genes associated with PPAR-alpha, and histopathological changes including hepatocellular hypertrophy and single cell necrosis.

PPAR-alpha is known to be active in human fetal tissues, including liver. Fetal liver weight was increased similarly in wild type and PPAR-alpha humanized mice after *in utero* exposure. Additionally, PFOA increased the expression of genes associated with PPAR-alpha in fetal liver to a greater degree in PPAR-alpha humanized mice than in wild type mice.

#### Immune system effects

Data from epidemiological studies suggest that the immune system is a sensitive target for PFOA in humans. PFOA suppresses the immune system in both non-human primates and mice. Data from mouse studies indicate that these effects on the immune system occur through both PPAR-alpha dependent and PPAR-alpha independent modes of action. Both PPAR-alpha dependent and independent effects on the immune system are considered relevant to humans for the purposes of risk assessment. Potentially relevant to this conclusion, lipid-lowering drugs that activate PPAR-alpha have recently been associated with decreased effectiveness of the influenza vaccine in humans, consistent with inhibition of human immune response by agents that activate PPAR-alpha such as PFOA.

### Developmental and reproductive effects

PFOA is associated with decreased fetal growth in humans. PPAR-alpha and other PPARs are present in human fetal tissues and are expected to have important roles in reproduction and development. Therefore, PPAR-alpha mediated effects of PFOA on development are considered relevant to humans for the purposes of risk assessment. Developmental effects of PFOA in rodents appear to occur primarily through PPAR-alpha dependent mechanisms, while some reproductive effects such as full litter resorptions appear to be PPAR-alpha independent. However, high affinity “pure” PPAR-alpha activators (WY and clofibrate) do not cause the developmental effects in mice that were caused by PFOA.

Delayed mammary gland development after developmental exposure is a sensitive endpoint for PFOA toxicity in mice. The rodent is considered a good model for human mammary gland development, and there is no mode of action evidence suggesting that the effects of PFOA on this endpoint are not relevant to humans.

PFOA also causes reproductive toxicity in male mice, and there is no mode of action information to suggest that these effects are not relevant to humans.

### Carcinogenicity

PFOA is associated with testicular and kidney cancer in communities with drinking water exposure after adjustment for smoking and other relevant factors. PFOA caused tumors of the liver, testicular Leydig cells, and pancreatic acinar cells in male rats. USEPA (2006) SAB did not dismiss the potential human relevance of the liver tumors in rats, based on similarities in hepatic effects of PFOA in monkeys and rodents and the limited evidence available at the time for hepatic effects of PFOA in PPAR-alpha null mice. Subsequent studies have provided substantial additional information showing hepatic effects of PFOA in PPAR-alpha null mice. Importantly, hepatic cell proliferation, a causal event for tumor formation, is increased similarly by PFOA in wild type and PPAR-alpha null mice. Although a carcinogenicity bioassay for PFOA has not been conducted in PPAR-alpha null mice, a recent study suggests that developmental exposures to PFOA may cause hepatic tumors in adulthood in this strain. Finally, studies in rainbow trout, a species used as a model for human liver cancer because it lacks PPAR-alpha, suggest that PFOA causes liver tumors through an estrogenic mode of action.

Because the modes of action for the testicular and pancreatic tumors caused by PFOA have not been established, these tumors are considered relevant to humans for the purposes of risk assessment.

### **DEVELOPMENT OF ISGWQC**

ISGWQC developed by NJDEP are intended to be protective for chronic (lifetime) exposure through drinking water. They are based on a one in one million lifetime cancer risk level for carcinogens and no adverse effects from lifetime ingestion for non-carcinogens.

### **Consideration of human epidemiological data**

Both the human epidemiology data and the animal toxicology data were considered as part of the overall weight of evidence for the potential human health effects of PFOA. As reviewed in the Epidemiology section, associations of PFOA with numerous health endpoints have been found in human populations, with evidence supporting multiple criteria for causality for some effects. These health endpoints include non-carcinogenic effects in the general population, and both non-carcinogenic effects and cancer in communities with drinking water exposure. The epidemiologic data for PFOA are notable because of the consistency between results among human epidemiologic studies in different populations, the concordance with toxicological findings in experimental animals, the use of serum concentrations as a measure of internal exposure, the potential clinical importance of the endpoints for which associations are observed, and the observation of associations within the exposure range of the general population and communities with contaminated drinking water. These features of the epidemiologic data distinguish PFOA from most other organic drinking water contaminants and justify concerns about exposures to PFOA through drinking water.

Although the data for some endpoints support multiple criteria for causality, the human epidemiology data have limitations and are therefore not used as the quantitative basis for the ISGWQC. Instead, the potential ISGWQCs developed below are based on sensitive and well-established animal toxicology endpoints that are considered relevant to humans based on mode of action data. Notwithstanding, continued exposure to even relatively low levels of PFOA in drinking water are known to cause substantial increases in PFOA in blood serum, to levels several fold higher than those found in the general population. The considerable evidence for increased risks of health effects from the low-level PFOA exposures prevalent in the general population (e.g. <10 ng/ml, NHANES 2007-2010; CDC 2015) and in communities with contaminated drinking water suggests a need for caution about drinking water exposures that will result in such elevations in serum PFOA level. The human epidemiological data thus support the use of a public health-protective approach in developing an ISGWQC based on animal toxicology data.

### **Weight of Evidence for Carcinogenicity**

PFOA caused tumors in male rats in two chronic studies (Sibinski et al., 1987, Butenhoff et al., 2012; and Biegel et al., 2001). It caused a statistically significant increase in testicular Leydig cell tumors in both studies, as well as a statistically significant increase in liver tumors and pancreatic tumors in Biegel et al. (2001). Human exposure to PFOA has also been associated with increased risk of cancer, including increased risk of kidney and testicular cancer in communities with contaminated drinking water after adjustment for smoking and other relevant factors (Barry et al, 2013; Vieira et al., 2013).

Based on the chronic animal data and mode of action studies available at the time, PFOA was described as “likely to be carcinogenic to humans” by the USEPA Science Advisory Board (USEPA, 2006). PFOA was classified as “possibly carcinogenic to humans” by IARC based on review of data from human epidemiology, animal toxicology, and mechanism of action studies

(Benbrahim-Tallaa et al., 2014). More recently, PFOA was described by the USEPA Office of Water (2016a) as having “suggestive evidence of carcinogenic potential”, a descriptor similar to the classification of “possibly carcinogenic to humans” used by IARC (2016).

The USEPA Guidelines for Carcinogen Risk Assessment (2005b) recommend dose-response modeling and low dose extrapolation for chemicals described as likely to be carcinogenic. For chemicals with suggestive evidence of carcinogenicity, dose-response modeling and low dose extrapolation may be performed when data to support it are available. The guidelines recommend that the risk-based estimates for suggestive carcinogens be used to obtain “a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities.” Risk-based drinking water concentrations based on both non-carcinogenic and carcinogenic endpoints are presented below.

### **Potential ISGWQCs based on non-carcinogenic endpoints**

#### Selection of toxicological endpoints for consideration as basis for potential ISGWQCs

As reviewed in the Toxicology section above, PFOA causes numerous systemic effects in experimental animals. These include liver toxicity, immune system toxicity, adverse developmental effects, and other adverse effects. It was concluded that delayed mammary gland development and increased relative liver weight are the most sensitive systemic endpoints with data appropriate for dose-response modeling.

Delayed mammary gland development from perinatal exposure is the most sensitive systemic endpoint for PFOA with data appropriate for dose-response modeling. It is a well-established toxicological effect of PFOA that is considered to be adverse and relevant to humans for the purposes of risk assessment. An RfD based on this endpoint is presented below.

It is believed that an RfD for delayed mammary gland development has not previously been used as the primary basis for health-based drinking water concentrations or other human health criteria for environmental contaminants. Because the use of this endpoint as the basis for human health criteria is a currently developing topic, it was decided not to recommend an ISGWQC with the RfD for delayed mammary gland development as its primary basis. However, the occurrence of this and other effects at doses far below those that cause increased relative liver weight (the endpoint used as the primary basis for the ISGWQC) clearly requires application of an uncertainty factor to protect for these more sensitive effects.

Increased relative liver weight is a well-established effect of PFOA that is more sensitive than most other toxicological effects such as immune system toxicity and most reproductive/developmental effects (Table 12 of Animal Toxicology section). An ISGWQC with increased liver weight as its primary basis is presented below.

### Selection of studies and data for dose-response modeling

Only those studies that provide serum PFOA data were considered for dose-response modeling of non-carcinogenic effects. A risk assessment approach based on measured serum PFOA levels is less uncertain than one based on pharmacokinetic modeling of estimated serum PFOA levels or an approach in which interspecies extrapolations is based on interspecies half-life differences. For example, as discussed above, serum PFOA concentrations from the same administered dose vary among strains and even between animals of the same strain obtained from different sources. Available approaches based on pharmacokinetic modeling or interspecies half-life conversions do not account for these intra-strain differences.

In some studies, serum PFOA was measured only at time points well after dosing had ended. Serum data from these later time points are not appropriate for dose-response modeling because PFOA body burdens would have decreased, and the serum levels thus do not represent the maximum exposures during the study. Therefore, only studies which provide serum PFOA levels close to the end of the dosing period were considered for dose-response modeling. Use of the maximum serum levels that occurred during the study is a non-conservative approach (i.e. would result in a less stringent, rather than more stringent, risk assessment than other potential approaches such as area under the curve modeling), since the observed toxicological effects could have resulted from the lower average exposures experienced over the entire dosing and post-dosing time periods.

Benchmark dose (BMD) modeling is a quantitative approach commonly used to estimate the lower 95% confidence limit (the BMDL) on the dose corresponding to a minimal response (the benchmark response, BMR) that is consistent with the observed data. The BMDL is considered to be an estimate of the NOAEL, but it is based on the entire dose-response curve for the endpoint of interest rather than just the fixed doses administered in the study. When the necessary data are available, BMD modeling can be performed using serum concentrations instead of administered doses. Serum concentrations are preferable to administered doses as the basis for BMD modeling because they are a better representation of the shape of the internal dose response curve. BMD modeling based on serum PFOA data was used to determine BMDLs for serum PFOA concentrations used as the points of departure (PODs) to develop RfDs for both increased relative liver weight and delayed mammary gland development.

### Reference Dose based on delayed mammary gland development

Delayed mammary gland development in mice from developmental exposures is a sensitive endpoint for PFOA's toxicity. This effect has been reported in nine separate studies presented in five publications (Table 14 of Toxicology section). Only one study (Albrecht et al., 2013), which has several general problematic issues (discussed in Toxicology section) did not find this effect. Gestational and/or lactational exposures to PFOA caused delayed mammary gland development in pregnant dams and/or female offspring in two strains of mice. In one study, this effect was statistically significant in mouse pups exposed to concentrations of 5000 ng/L (5 µg/L) in drinking water *in utero* and after birth. The serum PFOA levels (80 ng/ml in the dams and 20-70 ng/ml in the pups) that resulted in delayed mammary gland development in these mice are relevant to

human serum PFOA levels from contaminated drinking water. Histological changes in the mammary glands of exposed offspring persisted until adulthood and were considered permanent. Delayed mammary gland development occurs in a dose-related fashion, and there is no information indicating it is not relevant to humans. For these reasons, delayed mammary gland development from developmental exposures to PFOA is considered a sensitive and relevant endpoint for dose-response modeling.

Selection of study and data for dose-response modeling of delayed mammary gland development

The late gestational exposure study conducted by Macon et al. (2011) is the only developmental exposure study of mammary gland development that provides serum PFOA data from the end of the dosing period (PND 1) that can be used for dose-response modeling. In this study, pregnant dams were dosed with PFOA (0.01 to 1 mg/kg/day) on GD 10-17. Mammary gland development was assessed on PND 1, 4, 7, 14, and 21, and delays in development were most evident on PND 21. Of the several endpoints related to mammary gland development reported by Macon et al. (2011), two endpoints (decreased mammary gland developmental score and decreased number of terminal end buds) showed a statistically significant dose-related decrease at PND 21 over the dose range studied. These endpoints were selected for dose-response modeling (Table 15).

Table 15. Mammary gland development parameters selected for dose-response modeling from PND 21 female offspring after exposure on GD 10-17 (Macon et al., 2011)			
Dose (mg/kg/day)	Pup Serum PFOA, PND 1 (ng/ml)	Developmental score (1-4)	Number of Terminal End Buds
Control	22.6 ± 5.5	3.3 ± 0.3	40 ± 4
0.01	284.5 ± 21.0	2.2 ± 0.2	33 ± 4
0.1	2303.5 ± 114.1	1.8 ± 0.3	24 ± 4
1.0	16,305.5 ± 873.5	1.6 ± 0.1	15 ± 2

Determination of Point of Departure for delayed mammary gland development

USEPA Benchmark Dose Modeling Software 2.1.2 was used to perform BMD modeling of the data for mammary gland developmental score and number of terminal endbuds at PND 21 from Macon et al. (2011), using serum PFOA data from PND 1 as the dose. Continuous response models were used to obtain the BMD and the BMDL for a 10% change from the mean (the percent change typically used as the BMR) for the two endpoints. Modeling was based on serum levels at PND 1, since they were higher at this time than at later time points. In this study, the serum level in the control group was 22.6 ng/ml, indicating that some PFOA exposure occurred in these non-dosed animals. The BMD and BMDL values presented in Table 16 were derived using this value from the control group (22.6 ng/ml) as the baseline. The serum level BMDs and BMDLs may have been lower if the baseline serum level had been lower (Post et al., 2012).

Compared to controls, the developmental score at PND 21 significantly decreased at all three doses; the number of terminal end buds also decreased at all three doses, with significance at the two higher doses. The BMDLs for the two endpoints, 24.9 and 22.9 ng/ml (Table 16), are close to the NOAEL of 28.5 ng/ml that is estimated by applying a standard uncertainty factor of 10 for LOAEL-to-NOAEL extrapolation to the serum level of 285 ng/ml at the LOAEL, 0.01 mg/kg/day. Outputs of the BMD modeling are provided in Appendix 6.

Table 16. Benchmark Dose modeling of serum PFOA data (PND1) for mammary gland developmental effects (PND 21) in CD-1 mouse pups (Macon et al., 2011) <sup>a</sup>				
Model	Chi-square p-value <sup>b</sup>	Akaike Information Criterion (AIC) <sup>c</sup>	BMD (Serum PFOA, ng/ml)	BMDL (Serum PFOA, ng/ml)
<b>Decreased Mammary Gland Developmental Score</b>				
Hill (non-logged concs.)	0.70	-4.28	57.4	28.1
Polynomial - 2 <sup>nd</sup> deg. (logged concs.)	0.83	-4.39	25.9	24.0
<b>Exponential -model 2 (logged concs.)</b>	<b>0.87</b>	<b>-36.86</b>	<b>25.7<sup>d</sup></b>	<b>24.7<sup>d</sup></b>
Linear (logged concs.)	0.83	-6.05	28.0	26.5
<b>Decreased Number of Terminal End Buds</b>				
Hill (non-logged concs.)	0.47	88.43	235.1	78.5
Exponential - model 4 (non-logged concs.)	0.30	88.97	399.8	110.5
Power (non-logged concs.)	0.62	88.15	64.8	23.4
Power (logged concs.)	0.81	87.96	87.2	29.0
Polynomial – 2 <sup>nd</sup> deg. (logged concs.)	0.84	87.94	96.6	28.1
Linear (logged concs.)	0.12	89.47	27.0	25.7
<b>Exponential – model 3 (logged concs.)</b>	<b>0.98</b>	<b>87.90</b>	<b>25.1<sup>d</sup></b>	<b>22.9<sup>d</sup></b>

<sup>a</sup> Results are shown for all models that gave an acceptable visual fit.

<sup>b</sup> A larger Chi-square p-value indicates a better fit to the data.

<sup>c</sup> AIC: A measure of information loss from a dose-response model that can be used to compare a specified set of models. The AIC is defined as  $-2 \times (\text{LL} - p)$ , where LL is the log-likelihood of the model given the data, and p is the number of parameters estimated in the model. When comparing models, a lower AIC is preferable to a higher one (USEPA, 2012a).

<sup>d</sup> BMDs and BMDLs from the models with the lowest AIC statistic for each endpoint.

#### Application of uncertainty factors for delayed mammary gland development

The choice of uncertainty factors is consistent with current USEPA IRIS guidance (USEPA, 2012c) and previous risk assessments developed by NJDEP.

The BMDL for decreased number of terminal endbuds of 22.9 ng/ml in serum (derived above) was used as the POD for RfD development. Uncertainty factors (UFs) were applied to the POD to obtain the Target Human Serum Level. The Target Human Serum Level (ng/ml in serum) is analogous to an RfD but is expressed in terms of internal dose rather than administered dose.

The total of the uncertainty factors (UFs) applied to the POD serum level was 30, and included the following factors:

10 – UF for human variation, to account for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most sensitive to the effect.

3 – UF for animal-to-human extrapolation, to account for toxicodynamic differences between humans and mice.

The typical uncertainty factor of 3 for toxicokinetic variability between species is not included because the risk assessment is based on comparison of internal dose (serum levels) rather than administered dose.

1 – UF for LOAEL to NOAEL.

The point of departure is a BMDL, not a LOAEL. Therefore, this uncertainty factor is not applied.

1 – UF for duration of exposure.

Delayed mammary gland development occurs from exposures during development. Therefore, an UF to account for effects from longer term exposures is not used.

1 – UF for more sensitive effects that are not considered (e.g. incomplete database).

Because delayed mammary gland development is a sensitive endpoint for PFOA toxicity, the UF is not applied.

The target human serum level is:  $\frac{22.9 \text{ ng/ml}}{30} = 0.8 \text{ ng/ml}$  (800 ng/L).

As discussed above, the 2011-12 median and 95th percentile NHANES values for serum PFOA in the general population are 2.1 and 5.7 ng/ml (CDC, 2015). Therefore, the target human serum level for delayed mammary gland development, 0.8 ng/ml, is below the median serum PFOA level in the U.S. general population.

#### Development of Reference Dose based on delayed mammary gland development

USEPA (2016a) used a pharmacokinetic modeling approach to develop a species-independent clearance factor,  $1.4 \times 10^{-4}$  L/kg/day that relates serum PFOA level ( $\mu\text{g/L}$ ) to human PFOA dose ( $\mu\text{g/kg/day}$ ). As discussed in the Toxicokinetics section, this clearance factor predicts a serum:drinking water ration of 114:1 with average drinking water consumption, consistent with the empirically observed average serum:drinking water ratio of greater than 100:1 in human

populations exposed to PFOA through drinking water. The clearance factor can be used to calculate the RfD, as follows:

$$800 \text{ ng/L} \times 1.4 \times 10^{-4} \text{ L/kg/day} = 0.11 \text{ ng/kg/day}$$

Where: 800 ng/L = Target Human Serum Level

1.4 x 10<sup>-4</sup> L/kg/day = Clearance

0.11 ng/kg/day = RfD

#### ISGWQC based on increased relative liver weight

Increased relative liver weight is a well-established toxicological effect of PFOA in both non-human primates and rodents and is more sensitive than most other toxicological effects. Increased liver weight occurs in newborn animals after *in utero* exposure, during early life from lactational exposure, and from exposures during adulthood. As discussed in the Mode of Action section, PFOA may cause increased relative liver weight through multiple biochemical and cellular pathways. Increased relative liver weight can co-occur with and/or progress to other types of hepatic toxicity and is considered relevant to humans for the purposes of risk assessment.

According to USEPA IRIS guidance (USEPA, 2012c), endpoints that are “adverse, considered to be adverse, or a precursor to an adverse effect” are appropriate as the basis for non-cancer risk assessment. The increased relative liver weight caused by PFOA is usually accompanied by hepatocellular hypertrophy, and it can co-occur with and/or progress to more severe hepatic effects including hepatocellular necrosis, fatty liver, increased serum liver enzymes, and hyperplastic nodules. Additionally, PFOA caused hepatocellular adenomas in chronically exposed male rats in the study conducted by Biegel et al. (2001). Although these tumors were not reported to be increased in male rats in the earlier chronic study (Sibinski, 1987), Butenhoff et al. (2012) noted that these lesions represent a regenerative process and that diagnostic criteria for hepatic hyperplastic nodules have changed since the livers from the study were evaluated in 1986.

Increased relative liver weight in mice can result either from *in utero* exposure during the prenatal period or from lactational exposure during the neonatal period (Wolf et al., 2007; White et al., 2009). In other studies, ultrastructural and/or histopathological changes indicative of liver toxicity persisted until adulthood (age 3 months, Quist et al., 2015; age 18 months, Filgo et al., 2015) in offspring of dams dosed with PFOA during gestation. Hepatocellular hypertrophy and periportal inflammation occurred at doses below those that caused increased liver weight (Quist et al., 2015). It is not known whether these sensitive hepatic effects resulted from *in utero* exposure, lactational exposure, or both. Additionally, results from offspring at age 18 months suggest the possibility of an increased incidence of liver tumors from developmental exposures to PFOA, although the study was not designed as a carcinogenicity bioassay (Filgo et al., 2015). Although data from these studies are not amenable dose-response modeling, they support the conclusions that liver toxicity is a sensitive endpoint for PFOA, that the developmental period is a sensitive lifestage for PFOA’s hepatic effects, and that increased relative liver weight is a relevant and appropriate endpoint for PFOA’s toxicity.

### Selection of study and data for dose-response modeling of increased liver weight

Increased relative liver weight has been observed in many studies of PFOA in both rodents and non-human primates. The five publications reporting studies of relative liver weight that were considered for dose-response modeling are summarized in the first part of Table 10 of the Animal Toxicology section. Studies were included if they provide serum PFOA data from the end of the dosing period, and, for rodent studies, include relatively low doses (1 mg/kg/day or less). Rodent studies that meet these criteria were reported in four publications. The 90-day cynomolgus monkey study in which the lowest dose was 3 mg/kg/day is also included in Table 10 of the Animal Toxicology section for comparison purposes, since it used a non-human primate species and has been the focus of risk assessments by other groups.

The 90-day cynomolgus monkey study (Thomford et al., 2001b; Butenhoff et al., 2002) was not considered appropriate for dose-response modeling for several reasons (discussed in detail in Appendix 3). The study does not provide serum PFOA data that can be used for dose-response modeling because serum PFOA levels did not differ at the two lower doses (3 and 10 mg/kg/day); the high dose (30/20 mg/kg/day) group is excluded for use in dose-response modeling due to overt toxicity. Additionally, the death of one of four animals in the low dose group may have been due to PFOA toxicity. Aside from its lack of utility for dose-response modeling, this study provides no indication of the NOAEL for PFOA toxicity in this species because of the lack of a relationship between administered or internal dose and response, and because of the possibility of overt toxicity at the lowest dose.

Two of the four rodent studies (Loveless et al., 2006; Perkins et al., 2004) used adult male rats, and one of these (Loveless et al., 2006), also used adult male mice. Loveless et al. (2006) administered three different isomeric mixtures of PFOA (linear/branched, linear, and branched) to adult male mice and rats for 2 weeks, while Perkins et al. administered PFOA to adult male rats for 4, 7, or 13 weeks.

As discussed in the Toxicology section, increased relative liver weight associated with hepatocellular hypertrophy is an early manifestation of PFOA's hepatic toxicity. This effect does not appear to increase in magnitude over time, but rather it appears to progress over time to other more severe hepatic effects (Butenhoff et al., 2012). Relative liver weight data from male CD-1 mice after 14 day exposures (Loveless et al., 2006) and 29 day exposures (Loveless et al., 2008) were compared based on administered dose, as Loveless et al. (2008) does not provide serum PFOA levels. This comparison shows that the dose-response curves for increased relative liver weight are similar for the 14 day and 29 day exposure periods. Furthermore, dose-response curves for relative liver weight in male rats were similar after 4, 7, and 13 week exposures (Perkins et al., 2004).

Two additional developmental studies in mice (Lau et al., 2006; Macon et al., 2011) also met the criteria for inclusion in Table 10 of the Toxicology section. Lau et al. (2006) evaluated increased liver weight on GD 18 in pregnant mice dosed with PFOA on GD 1-18. The data for liver weight

and serum PFOA levels in pregnant mice in this publication are not presented in a form that is appropriate for dose-response modeling of increased relative liver weight. Data on absolute liver weight and serum PFOA levels are presented in graphical form in the publication; numerical data for absolute liver weight, and liver weight relative to body weight minus weight of gravid uterus, were obtained from the investigator.

Macon et al. (2011) evaluated relative liver weight on PND 1 in female offspring exposed *in utero* on GD 10-17. Comparison of serum PFOA level LOAELs for increased relative liver weight in neonatal female mice in Macon et al. (2011) and in adult male mice (Loveless et al., 2006) suggest similar sensitivity to this effect at both life stages.

The relative liver weight data from male mice exposed to branched/linear PFOA for 14 days (Loveless et al., 2006) were selected for dose-response modeling. These data are shown in Table 17. The branched/linear isomeric mixture is relevant to environmental contamination and human exposure, and almost all toxicological studies of PFOA used the branched/linear isomeric mixture. An increasing response with dose was observed in mice for increased relative liver weight from branched/linear PFOA over the range of doses used in this study. Data from both the standard strain and PPAR-alpha null strains of mice demonstrate that increased liver weight and other types of hepatic toxicity occur through both PPAR-alpha dependent and independent modes of action in mice, and these effects are considered relevant to humans. As shown in Figure 13 in the Mode of Action section, increased liver weight was not correlated with PPAR-alpha activity in mice in Loveless et al. (2006). As discussed above, relative liver weight does not appear to increase in magnitude with longer exposure durations. Therefore, 14 days is considered to be of sufficient duration, particularly since dose-response modeling is based on serum PFOA level, rather than administered dose, thus avoiding uncertainties about whether internal dose increases with exposures longer than 14 days.

Table 17: Serum PFOA and relative liver weight in Male CD-1 mice dosed with branched/linear PFOA for 14 days (Loveless et al., 2006)		
<b>Dose (mg/kg/day)</b>	<b>Serum PFOA (µg/ml)</b>	<b>Relative Liver Weight (g/100 g)</b>
0	0.04±0.02	5.14±0.27
0.3	10±1.4	6.12±0.25
1	27±5.0	7.92±0.49
3	66±8.6	10.72±0.63
10	190±29	16.27±1.05
30	241±28	18.28±1.57

Determination of Point of Departure (POD) for increased relative liver weight

USEPA Benchmark Dose Modeling Software 2.6.0.88 was used to perform BMD modeling of the data on increased relative liver weight in male mice exposed to linear/branched PFOA from Loveless et al. (2006). BMD and BMDL serum levels were determined for a BMR of a 10% increase in

mean relative liver weight from the control values. All models for continuous data included in the software were run.

Results of the BMD modeling are shown in Table 18, and a more detailed explanation and the complete output of the BMDS software for each model are presented in Appendix 7. Both of the exponential models (models 4 and 5) gave identical fits. These exponential models and the 3<sup>rd</sup> degree polynomial model gave acceptable fits to these data. The 3<sup>rd</sup> degree polynomial model over-fits the data at the high dose, forcing a fit and resulting in a biologically unlikely fit in this area of the dose-response curve. However, the fit of the 3<sup>rd</sup> degree polynomial model at the lower doses (i.e., in the range of the BMD) is regular and biologically appropriate. It is unlikely that the forced fit at the high dose has any significant influence on the fit of the model at the BMD. Although the 3<sup>rd</sup> degree polynomial model gave a slightly better fit than the exponential models and also yielded a slightly lower BMDL, the exponential models produced a highly comparable fit and a similar BMDL. As neither model appears to have a claim to greater biological significance, the point-of-departure was derived as the average of the BMDLs for both of these models. **This yielded an average BMDL of 4,351 ng/ml.**

**Table 18.** Benchmark Dose analysis for a 10% increase in relative liver weight from linear/branched PFOA in male mice (Loveless et al., 2006)<sup>a</sup>

<b>Model</b>	<b>Chi-square p-value<sup>b</sup></b>	<b>AIC<sup>c</sup></b>	<b>BMD (Serum PFOA, ng/ml)</b>	<b>BMDL (Serum PFOA, ng/ml)</b>
<b>Exponential (Models 4 and 5)</b>	<b>0.2636</b>	<b>2.12782</b>	<b>4,904<sup>d</sup></b>	<b>4,466<sup>d</sup></b>
Hill	-	-	-	-
Linear	-	-	-	-
Polynomial (2 <sup>nd</sup> degree)	0.03245 <sup>c</sup>	6.92134	5,317	4,896
<b>Polynomial (3<sup>rd</sup> degree)</b>	<b>0.4678</b>	<b>1.66669</b>	<b>4,682<sup>d</sup></b>	<b>4,236<sup>d</sup></b>
<i><b>Average of Exponential (Models 4 and 5) and Polynomial (3rd degree)</b></i>			<b>4,793</b>	<b>4,351</b>

<sup>a</sup> Results are shown for all models that gave an acceptable visual fit.

<sup>b</sup> A larger Chi-square p-value indicates a better fit to the data.

<sup>c</sup> AIC: A measure of information loss from a dose-response model that can be used to compare a specified set of models. The AIC is defined as  $-2 \times (\text{LL} - p)$ , where LL is the log-likelihood of the model given the data, and p is the number of parameters estimated in the model. When comparing models, a lower AIC is preferable to a higher one (USEPA, 2012a).

<sup>d</sup> BMDs and BMDLs from the models used to derive the point of departure, as discussed in text.

Application of uncertainty factors for increased relative liver weight

The choice of UFs is consistent with current USEPA IRIS guidance (USEPA, 2012c) and previous risk assessments developed by NJDEP.

The BMDL of 4,351 ng/ml was used as the POD for RfD development. UFs were applied to the POD serum level of 4,351 ng/ml to obtain the Target Human Serum Level. The Target Human Serum level (ng/ml serum) is analogous to the RfD but is expressed in terms of internal, rather than administered, dose.

The total of the UFs applied to the POD serum level was 300, and included the following factors:

10 - UF for human variation, to account for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most sensitive to the effect.

3 - UF for animal-to-human extrapolation, to account for toxicodynamic differences between humans and mice.

The typical uncertainty factor of 3 for toxicokinetic variability between species is not included because the risk assessment is based on comparison of internal dose (serum levels) rather than administered dose.

1 - UF for LOAEL to NOAEL.

The point of departure is a BMDL, not a LOAEL. Therefore, an adjustment for use of a LOAEL is not necessary.

1 - UF for duration of exposure.

The POD is based on increased liver weight resulting from exposure for 2 weeks, while the ISGWQC is intended to protect for chronic exposure. However, increased liver weight, usually associated with hepatocellular hypertrophy, is an early manifestation of PFOA's hepatic toxicity. Data from the relevant studies (reviewed above) indicate that the dose-response for this effect, on an internal dose (serum PFOA level) basis, is similar after 2 weeks of exposure and from longer exposures, and that this effect does not appear to occur at lower internal doses (serum PFOA levels) or increase in magnitude with chronic exposures. Rather, the initial effect (increased liver weight accompanied by hepatocellular hypertrophy) appears to progress over time to other more severe hepatic types of effects. Therefore, an adjustment based on duration of exposure is not necessary.

10 - UF for more sensitive effects that are not otherwise considered (e.g. incomplete database).

USEPA IRIS guidance (USEPA, 2012c) states that: "If an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or lifestage, the assessment may apply a database uncertainty factor." Adverse effects on mammary gland development occur at doses much more than 10-fold lower than those that cause increased relative liver weight. Additionally,

hepatic toxicity not associated with increased liver weight occurs at similarly low doses after developmental exposures. Therefore, a UF of 10 to account for more sensitive effects was applied.

The target human serum level is:  $\frac{4351 \text{ ng/ml}}{300} = 14.5 \text{ ng/ml}$  (14,500 ng/L)

#### Development of Reference Dose for increased relative liver weight

As above, the clearance factor ( $1.4 \times 10^{-4}$  L/kg/day; USEPA, 2016a) was used to derive the RfD from the Target Human Serum Level. This factor was used to develop the RfD that is the basis for the ISGWQC. As discussed in the Toxicokinetics section, the clearance factor is consistent with empirical data on the serum:drinking water ratio from communities with contaminated drinking water. It should be noted that health-based drinking water values may also be developed from target human serum levels for PFOA and other PFCs using an approach based on this ratio.

$$14,500 \text{ ng/L} \times 1.4 \times 10^{-4} \text{ L/kg/day} = 2 \text{ ng/kg/day}$$

Where: 14,500 ng/L = Target Human Serum Concentration

$1.4 \times 10^{-4}$  L/kg/day = Clearance Factor

2 ng/kg/day = RfD

#### Relative Source Contribution factor

A Relative Source Contribution (RSC) factor that accounts for non-drinking water sources including food, soil, air, water, and consumer products is used in risk assessments based on non-carcinogenic effects from drinking water exposures. An RSC is used by NJDEP in development of ISGWQCs, as well as by the DWQI for Health-based MCLs, by USEPA for Maximum Contaminant Level Goals, and by other states in development of similar health-based drinking water values. The RSC is intended to prevent total exposure from all sources from exceeding the RfD (USEPA, 2000). When sufficient chemical-specific information on non-drinking water exposures is not available, a default RSC of 0.2 (20%) is used. This default value assumes that 20% of exposure comes from drinking water and 80% from other sources (USEPA, 2000). When sufficient chemical-specific exposure data are available, a less stringent chemical-specific RSC may be derived, with floor and ceiling RSC values of 20% and 80% (USEPA, 2000).

It was concluded that there are insufficient data to develop a chemical-specific RSC for PFOA. There are no New Jersey-specific biomonitoring data for PFOA, and its frequent occurrence in NJ PWS suggests that New Jersey residents may also have higher exposure from non-drinking sources than the U.S. general population (e.g. NHANES). Elevated levels of PFOA were detected in PWS located throughout NJ in USEPA UCMR3 and other monitoring studies; PFOA was detected much more frequently at  $> 20 \text{ ng/L}$  in NJ PWS (10.5%) than nationwide (1.9%) in UCMR3 (discussed in the Drinking Water Occurrence section). Potential sources of this contamination have been identified in some instances, while sources are unknown in other locations. Environmental contamination with PFOA that results in its presence in drinking water can arise from a number of

different types of sources (reviewed in Fate and Transport Relevant to Drinking Water Contamination). These include releases to air, soil, and water from fluoropolymer telomer manufacturing facilities, on-site and off-site disposal from smaller industrial facilities that make products from fluoropolymer dispersions containing PFOA, releases of aqueous firefighting foams, and land application of biosolids from wastewater treatment plants treating waste containing PFOA, among others. These various sources may potentially result in human exposures through contamination of nearby soils, house dust, or other environmental media. In communities with drinking water contamination, consumption of produce from home gardens or grown locally was associated with higher serum levels of PFOA (Emmett et al., 2006a; Holzer et al., 2008; Steenland et al., 2009a).

The exposure factors used to develop the ISGWQC (below) are based on an adult drinking water consumption rate and body weight. The default RSC of 20%, while not explicitly intended for this purpose, also partially accounts for the higher PFOA exposures in infants. Exposures to infants, both breastfed and consuming formula prepared with contaminated drinking water, are much higher than in than older individuals. Infants consume much more fluid (breast milk or formula) than older individuals on a body weight basis; about 10-fold more from birth to 1 month of age, and 4-6 fold more between ages 6-12 months. Additionally, PFOA concentrations in breast milk are similar or higher than in the mother's drinking water source (Post et al., 2012).

For these reasons, although serum levels in infants are similar to their mother's at birth (Post et al., 2012), they increase rapidly by several-fold shortly after birth for a period of at least several months. As shown in Figure 16, this increase was five-fold or greater in a considerable portion of infants evaluated in two studies (Fromme et al., 2010; Mogensen et al., 2015). Additionally, Monte Carlo simulations of results of a pharmacokinetic model predict median, 95<sup>th</sup> percentile, and maximum infant:mother plasma PFOA ratios of 4.5-fold, 7.8-fold, and 15.3-fold, respectively, during the period of greatest infant exposure (Verner et al., 2016a; Figure 17).

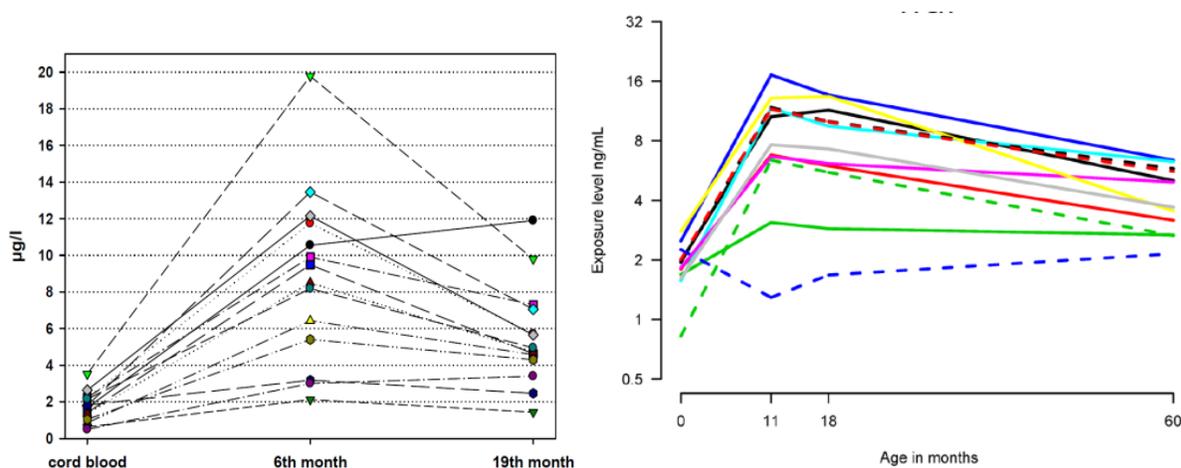


Figure 16. Changes in PFOA levels in breast-fed infants from birth to later timepoints (Fromme et al., 2010; Mogensen et al., 2015)

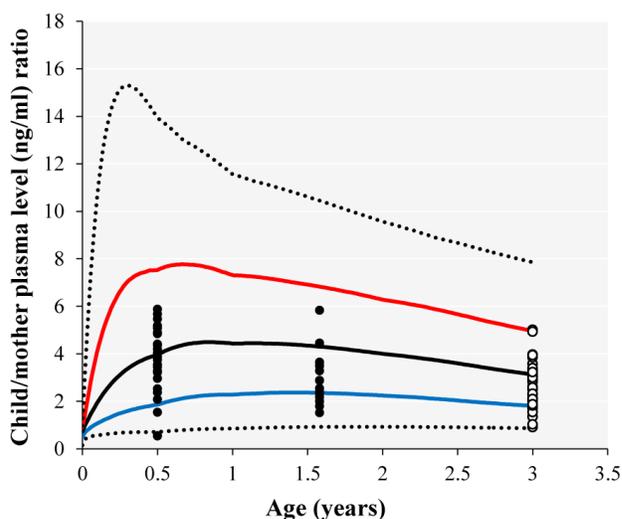


Figure 17. Monte Carlo simulations of child/mother ratios of plasma PFOA levels (ng/ml) a breastfeeding period of 30 months. Black line - 50th percentile; blue line - 5th percentile; red line - 95th percentile; dotted lines - minimum and maximum values (Verner et al., 2016).

These higher infant exposures must be considered because the toxicological effects of concern (delayed mammary gland development and increased relative liver weight) occur from short term exposures relevant to elevated exposures in infancy. Cross-fostering studies (discussed in Toxicology section) show that lactational exposure causes increased relative liver weight and delayed mammary gland development (White et al., 2007; White et al., 2009) in animals with no *in utero* exposure. Additionally, hepatic toxicity that persists until adulthood occurs in offspring of dams exposed to low doses of PFOA during gestation (Filgo et al., 2015; Quist et al., 2015). These effects could result from prenatal or lactational exposure, or both.

For the reasons discussed above, the default RSC of 20% is used to develop the ISGWQC.

Development of the health-based water concentration based on hepatic effects

$$\frac{2 \text{ ng/kg/day} \times 70 \text{ kg} \times 0.2}{2 \text{ L/day}} = 14 \text{ ng/L} \text{ (} 0.014 \text{ } \mu\text{g/L)}$$

Where:

- 2 ng/kg/day = Reference Dose
- 70 kg = assumed adult body weight
- 0.2 = Relative Source Contribution from drinking water
- 2 L/day = assumed adult daily drinking water intake

### **ISGWQC based on carcinogenicity**

PFOA caused tumors in male rats in two chronic studies (Sibinski et al., 1987, Butenhoff et al., 2012; and Biegel et al., 2001). It caused a statistically significant increase in testicular Leydig cell tumors in both studies, as well as a statistically significant increase in liver tumors and pancreatic tumors in Biegel et al. (2001). The testicular tumor data from the chronic dietary exposure rat study reported by Sibinski et al. (1987) and Butenhoff et al. (2012) are appropriate for BMD modeling and can be used as the basis for development of a cancer potency factor. This study used two PFOA dose levels, and the incidence of testicular tumors increased in a dose-related fashion. Because Biegel et al. (2001) used only one dose, data from this study are not appropriate for dose-response modeling. As discussed above, the mode of action for rat testicular tumors has not been established and they are considered relevant to humans for the purposes of risk assessment. USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005b) state that linear low-dose extrapolation should be used for dose-response modeling if the mode of action has not been established. Therefore, the linear low-dose extrapolation approach is appropriate for dose-response modeling of these testicular tumors. The linear low-dose extrapolation approach is based on the assumption that exposure to any dose of a carcinogen results in some risk of cancer.

The incidence of testicular tumors (Sibinski et al., 1987; Butenhoff et al., 2012) was 0/49, 2/50, and 7/50 in the control, 30 ppm (1.3 mg/kg/day), and 300 ppm (14.2 mg/kg/day) groups. Modeling was performed using EPA BMD software (version 2.6.0.86). Because serum PFOA levels were not measured in this study, the BMDL and slope factor were modeled in terms of dose administered to rats. The value based on administered dose to rats was then converted to the equivalent human dose, based on pharmacokinetic differences (ratio of half-lives) between rats and humans. A benchmark response (BMR) of 0.05 (5%) tumor incidence was selected for consistency with the recommendations for selection of the POD for cancer potency slope derivation in the USEPA (2005b) Guidance for Carcinogen Risk Assessment. This value is close to the 4% response at the lowest dose in this data set. Results of the modeling are shown in Table 21, and the complete output from the modeling is found in Appendix 8.

Table 19. BMD modeling (0.05 BMR; 5% response) of rat testicular tumor data (Butenhoff et al., 2012)<sup>a</sup>

<i>Model</i>	<i>Chi-square p-value</i> <sup>b</sup>	<i>AIC</i> <sup>c</sup>	<i>BMD (mg/kg/day)</i>	<i>BMDL (mg/kg/day)</i>	<i>Cancer Potency Slope (mg/kg/day)<sup>-1</sup></i>	<i>Rat Dose at 1 x 10<sup>-6</sup> risk (mg/kg/day)</i>
<b>Gamma (power restricted to <math>\geq 1</math>)</b> <b>Multistage (betas <math>\geq 0</math>)</b> <b>Weibull (power restricted to <math>\geq 1</math>)</b> <b>Quantal linear</b>	<b>0.2292</b>	<b>62.6851</b>	<b>4.42913<sup>d</sup></b>	<b>2.50664<sup>d</sup></b>	<b>0.020</b>	<b>5.0 x 10<sup>-5</sup></b>
Gamma (power unrestricted)	1.00	61.2908	4.42913	1.36483e-6		
Log-logistic (power unrestricted)	1.00	61.2908	1.95859	2.00091e-6		
Logistic	0.1905	63.6843	8.85708	6.49805		
<b>Log-logistic (slope restricted to <math>\leq 1</math>)</b>	<b>0.2338</b>	<b>62.5526</b>	<b>4.02707<sup>d</sup></b>	<b>2.2101<sup>d</sup></b>	<b>0.023</b>	<b>4.3 x 10<sup>-5</sup></b>
Probit	0.1948	63.625	8.32341	5.95965		
Weibull (power unrestricted)	1.00	61.2908	1.97407	1.65976e-6		
<b>Average of Gamma (and other identical models) and Log-logistic (slope restricted to <math>\leq 1</math>)</b>				<b>2.36</b>	<b>0.021</b>	<b>4.8 x 10<sup>-5</sup></b>

<sup>a</sup> Results are shown for all models that gave an acceptable visual fit.

<sup>b</sup> A larger Chi-square p-value indicates a better fit to the data.

<sup>c</sup> AIC: A measure of information loss from a dose-response model that can be used to compare a specified set of models. The AIC is defined as  $-2 \times (\text{LL} - p)$ , where LL is the log-likelihood of the model given the data, and p is the number of parameters estimated in the model. When comparing models, a lower AIC is preferable to a higher one (USEPA, 2012a).

<sup>d</sup> BMDs and BMDLs from the models used to derive the slope factor, as discussed in the text.

The Gamma model with power restricted to  $\geq 1$  (and the other models shown in the same cell in Table 19) and the Log-logistic model with slope restricted to  $\leq 1$  fit the data very similarly and yielded very similar BMDLs. Because neither of these models has a form that is obviously more biologically accurate, it is appropriate to average their BMDLs. The average BMDL for these models is **2.36 mg/kg/day**. For a 5% BMR, the corresponding cancer potency slope is **0.021 (mg/kg/day)<sup>-1</sup>**, and the dose in rats corresponding to a  $1 \times 10^{-6}$  risk is estimated as **4.8 x 10<sup>-5</sup> mg/kg/day**. These values are shown in the last row of Table 19.

As above, the dose-response modeling was based on administered PFOA dose to rats (mg/kg/day) instead of internal dose (serum PFOA level) since serum PFOA levels were not measured in the study. Thus, the rat doses derived through the modeling must be converted to equivalent human doses. As per USEPA (2005b) guidelines for carcinogen risk assessment, this adjustment is made

based on pharmacokinetic differences between species instead of through the default adjustment based on body weight<sup>3/4</sup>. To make the interspecies adjustment, the dose in male rats corresponding to a  $1 \times 10^{-6}$  cancer risk is converted to the human equivalent dose based on the ratio of half-lives in the two species. This approach accounts for the much longer half-life of PFOA in humans than male rats, although it is associated with more uncertainty than an approach using measured serum PFOA levels.

The half-lives used for this adjustment were 7 days for male rats and 2.3 years (840 days) for humans. The half-life in male Sprague-Dawley rats after a single gavage dose of 0.1 to 25 mg/kg PFOA was about 7 days and was independent of dose (Kemper et al., 2003). Bartell et al. (2010a) estimated a human half-life of 2.3 years for a one-year period after exposure to contaminated drinking water ceased; elimination rate was not affected by age or gender. The ratio of these human and rat half-lives (840 days/7 days) is 120.

Therefore, the human dose corresponding to a  $1 \times 10^{-6}$  lifetime cancer risk is estimated as:

$$(4.8 \times 10^{-5} \text{ mg/kg/day}) / 120 = 4 \times 10^{-7} \text{ mg/kg/day} (4 \times 10^{-4} \text{ } \mu\text{g/kg/day}; 0.4 \text{ ng/kg/day})$$

Using default drinking water assumptions (2 L/day water consumption; 70 kg body weight), the health-based drinking water concentration at the  $1 \times 10^{-6}$  lifetime cancer risk level is:

$$\frac{0.4 \text{ ng/kg/day} \times 70 \text{ kg}}{2 \text{ L}} = \mathbf{14 \text{ ng/L}} (0.014 \text{ } \mu\text{g/L})$$

This value is identical to the health-based water concentration based on non-cancer endpoints developed above.

### **ISGWQC**

An ISGWQC based on the RfD for delayed mammary gland development was not developed, for reasons discussed above. The health-based water concentration based on the RfD for increased relative liver weight is 14 ng/L. The health-based water concentration based on a lifetime carcinogenic risk one in one million ( $1 \times 10^{-6}$ ), the cancer risk goal for ISGWQC, is also 14 ng/L.

**Since interim ground water criteria are rounded to one significant figure, the ISGWQC for PFOA is 10 ng/L (0.01  $\mu$ g/L).**

### **DISCUSSION OF UNCERTAINTIES**

- PFOA is associated with multiple human health effects in epidemiology studies of the general population and communities with drinking water exposure. There is evidence to support multiple criteria for causality for some of these endpoints. Although causality cannot be definitively proven for these associations of PFOA with human health effects, these numerous findings indicate the need for caution about drinking water exposures that will increase serum PFOA to levels substantially higher than in the general population. This is particularly true because elevated

serum PFOA levels persist for many years after exposure ends, due to its long human half-life (several years).

Ongoing exposure to the health-based water concentration of 14 ng/L is expected to increase serum PFOA levels, on average, by about 1.1 ng/ml (ppb) with average daily water consumption and 2 ng/ml (ppb) with upper percentile daily water consumption in adults. Increases in serum PFOA levels are predicted to be several-fold higher than in infants than in adults, including both breastfed infants whose mothers ingest PFOA in drinking water or from formula prepared with water contaminated with PFOA.

- The potential for prenatal and early life exposures to environmental contaminants to cause adverse health effects later in life is currently a focus of high interest in both epidemiology and toxicology (Boekelheide et al., 2012; Heindel and Vandenberg, 2015). Developmental exposures to PFOA caused effects in mice, with no threshold (NOAEL) identified, at doses far below those that caused effects in older animals. These effects include persistent changes in the mammary gland, persistent damage to hepatic cells, persistent neurobehavioral effects from a single relatively low dose to the pregnant dam, and obesity and metabolic changes in adulthood. Some of these effects were not evident until later in life and/or adulthood, long after the administered PFOA has been eliminated from the body. Some of these effects (obesity/overweight later in life after prenatal exposure; neurobehavioral effects) have been evaluated in humans, with both positive and negative findings among the studies. As discussed in the Development of ISGWQC section, the Target Human Serum Level based on delayed mammary gland development in mice is below the serum PFOA levels prevalent in the general population. The ISGWQC includes an uncertainty factor to protect for more sensitive developmental effects. However, there is still uncertainty about whether it is sufficiently protective for subtle effects later in life that may result from very low exposures during the developmental period.

- Chronic toxicity and carcinogenicity of PFOA have been studied only in the rat, a species in which PFOA is rapidly excreted by females. There is uncertainty about chronic effects including carcinogenicity in other species such as mice in which PFOA is persistent in both sexes. Furthermore, the chronic studies did not assess effects including carcinogenicity which might result from exposures during the critical developmental stages now known to be sensitive periods for PFOA toxicity.

- Uncertainties about the human relevance of effects seen in animals are inherent to all risk assessments based on animal data. As reviewed in detail in this document, the available information indicates that the effects of PFOA observed in experimental animals are relevant to humans for the purposes of risk assessment.

- Available information indicates that the target organs and modes of action are generally similar for PFOA and some other PFCs, including PFNA (DWQI, 2015c). Therefore, the toxicity of PFOA and other PFCs may be additive. Although PFOA and other PFCs, including PFNA, are

known to co-occur in some NJ public water supplies, the potential for additive toxicity of PFOA and other PFCs was not considered in development of the ISGWQC.

**The ISGWQC for PFOA is 10 ng/L (0.01 µg/L).**

## **CITATIONS**

Abbott, B. D., Wolf, C. J., Schmid, J. E., Das, K. P., Zehr, R. D., Helfant, L., Nakayama, S., Lindstrom, A. B., Strynar, M. J., Lau, C. (2007). Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha. *Toxicol. Sci.* 98: 571–581.

Abbott, B.D. (2009). Review of the expression of peroxisome proliferator-activated receptors alpha (PPAR alpha), beta (PPAR beta), and gamma (PPAR gamma) in rodent and human development. *Reprod. Toxicol.* 27: 246-257.

Abbott, B.D., Wood, C.R., Watkins, A.M., Das, K.P., Lau, C.S. (2010). Peroxisome proliferator-activated receptors alpha, beta, and gamma mRNA and protein expression in human fetal tissues. *PPAR Res.* Volume 2010, Article ID 690907

Abbott, B.D., Wood, C.R., Watkins, A.M, Tatum-Gibbs, K., Das, K.P., Lau, C. (2012). Effects of perfluorooctanoic acid (PFOA) on expression of peroxisome proliferator-activated receptors (PPAR) and nuclear receptor-regulated genes in fetal and postnatal CD-1 mouse tissues. *Reprod Toxicol.* 33: 491-505

Abdellatif, A.G., Preat, V., Taper, H.S., Roberfroid, M. (1991). The modulation of rat liver carcinogenesis by perfluorooctanoic acid, a peroxisome proliferator. *Toxicol. Appl. Pharmacol.* 111: 530-537.

Abdelmegeed, M.A., Yoo, S.H., Henderson L.E., Gonzalez, F.J., Woodcroft, K.J., Song, B.J. (2011). PPAR-alpha expression protects male mice from high fat-induced nonalcoholic fatty liver. *J. Nutr.* 141: 603-10.

Albrecht, P. P., Torsell, N. E., Krishnan, P., Ehresman, D. J., Frame, S. R., Chang, S. C., Butenhoff, J. L., Kennedy, G. L., Gonzalez, F. J., Peters, J. M. (2013). A species difference in the peroxisome proliferator-activated receptor alpha-dependent response to the developmental effects of perfluorooctanoic acid. *Toxicol. Sci.* 131: 568-582.

Alexander, B.H., Olsen, G.W., Burris, J.M., Mandel, J.H., Mandel, J.S. (2003). Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. *Occup. Environ. Med.* 60: 722-729.

Alexander, B.H., Olsen, G.W. (2007). Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers. *Ann. Epidemiol.* 17: 471-478.

Andersen, M.E., Clewell, H.J., Tan, Y., Butenhoff, J. L., Olsen, G. W. (2006). Pharmacokinetic modeling of saturable, renal resorption of Perfluoroalkyl acids in monkeys—Probing the determinants of long plasma half-lives. *Toxicology* 227: 156–164.

Andersen, C.S., Fei, C., Gamborg, M., Nohr, E.A., Sørensen, T.I., Olsen, J. (2010). Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy. *Am. J. Epidemiol.* 172: 1230-1337.

Andersen, C. S., Fei, C., Gamborg, M., Nohr, E. A., Sorensen, T. I., Olsen, J. (2013). Prenatal exposures to perfluorinated chemicals and anthropometry at 7 years of age. *Am. J. Epidemiol.* 178: 921-927.

Anderson-Mahoney, P., Kotlerman, J., Takhar, H., Gray, D., Dahlgren, J.C. (2008). Self-reported health effects among community residents exposed to perfluorooctanoate. *New Solut.* 18: 129-143.

Apelberg, B.J., Witter, F.R., Herbstman, J.B., Calafat, A.M., Halden, R.U., Needham, L.L., Goldman, L.R. (2007). Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ. Health Perspect.* 115: 1670-1676.

Appleman, T.D., Higgins, C. P., Quinones, O., Vanderford, B.J, Kolstad, C., Zeigler-Holady, J.C., Dickenson, E.R. (2014). Treatment of poly- and perfluoroalkyl substances in U.S. full-scale water treatment systems. *Water Res.* 51: 246-255.

ATSDR (2013). Agency for Toxics Substances and Disease Registry. Health Consultation. Exposure Investigation Report. Perfluorochemical serum sampling in the vicinity of Decatur, Alabama. Morgan, Lawrence, and Limestone Counties. April 1, 2013. [http://www.atsdr.cdc.gov/HAC/pha/Decatur/Perfluorochemical\\_Serum%20Sampling.pdf](http://www.atsdr.cdc.gov/HAC/pha/Decatur/Perfluorochemical_Serum%20Sampling.pdf)

ATSDR (2015). Agency for Toxics Substances and Disease Registry. Toxicological Profile for Perfluoroalkyls. Draft for Public Comment. August 2015.

ATSDR. 2018. Agency for Toxics Substances and Disease Registry. Toxicological Profile for Perfluoroalkyls. Draft for Public Comment. June 2018. <https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf>

Backe W.J., Day, T.C., Field, J.A. (2013). Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS. *Environ. Sci. Technol.* 47: 5226-34.

Balakumar, P. and Mahadevan, N. (2015). Interplay between statins and PPARs in improving cardiovascular outcomes: a double-edged sword? *Br. J. Pharmacol.* 165: 373-9.

Barry, V., Winqvist, A., Steenland, K. (2013). Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. *Environ. Health Perspect.* 121: 1313-1318.

Barry, V., Darrow, L.A., Klein, M., Winqvist, A., Steenland, K. (2014). Early life perfluorooctanoic acid (PFOA) exposure and overweight and obesity risk in adulthood in a community with elevated exposure. *Environ. Res.* 132: 62-69.

Bartell, S.M., Calafat, A.M., Lyu, C., Kato, K., Ryan, P.B., Steenland, K. (2010a). Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. *Environ. Health Perspect.* 118: 222-8.

Bartell, S.M., Lyu, C., Ryan, P.B., Steenland, K. (2010b). Decline of perfluorooctanoic acid in human serum before and after granular activated carbon filtration in two public water supplies, and implications for half-life estimation. Presented at PFAA Days III, Research Triangle, Park, NC. June 2010. <http://www.health.state.mn.us/divs/eh/hazardous/topics/pfcs/pfaadays3.pdf> Accessed 1/17/12.

Beesoon, S., Webster G.M., Shoeib, M., Harner, T., Benskin, J.P., Martin, J.W. (2011). Isomer profiles of perfluorochemicals in matched maternal, cord and house dust samples: manufacturing sources and transplacental transfer. *Environ. Health Perspect.* 119: 1659-1664.

Beesoon, S., Martin, J.W. (2015). Isomer-specific binding affinity of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) to serum proteins. *Environ. Sci. Technol.* 49: 5722-31.

Benbrahim-Tallaa, L., Lauby-Secretan, B., Loomis, D., Guyton, K. Z., Grosse, Y., Ghissassi, V., Bouvard, F.E., Guha, N., Mattock, H., Straif, K. (2014). Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone. *Lancet Oncol.* 15: 924-925.

Benninghoff, A.D., Bisson, W.H., Koch, D.C., Ehresman, D.J., Kolluri, S.K., Williams, D.E. (2011). Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro. *Toxicol. Sci.* 120: 42-58.

Benninghoff A.D., Orner, G.A., Buchner, C.H., Hendricks, J.D., Duffy, A.M., Williams, D.E. (2012). Promotion of hepatocarcinogenesis by perfluoroalkyl acids in rainbow trout. *Toxicol Sci.* 125: 69-78.

Benskin, J.P., De Silva, A.O., Martin, L.J., Arsenault, G., McCrindle, R., Riddell, N., Mabury, S.A., Martin, J.W. (2009). Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 1: single dose. *Environ. Toxicol. Chem.* 28: 542-554.

Berthiaume, J. and K. B. Wallace (2002). Perfluorooctanoate, perfluorooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. *Toxicol. Lett.* 129: 23-32

Biegel, L.B., Hurtt, M.E., Frame, S.R., O'Connor, J.C., Cook, J.C. (2001). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol. Sci.* 60: 44-55.

Black, S., Nicolay, U., Del Giudice, G., Rappuoli, R. (2016). Influence of statins on influenza vaccine response in elderly individuals. *J. Infect. Dis.* 213: 1224-8.

Bloom, M. S., Kannan, K. Spliethoff, H. M., Tao, L., Aldous, K. M, Vena, J.E. (2010). Exploratory assessment of perfluorinated compounds and human thyroid function. *Physiol. Behav.* 99: 240-245.

Boekelheide K, Blumberg B, Chapin R.E., Cote I., Graziano J.H., Janesick, A., Lane, R., Lillycrop, K, Myatt, L., States J.C., Thayer, K.A., Waalkes, M.P., Rogers, J.M. (2012). Predicting later-life outcomes of early-life exposures. *Environ. Health Perspect.* 120: 1353-61.

Botelho S.C., Saghafian M, Pavlova S, Hassan M, DePierre J.W., Abedi-Valugerdi M. (2015). Complement activation is involved in the hepatic injury caused by high-dose exposure of mice to perfluorooctanoic acid. *Chemosphere* 129: 225-31.

Braun, J.M., Chen, A., Romano, M.E., Calafat, A.M., Webster, G.M., Yolton, K., Lanphear, B.P. (2016). Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME study. *Obesity (Silver Spring)* 24: 231-7.

Brede, E., Wilhelm, M., Göen, T., Müller, J., Rauchfuss, K., Kraft, M., Hölzer, J. (2010). Two-year follow-up biomonitoring pilot study of residents' and controls' PFC plasma levels after PFOA reduction in public water system in Arnsberg, Germany. *Int. J. Hyg. Environ. Health* 213: 217-223.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr. Environ. Assess. Manag.* 7: 513-541.

Budd, A., Alleva, L., Alsharifi, M., Koskinen, A., Smythe, V., Müllbacher, A., Wood, J., Clark, I. (2007). Increased survival after gemfibrozil treatment of severe mouse influenza. *Antimicrob. Agents. Chemother.* 51: 2965-8.

Buhrke, T., Krüger, E., Pevny, S., Rößler, M., Bitter, K., Lampen, A. (2015). Perfluorooctanoic acid (PFOA) affects distinct molecular signalling pathways in human primary hepatocytes. *Toxicology* 333: 53-62.

Butenhoff, J., Costa, G., Elcombe, C., Farrar, D., Hansen, K., Iwai, H., Jung, R., Kennedy, G., Lieder, P., Olsen, G., Thomford, P. (2002). Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. *Toxicol. Sci.* 69: 244-257.

Butenhoff, J.L., Kennedy, Jr, G.L., Hinderliter, P.M., Lieder, P.H., Jung, R., Hansen, K.J, Gorman, G.S., Noker, P.E., Thomford, P.J. (2004a). Pharmacokinetics of perfluorooctanoate in Cynomolgus monkeys. *Toxicol. Sci.* 82: 394-406.

Butenhoff, J.L., Kennedy, G.L., Frame, S.R., O'Conner, J.C., York, R.G. (2004b). The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. *Toxicology* 196: 95-116.

Butenhoff, J. L., Gaylor, D. W., Moore, J. A., Olsen, G. W., Rodricks, J., Mandel, J. H., Zobel, L. R. (2004c). Characterization of risk for general population exposure to perfluorooctanoate. *Regul. Tox. Pharmacol.* 39: 363-380.

Butenhoff, J. L., Kennedy, G. L. Jr., Chang, S. C. Olsen, G. W. (2012). Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. *Toxicology* 298: 1-13.

Butenhoff, J.L., Kennedy, G.L., Jung, R., Chang, S.C. (2014). Evaluation of perfluorooctanoate for potential genotoxicity. *Toxicology Reports* 1: 252-270.

Butt, C.M., Berger, U., Bossi, R., Tomy, G.T. (2010). Review: Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Sci. Total Environ.* 408: 2936-2965.

Butt, C. M., Muir, D. C., Mabury, S. A. (2014). Biotransformation pathways of fluorotelomer-based polyfluoroalkyl substances: a review. *Environ. Toxicol. Chem.* 33: 243-267.

C8 Science Panel (undated, a). Background Information on Lawsuit Settlement. [http://www.c8sciencepanel.org/panel\\_background.html](http://www.c8sciencepanel.org/panel_background.html) (accessed March 15, 2016).

C8 Science Panel (undated, b). The Science Panel Website. <http://www.c8sciencepanel.org/> (Accessed March 15, 2016).

C8 Science Panel. (2011). Probable Link Evaluation of Preterm Birth and Low Birthweight. [http://www.c8sciencepanel.org/pdfs/Probable\\_Link\\_C8\\_Preterm\\_and\\_LBW\\_birth\\_5Dec2011.pdf](http://www.c8sciencepanel.org/pdfs/Probable_Link_C8_Preterm_and_LBW_birth_5Dec2011.pdf), (accessed March 18, 2016)

C8 Science Panel. (2012). "Probable Link Evaluation of Thyroid Disease." Retrieved February 12, 2016, [http://www.c8sciencepanel.org/pdfs/Probable\\_Link\\_C8\\_Thyroid\\_30Jul2012.pdf](http://www.c8sciencepanel.org/pdfs/Probable_Link_C8_Thyroid_30Jul2012.pdf) (accessed February 12, 2016)

Calafat, A.M., Wong, L.Y., Kuklenyik, Z., Reidy, J.A., Needham, L.L. (2007). Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. *Environ. Health Perspect.* 115: 1596–1602.

California Environmental Protection Agency. 2018. Recommendation for Interim Notification Levels for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS). Office of Environmental Health Hazard Assessment. June 26, 2018. [https://www.waterboards.ca.gov/drinking\\_water/certlic/drinkingwater/documents/pfos\\_and\\_pfoa/OEHHA\\_Recommended\\_Int\\_NL\\_Jun\\_26\\_2018.pdf](https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/documents/pfos_and_pfoa/OEHHA_Recommended_Int_NL_Jun_26_2018.pdf)

California State Water Resources Control Board, 2018. Perfluorooctanoic acid (PFOA) and Perfluorooctanesulfonic acid (PFOS). July 13, 2018. [https://www.waterboards.ca.gov/drinking\\_water/certlic/drinkingwater/PFOA\\_PFOS.html](https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/PFOA_PFOS.html)

CDC (2015). Centers for Disease Control and Prevention. Fourth National Report on Human Exposure to Environmental Chemicals. [http://www.cdc.gov/biomonitoring/pdf/FourthReport\\_UpdatedTables\\_Feb2015.pdf](http://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf).

CDC (2016). Centers for Disease Control and Prevention. National Center for Health Statistics. Diet/Nutrition. Last updated April 27, 2016. <http://www.cdc.gov/nchs/fastats/diet.htm>

Chan, E., Burstyn, I., Cherry, N. Bamforth, F., Martin, J. W. (2011). Perfluorinated acids and hypothyroxinemia in pregnant women. *Environ. Res.* 111: 559-564.

Chang ET, Adami HO, Boffetta P, Cole P, Starr TB, Mandel JS. A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans. *Crit Rev Toxicol.* 2014 May;44 Suppl 1:1-81.

Cheng, X., Klaassen, C. D. (2008). Critical role of PPAR-alpha in perfluorooctanoic acid- and perfluorodecanoic acid-induced downregulation of Oatp uptake transporters in mouse livers. *Toxicol. Sci.* 106: 37-45.

Clarke, B.O., Smith, S.R. (2011). Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environ. Int.* 37: 226-247.

Clewell, H.J. (2006). Application of pharmacokinetic modeling to estimate PFOA exposures associated with measured blood concentrations in human populations. Society for Risk Analysis 2006 Annual Meeting (PowerPoint Presentation).

Clewell, H. (2009). Pharmacokinetic modeling of PFOA and PFOS. PowerPoint presentation to USEPA, Washington DC, September 2009.

Conder, J.M., Hoke, R.A., De Wolf, W., Russell, M.H., Buck, R.C. (2008). Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. *Environ. Sci. Technol.* 42: 995-1003.

Connecticut Department of Public Health. 2016. Drinking Water Action Level for Perfluorinated Alkyl Substances (PFAS). December 12, 2016. [https://portal.ct.gov/-/media/Departments-and-Agencies/DPH/dph/environmental\\_health/eoha/Toxicology\\_Risk\\_Assessment/DrinkingWaterActionLevelPerfluorinatedAlkylSubstances-PFAS.pdf?la=en](https://portal.ct.gov/-/media/Departments-and-Agencies/DPH/dph/environmental_health/eoha/Toxicology_Risk_Assessment/DrinkingWaterActionLevelPerfluorinatedAlkylSubstances-PFAS.pdf?la=en)

Cornelis, C., D'Hollander, W., Roosens, L., Covac, A., Smolders, R., Van Den Heuvel, R., Govarts, E., Van Campenhout, K., Reynders, H., Bervoets, L. (2012). First assessment of population exposure to perfluorinated compounds in Flanders, Belgium. *Chemosphere* 86: 308-314.

Corsini, E., Avogadro, A., Galbiati, V., dell'Agli, M., Marinovich, M., Galli, C. L., Germolec, D. R. (2011). In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). *Toxicol. Appl. Pharmacol.* 250: 108-116.

Corton, J.C. (2010). Mode of action analysis and human relevance of liver tumors induced by PPAR-alpha activation. In: Hsu, C-H, Stedeford, T. (Eds.), *Cancer Risk Assessment: Chemical Carcinogenesis from Biology to Standards Quantification*, John Wiley & Sons, Inc., Hoboken, NJ.

Costa, G., Sartori, S., Consonni, D. (2009). Thirty years of medical surveillance in perfluooctanoic acid production workers. *J. Occup. Environ. Med.* 51: 364-372.

Cui, L., Zhou, Q. F., Liao, C. Y., Fu, J. J., Jiang, G. B. (2009). Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. *Arch. Environ. Contam. Toxicol.* 56: 338-349.

Cui, L., Liao, C. Y., Zhou, Q. F., Xia, T. M., Yun, Z. J., Jiang, G. B. (2010). Excretion of PFOA and PFOS in male rats during a subchronic exposure. *Arch. Environ. Contam. Toxicol.* 58: 205-213.

Daniel, C.W., Silberstein, G.B. (1987). Chapter 1: Postnatal development of the rodent mammary gland. In: *The Mammary Gland Development, Regulation and Function*. Editors: Margaret C. Neville and Charles W. Daniel, Plenum Press, NY, London.

Darrow, L. A., Groth, A. C., Winquist, A. Shin, H. M., Bartell, S. M., Steenland, K. (2016). Modeled perfluorooctanoic acid (PFOA) exposure and liver function in a Mid-Ohio Valley community. *Environ. Health. Perspect.* (in press).

Das, K.P., Zehr, D., Strynar, M., Lindstrom, A., Wambaugh, J., Lau, C. (2010). Pharmacokinetic profiles of perfluorooctanoic acid in mice after chronic exposure. *Toxicologist* 114: 47.

Davis, K.L., Aucoin, M.D., Larsen, B.S., Kaiser, M.A., Hartten, A.S. (2007). Transport of ammonium perfluorooctanoate in environmental media near a fluoropolymer manufacturing facility. *Chemosphere* 67: 2011-2019.

de Cock, M., de Boer, M. R., Lamoree, M., Legler, J., van de Bor, M. (2014). Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants - a Dutch prospective cohort study. *Environ. Health* 13: 106

D'eon, J.C., Crozier, P.W., Furdui, V.I., Reiner, E.J., Libelo, E.L., Mabury, S.A. (2009). Observation of a commercial fluorinated material, the polyfluoroalkyl phosphoric acid diesters, in human sera, wastewater treatment plant sludge, and paper fibers. *Environ. Sci. Technol.* 43: 4589-4594.

D'eon, J.C., Mabury, S.A. (2011a). Exploring indirect sources of human exposure to perfluoroalkyl carboxylates (PFCAs): Evaluating uptake, elimination, and biotransformation of polyfluoroalkyl phosphate esters (PAPs) in the rat. *Environ. Health Perspect.* 119: 344-350.

D'eon, J.C., Mabury, S.A. (2011b). Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? *Environ. Sci. Technol.* 45: 7974-7984.

De Silva, A.O., Benskin, J.P., Martin, L.J., Arsenault, G., McCrindle, R., Riddell, N., Martin, J.W., Mabury, S.A. (2009b). Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 2: Subchronic dose. *Environ. Toxicol. Chem.* 28: 555-567.

Dewitt, J.C., Copeland, C.B., Strynar, M.J., Luebke, R.W. (2008). Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environ. Health Perspect.* 116: 644-650.

Dewitt, J.C., Shnyra, A., Badr, M.Z., Loveless, SE., Hoban, D., Frame, S.R., Cunard, R., Anderson, S.E., Meade, B.J., Peden-Adams, M.M., Luebke, R.W., Luster M.I. (2009a).

Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. *Crit. Rev. Toxicol.* 39: 76-94.

DeWitt, J. C., Copeland, C. B., & Luebke, R. W. (2009b). Suppression of humoral immunity by perfluorooctanoic acid is independent of elevated serum cortisone concentration in mice. *Toxicol. Sci.*, 109: 106-112.

Dewitt, J.C., Peden-Adams, M.M., Keller, J.M., Germolec, D.R. (2012). Immunotoxicity of perfluorinated compounds: recent developments. *Toxicol. Pathol.* 40: 300-11.

DeWitt, J.C., Williams, W.C., Creech, N.J., Luebke, R.W. (2016). Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPAR $\alpha$  and T- and B-cell targeting. *J. Immunotoxicol.* 13: 38-45.

D'Hollander, W., de Voogt, P., De Coen, W., Bervoets, L. (2010). Perfluorinated substances in human food and other sources of human exposure. *Rev. Environ. Contam. Toxicol.* 208: 179-215.

Dixon, D., Reed, C.E., Moore, A.B., Gibbs-Flournoy, E.A., Hines, E.P., Wallace, E.A., Stanko, J.P., Lu, Y., Jefferson, W.N., Newbold, R.R., Fenton, S.E. (2012). Histopathologic changes in the uterus, cervix and vagina of immature cd-1 mice exposed to low doses of perfluorooctanoic acid (PFOA) in a uterotrophic assay. *Reproductive Toxicology* 33:506-12

Domingo, J. L. (2012). Health risks of dietary exposure to perfluorinated compounds. *Environ. Int.* 40: 187-195.

DuPont and URS Diamond Corporate Remediation Group, 2008. Data Assessment Dupont Washington Works (OPPT-2004-0113 PFOA Site-Related Environmental Assessment Program). <http://itp-pfoa.ce.cmu.edu/docs/Data%20Assessment/DA%20Report-1%20100208%20Text%20and%20Tables.pdf>. Accessed 6/16/11.

DuPont (2009). Private Drinking Water Well Survey and Sampling Update. DuPont Chambers Works Facility, Deepwater, New Jersey (Table 1). Data submitted to NJDEP, May 12, 2009.

DWQI (1987). New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendations for Hazardous Contaminants in Drinking Water. March 26, 1987.

DWQI (1994). New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendations for Hazardous Contaminants in Drinking Water. March 26, 1987.

DWQI (2009). New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendations for Hazardous Contaminants in Drinking Water. March, 2009a.

DWQI (2009b). New Jersey Drinking Water Quality Institute. Minutes of January 27, 2009 meeting. <http://www.nj.gov/dep/watersupply/pdf/minutes090127.pdf>

DWQI (2010). New Jersey Drinking Water Quality Institute. Minutes of September 10, 2010 meeting. <http://www.nj.gov/dep/watersupply/pdf/minutes100910.pdf>

DWQI (2015a). New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendations for Perfluorononanoic Acid in Drinking Water. July 1, 2015. <http://www.nj.gov/dep/watersupply/pdf/pfna-recommend-final.pdf>

DWQI (2015b). New Jersey Drinking Water Quality Institute. Recommendation on Perfluorinated Compound Treatment Options for Drinking Water. New Jersey Drinking Water Quality Institute Treatment Subcommittee. June 2015. <http://www.nj.gov/dep/watersupply/pdf/pfna-pfc-treatment.pdf>

DWQI (2015c). New Jersey Drinking Water Quality Institute. Health-Based Maximum Contaminant Level Support Document: Perfluorononanoic Acid (PFNA). New Jersey Drinking Water Quality Institute Health Effects Subcommittee. June 22, 2015. <http://www.nj.gov/dep/watersupply/pdf/pfna-health-effects.pdf>

DWQI (2017). New Jersey Drinking Water Quality Institute. Health-Based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA). February 15, 2017. <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>

ECHA (2013). European Chemical Agency. Member State Committee Support Document for Identification of Pentadecafluorooctanoic Acid (PFOA) as a Substance of Very High Concern Because of its CMR1 and PBT2 Properties. June 14, 2013. <http://echa.europa.eu/documents/10162/8059e342-1092-410f-bd85-80118a5526f5>

ECHA (2015). European Chemical Agency. RAC concludes on PFOA restriction. September 30, 2015. [http://echa.europa.eu/view-article/-/journal\\_content/title/rac-concludes-on-pfoa-restriction](http://echa.europa.eu/view-article/-/journal_content/title/rac-concludes-on-pfoa-restriction)

EFSA (2008) European Food Safety Authority. Opinion of the Scientific Panel on Contaminants in the Food Chain on Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA) and their Salts. EFSA Journal, 2008, Journal number 653: 1-131; available at [http://www.efsa.europa.eu/EFSA/efsa\\_locale-1178620753812\\_1211902012410.htm](http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902012410.htm)

Eggen, T., Moeder, M., Arukwe, A. (2010). Municipal landfill leachates: a significant source for new and emerging pollutants. Sci. Total. Environ. 408: 5147-5157.

Elcombe, C.R., Elcombe, B.M, Foster, J.R., Farrar, D.G., Jung, R., Chang, S.C., Kennedy, G.L., Butenhoff, J.L. (2010). Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats

- following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARalpha and CAR/PXR. *Arch. Toxicol.* 84: 787-798.
- Emmett, E.A., Shofer, F.S., Zhang, H., Freeman, D., Desai, C., Shaw, L.M. (2006a). Community exposure to perfluorooctanoate: Relationships between serum concentrations and exposure sources. *J. Occ. Environ. Med.* 48: 759-770.
- Emmett, E. A., Zhang, H., Shofer, F. S., Freeman, D., Rodway, N. V., Desai, C., Shaw, L. M. (2006b). Community exposure to perfluorooctanoate: relationships between serum levels and certain health parameters. *J. Occup. Environ. Med.* 48: 771-779.
- Eriksen, K. T., Raaschou-Nielsen, O., McLaughlin, K., Lipworth, L., Tjønneland, A., Overvad, K., Sorensen, M. (2013). Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. *PLoS One* 8: e56969.
- Eschauzier, C., Beerendonk, E., Scholte-Veenendaal, P., De Voogt, P. (2012). Impact of treatment processes on the removal of perfluoroalkyl acids from the drinking water production chain. *Environ. Sci. Technol.* 46: 1708-1715.
- Fang, J., Alderman, M. H. (2000). Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971-1992. National Health and Nutrition Examination Survey. *JAMA* 283: 2404-2410.
- Fairley, K.J., Purdy, R., Kearns, S., Anderson, S.E., Meade, B.J. (2007). Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. *Toxicol. Sci.* 97: 375-383.
- Fasano, W.J., Kennedy, G.L., Szostek, B., Farrar, D.G., Ward, R.J., Haroun, L., Hinderliter, P.M. (2005). Penetration of ammonium perfluorooctanoate through rat and human skin in vitro. *Drug Chem. Toxicol.* 28: 79-90.
- Fei, C., McLaughlin, J.K., Tarone, R.E., Olsen, J. (2007). Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. *Environ. Health Perspect.* 115: 1677-1682.
- Fei, C., McLaughlin, J. K., Lipworth, L., Olsen, J. (2010). Maternal concentrations of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) and duration of breastfeeding. *Scand. J. Work Environ. Health* 36: 413-421.
- Fenton, S.E. (2006). Endocrine-disrupting compounds and mammary gland development: early exposure and later life consequences. *Endocrinology* 147: 518-524.

Fenton, S.E., Reiner, J.L., Nakayama, S.F., Delinsky, A.D., Stanko, J.P., Hines, E.P., White, S.S., Lindstrom, A.B., Strynar, M.J., Petropoulou, S.S. (2009). Analysis of PFOA in dosed CD-1 mice. Part 2. Disposition of PFOA in tissues and fluids from pregnant and lactating mice and their pups. *Reprod. Toxicol.* 27: 365-732.

Fenton, S.E., Reed, C., Newbold, R.R. (2012). Perinatal environmental exposures affect mammary development, function, and cancer risk in adulthood. *Annu. Rev. Pharmacol. Toxicol.* 52: 455-79.

Fenton, S.E., Birnbaum, L.S. (2015). Timing of environmental exposures as a critical element in breast cancer risk. *J. Clin. Endocrinol. Metab.* 100: 3245-50.

Filgo, A.J., Quist, E.M., Hoenerhoff, M.J., Brix, A.E., Kissling, G.E., Fenton, S.E. (2015). Perfluorooctanoic acid (PFOA)-induced liver lesions in two strains of mice following developmental exposures: PPAR $\alpha$  is not required. *Toxicol. Pathol.* 43:558-68.

Fisher, M., Arbuckle, T. E., Wade, M., Haines, D. A. (2013). Do perfluoroalkyl substances affect metabolic function and plasma lipids?--Analysis of the 2007-2009, Canadian Health Measures Survey (CHMS) Cycle 1. *Environ. Res.* 121: 95-103.

Fitz-Simon, N., Fletcher, T., Luster, M. I., Steenland, K. Calafat, A. M., Kato, K., Armstrong, B. (2013). Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid. *Epidemiology* 24: 569-576.

Fletcher, T., Steenland, K., Savitz, D. (2009). Status Report: PFOA and immune biomarkers in adults exposed to PFOA in drinking water in the Mid Ohio Valley. [http://www.c8sciencepanel.org/pdfs/Status\\_Report\\_C8\\_and\\_Immune\\_markers\\_March2009.pdf](http://www.c8sciencepanel.org/pdfs/Status_Report_C8_and_Immune_markers_March2009.pdf). Accessed April 12, 2016.

Fletcher, T., Galloway, T. S., Melzer, D., Holcroft, P., Cipelli, R. Pilling, L. C., Mondal, D., Luster, M., Harries, L. W. (2013). Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. *Environ. Int.* 57-58: 2-10.

Frame, S.R., McConnell, E.E. (2003). Review of proliferative lesions of the exocrine pancreas in two chronic studies in rats with ammonium perfluorooctanoate. DuPont-13788. Oct. 16, 2003. (Cited in USEPA, 2005a).

Franko J., Meade, B.J., Frasc, H.F., Barbero, A.M., Anderson, S.E. (2012). Dermal penetration potential of perfluorooctanoic acid (PFOA) in human and mouse skin. *J. Toxicol. Environ. Health A* 75: 50-62.

Fraser, A.J., Webster, T.F., Watkins, D.J., Nelson, J.W., Stapleton, H.M., Calafat, A.M., Kato, K., Shoeib, M., Vieira, V.M., McClean, M.D. (2012). Perfluorinated compounds in serum linked to indoor air in office environments. *Environ. Sci. Technol.* 46: 1209-1215.

Fraser, A. J., Webster, T. F., Watkins, D. J., Strynar, M. J. Kato, K., Calafat, A. M., Vieira, V. M. McClean, M. D. (2013). Polyfluorinated compounds in dust from homes, offices, and vehicles as predictors of concentrations in office workers' serum. *Environ. Int.* 60: 128-136.

Freberg, B.I., Haug, L.S., Olsen, R., Daae, H.L., Hersson, M., Thomsen, C., Thorud, S., Becher, G., Molander, P., Ellingsen, D.G. (2010). Occupational exposure to airborne perfluorinated compounds during professional ski waxing. *Environ. Sci. Technol.* 44: 7723-7728.

Freedman, D. S., Williamson, D. F., Gunter, E. W., Byers, T. (1995). Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study. *Am. J. Epidemiol.* 141: 637-644.

Frisbee, S. J., Brooks, Jr., A. P., Maher, A., Flensburg, P., Arnold, S., Fletcher, T., Steenland, K., Shankar, A., Knox, S. S., Pollard, C., Halverson, J. A., Vieira, V. M., Jin, C., Leyden, K. M., Ducatman, A. M. (2009). The C8 health project: design, methods, and participants. *Environ. Health Perspect.* 117: 1873-1882.

Frisbee, S. J., Shankar, A., Knox, S. S., Steenland, K. D., Savitz, A., Fletcher, T., Ducatman, A. M. (2010). Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project. *Arch. Pediatr. Adolesc. Med.* 164: 860-869.

Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D. (2009). Perfluorinated compounds-exposure assessment for the general population in Western countries. *Int. J. Hyg. Environ. Health.* 212: 239-270.

Fromme, H., Mosch, C., Morovitz, M., Alba-Alejandre, I., Boehmer, S., Kiranoglu, M., Faber, F., Hannibal, I., Genzel-Boroviczény, O., Koletzko, B., Völkel, W. (2010). Pre- and postnatal exposure to perfluorinated compounds (PFCs). *Environ. Sci. Technol.* 44: 7123-7129.

Fujii, Y., Harada, K. H., Koizumi, A. (2013). Occurrence of perfluorinated carboxylic acids (PFCAs) in personal care products and compounding agents. *Chemosphere* 93: 538-544.

Fu, Y., Wang, T., Fu, Q., Wang, P., Lu, Y. (2014). Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. *Ecotoxicol. Environ. Saf.* 106: 246-252.

Fujii, Y., Niisoe, T., Harada, K. H., Uemoto, S., Ogura, Y., Takenaka, K., A. Koizumi, K. (2015). Toxicokinetics of perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans. *J. Occup. Health* 57: 1-12.

Gallo, V., Leonardi, G., Genser, B., Lopez-Espinosa, M. J., Frisbee, S. J., Karlsson, L., Ducatman, A. M., Fletcher, T. (2012). Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate

(PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. *Environ. Health Perspec.* 120: 655-660.

Gao, Y, Fu, J., Cao, H, Wang, Y, Zhang A, Liang Y, Wang, T., Zhao, C, Jiang, G. (2015). Differential accumulation and elimination behavior of perfluoroalkyl Acid isomers in occupational workers in a manufactory in China. *Environ. Sci. Technol.* 49: 6953-62.

Gebbink, W. A., Glynn, A, Darnerud, P. O., Berger, U. (2015a). Perfluoroalkyl acids and their precursors in Swedish food: The relative importance of direct and indirect dietary exposure. *Environ. Pollut.* 198: 108-115.

Gebbink, W. A., Berger, U., Cousins, I. T. (2015b). Estimating human exposure to PFOS isomers and PFCA homologues: the relative importance of direct and indirect (precursor) exposure. *Environ. Int.* 74: 160-169.

Geiger, S. D., Xiao, J., Ducatman, A., Frisbee, S., Innes, K., Shankar, A. (2014). The association between PFOA, PFOS and serum lipid levels in adolescents. *Chemosphere* 98: 78-83.

Geiger, S. D., Xiao, J., Shankar, A. (2013). Positive association between perfluoroalkyl chemicals and hyperuricemia in children. *Am. J. Epidemiol.* 177: 1255-1262.

Genser, B., Teles C.A., Barreto M.L., Fischer, J.E. (2015). Within- and between-group regression for improving the robustness of causal claims in cross-sectional analysis. *Environ. Health* 10: 14:60.

Gewurtz, S.B., Bhavsar, S.P., Crozier, P.W., Diamond, M.L., Helm, P.A., Marvin, C.H. Reiner, E.J. (2009). Perfluoroalkyl contaminants in window film: indoor/outdoor, urban/rural, and winter/summer contamination and assessment of carpet as a possible source. *Environ. Sci. Technol.* 43: 7317–7323.

Gibson, H. M. (1973) Plasma volume and glomerular filtration rate in pregnancy and their relation to differences in fetal growth. *J. Obstet. Gynaecol. Br. Commonw.* 80: 1067-1074.

Gibson, S.J., Johnson, J.D. (1983). Extent and route of excretion of total carbon-14 in pregnant rats after a single oral dose of ammonium 14C-PFOA. Riker Laboratories, St. Paul, MN. USEPA AR-226-0458. (Cited in Hinderliter et al., 2005).

Gilliland, F. D., Mandel, J. S. (1996). Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins, and cholesterol: a study of occupationally exposed men. *Am. J. Ind. Med.* 29: 560-568.

Gleason, J. A., Post, G. B., Fagliano, J. A. (2015). Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. *Environ. Res.* 136: 8-14.

Goldenthal, E.I. (1978). Final report. Ninety-day subacute rhesus monkey toxicity study. International Research and Development Corporation. Study No. 137-090. November 10, 1978. USEPA AR226-0447 (Cited in USEPA, 2005a).

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari J., Zoeller, R.T. (2015). EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocr Rev.* 36: E1-E150.

Gortner, E.G. (1981). Oral teratology study of T-2998CoC in rats. Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment Number: 0681TR0110, December 1981 (Cited in USEPA, 2005).

Gortner, E.G. (1982). Oral teratology study of T-3141CoC in rabbits. Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment Number: 0681TB0398, February 1982 (Cited in USEPA, 2005).

Goss, K.U. (2008). The pKa values of PFOA and other highly fluorinated carboxylic acids. *Environ. Sci. Technol.* 42: 456–458.

Grandjean, P., E. Andersen, W., Budtz-Jorgensen, E., Nielsen, F., Molbak, K., Weihe, P. Heilmann, C. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA* 307: 391-397.

Granum, B., L. Haug, S. Namork, E. Stolevik, S. B., Thomsen, C., Aaberge, I. S., H. van Loveren, I. S., Lovik, M., Nygaard, U. C. (2013). Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J. Immunotoxicol.* 10: 373-379.

Griffith, F.D., Long, J.E. (1980). Animal toxicity studies with ammonium perfluorooctanoate. *Am. Ind. Hyg. Assoc. J.* 41: 576-583.

Guo, Z., Lin, X., Krebs, K.A., Roache, N. (2009). Perfluorocarboxylic acid content in 116 articles of commerce. ORD, USEPA, RTP, NC. EPA/600/R-09/033. March 2009. <http://www.oecd.org/dataoecd/47/50/48125746.pdf>. Accessed 1/17/12.

Guruge, K.S., Taniyasu, S., Yamashita, N., Wijeratna, S., Mohotti, K.M., Seneviratne, H.R., Kannan, K., Yamanaka, N., Miyazaki, S. (2005). Perfluorinated organic compounds in human blood serum and seminal plasma: a study of urban and rural tea worker populations in Sri Lanka. *J. Environ. Monit.* 7: 371-7.

- Gützkow, K.B., Haug, L.S., Thomsen, C., Sabaredzovic, A., Becher, G., Brunborg, G. (2012). Placental transfer of perfluorinated compounds is selective - A Norwegian Mother and Child sub-cohort study. *Int. J. Hyg. Environ. Health* 215: 216-19.
- Guyton, K.Z., Chiu, W.A., Bateson, T. F., Jinot, J., Scott, C.S., Brown, R.C., Caldwell, J. C. (2009). A reexamination of the PPAR- $\alpha$  activation mode of action as a basis for assessing human cancer risks of environmental contaminants. *Environ. Health Perspect.* 117: 1664–1672.
- Halldorsson, T.I., Rytter, D., Haug, L.S., Bech, B.H., Danielsen, I., Becher, G., Henriksen, T.B., Olsen, S.F. (2012). Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. *Env. Health Perspect.* 120: 668-673.
- Hamm, M.P., Cherry, N.M., Chan, E., Martin, J.W., Burstyn, I. (2010). Maternal exposure to perfluorinated acids and fetal growth. *J. Expo. Sci. Environ. Epidemiol.* 20: 589-597.
- Han, H., Snow, T.A., Kemper, R.A., Jepson, G.W. (2003). Binding of perfluorooctanoic acid to rat and human plasma proteins. *Chem. Res. Toxicol.* 16: 775-781.
- Han, X, Kemper, R.A, Jepson, G.W. (2005). Subcellular distribution and protein binding of perfluorooctanoic acid in rat liver and kidney. *Drug Chem. Toxicol.* 28: 197-209.
- Han, X., Nabb, D.L., Russell, M.H., Kennedy, G.L., Rickard, R.W. (2012). Renal elimination of perfluorocarboxylates (PFCAs). *Chem. Res. Toxicol.* 25: 35-46.
- Hanhijarvi, H., Ylinen, M., Haaranen, T., Nevalainen, T. (1988). A proposed species difference in the renal excretion of perfluorooctanoic acid in the beagle dog and rat. In: Beynen, A.C. Solleveld, H.A. (Eds.), *New Development in Biosciences: Their Implications for Laboratory Animal Sciences*, Martinus Nijhoff Publishers, Dodrecht, The Netherlands, pp. 409–412. (Cited in Lau et al., 2007).
- Hansen, K.J., Johnson, H.O., Eldridge, J.S., Butenhoff, J.L., Dick, L.A. (2002). Quantitative characterization of trace levels of PFOS and PFOA in the Tennessee River. *Environ. Sci. Technol.* 36: 1681-1685.
- Hanssen, L., Röllin, H., Odland, J.O., Moe, M.K., Sandanger, T.M. (2010). Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: results of a pilot study. *J. Environ. Monit.* 12: 1355 – 1361.
- Harada, K.H., Koizumi, A. (2009). Environmental and biological monitoring of persistent fluorinated compounds in Japan and their toxicities. *Environ Health Prev Med.* 14, 7-19.

Hardisty, J.F., Willson, G.A., Brown, W.R., McConnell, E.E., Frame, S.R., Gaylor, D.W., Kennedy, G.L., Butenhoff, J.L. 2010. Pathology Working Group review and evaluation of proliferative lesions of mammary gland tissues in female rats fed ammonium perfluorooctanoate (APFO) in the diet for 2 years. *Drug Chem. Toxicol.* 33: 131-137.

Haug, L.S., Thomsen, C., Becher, G. (2009). Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. *Environ. Sci. Technol.* 43: 2131-2136.

Haug, L.S., Salihovic, S., Jogsten, I.E., Thomsen, C., van Bavel, B., Lindström, G., Becher, G., (2010a). Levels in food and beverages and daily intake of perfluorinated compounds in Norway. *Chemosphere* 80: 1137-1143.

Haug, L.S., Thomsen, C., Brantsaeter, A.L., Kvalem, H.E., Haugen, M., Becher, G., Alexander, J., Meltzer, H.M., Knutsen, H.K. (2010b). Diet and particularly seafood are major sources of perfluorinated compounds in humans. *Environ. Int.* 36: 772-778.

Haughom, B., Spydevold, O. (1992). The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibrilic acid. *Biochim. Biophys. Acta* 1128: 65-72.

Haug, L.S., Huber, S., Becher, G., Thomsen, C. (2011). Characterisation of human exposure pathways to perfluorinated compounds - comparing exposure estimates with biomarkers of exposure. *Environ. Int.* 37: 687-693.

Heindel, J.J., Vandenberg, L.N. (2015) Developmental origins of health and disease: a paradigm for understanding disease cause and prevention. *Curr. Opin. Pediatr.* 27: 248-53.

Hemat, H., Wilhelm, M., Völkel, W., Mosch, C., Fromme, H., Wittsiepe, J. (2010). Low serum levels of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) in children and adults from Afghanistan. *Sci. Total Environ.* 408: 3493-3495.

Hinderliter, P.M., Mylchreest, E., Gannon, S.A., Butenhoff, J.L., Kennedy, G.L. Jr. (2005). Perfluorooctanoate: Placental and lactational transport pharmacokinetics in rats. *Toxicology*. 211: 139-48.

Hinderliter, P.M., Han, X., Kennedy, G.L., Butenhoff, J.L. (2006). Age effect on perfluorooctanoate (PFOA) plasma concentration in post-weaning rats following oral gavage with ammonium perfluorooctanoate (APFO). *Toxicology* 225: 195-203.

Hines, E.P., White, S.S., Stanko, J.P., Gibbs-Flournoy, E.A., Lau, C., Fenton, S.E. (2009). Phenotypic dichotomy following developmental exposure to perfluorooctanoic acid (PFOA) in

female CD-1 mice: Low doses induce elevated serum leptin and insulin, and overweight in mid-life. *Mol. Cell. Endocrinol.* 304: 97-105.

Hoffman, K., Webster, T.F., Weisskopf, M.G., Weinberg, J., Vieira, V.M. (2010). Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children aged 12-15 years. *Environ. Health Perspect.* 118: 1762-1767.

Hoffman, K., Webster, T.F., Bartell, S.M., Weisskopf, M.G., Fletcher, T., Vieira, V.M. (2011). Private drinking water wells as a source of exposure to PFOA in communities surrounding a fluoropolymer production facility. *Environ. Health Perspect.* 119: 92-97.

Hölzer, J., Midasch, O., Rauchfuss, K., Kraft, M., Reupert, R., Angerer, J., Kleeschulte, P., Marschall, N., Wilhelm, M. (2008). Biomonitoring of perfluorinated compounds in children and adults exposed to perfluorooctanoate-contaminated drinking water. *Environ. Health Perspect.* 116: 651-657.

Hölzer, J., Göen T, Just, P., Reupert, R., Rauchfuss, K., Kraft, M., Müller, J., Wilhelm, M. (2011). Perfluorinated compounds in fish and blood of anglers at Lake Möhne, Sauerland area, Germany. *Environ. Sci. Technol.* 45: 8046-8052.

Howard, B.A., Gusterson, B.A. (2000). Human breast development. *J Mammary Gland Biol Neoplasia.* 5: 119-37.

Hu, Q., Strynar, M. J., DeWitt, J. C. (2010). Are developmentally exposed C57BL/6 mice insensitive to suppression of TDAR by PFOA? *J. Immunotoxicol.* 7: 344-349.

Hu, Q., Franklin, J. N., Bryan, I., Morris, E., Wood, A., DeWitt, J. C. (2012). Does developmental exposure to perfluorooctanoic acid (PFOA) induce immunopathologies commonly observed in neurodevelopmental disorders? *Neurotoxicology* 33: 1491-1498.

Hundley, S.G., Sarrif, A.M., Kennedy, G.L. (2006). Absorption, distribution, and excretion of ammonium perfluorooctanoate (APFO) after oral administration to various species. *Drug Chem. Toxicol.* 29: 137-145.

Hurley, S., Houtz, E., Goldberg, D., Wang, M., Park, J-S., Nelson, D.O., Reynolds, P., Bernstein, L., Anton-Culver, H., Horn-Ross, P., Petreas, M. (2016). Preliminary associations between the detection of perfluoroalkyl acids (PFAAs) in drinking water and serum concentrations in a sample of California women. In press. DOI: 10.1021/acs.estlett.6b00154

IARC (2016). International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 110. Perfluorooctanoic acid. p. 37-110.

IOM (2011). *Breast Cancer and the Environment: A Life Course Approach.* Institute of Medicine of the National Academies. The National Academies Press, Washington, DC. Prepublication

copy, December 7, 2011. [https://download.nap.edu/catalog.php?record\\_id=13263](https://download.nap.edu/catalog.php?record_id=13263). Accessed December 7, 2011.

ITRC. 2018. Interstate Technology and Regulatory Council. PFAS Fact Sheets. <https://pfas-1.itrcweb.org/fact-sheets/> Last updated 7/16/18.

Iwai, H., Yamashita, K. (2006). A fourteen-day repeated dose oral toxicity study of APFO in rats. *Drug Chem. Toxicol.* 29: 323-332.

Jain, R. B. (2013). Association between thyroid profile and perfluoroalkyl acids: data from NHNAES 2007-2008. *Environ. Res.* 126: 51-59.

Ji, K., Kim, S., Kho, Y., Paek, D., Sakong, J., Ha, J., Kim, S., Choi, K. (2012). Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones." *Environ. Int.* 45: 78-85.

Jiang, W., Zhang, Y., Zhu, L., Deng, J. (2014). Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. *Environ. Int.* 64: 40-47.

Joensen, U.N., Bossi, R., Leffers, H., Jensen, A.A., Skakkebaek, N.E., Jørgensen N. (2009). Do perfluoroalkyl compounds impair human semen quality? *Environ. Health Perspect.* 117: 923-927.

Johansson, N., Fredriksson, A., Eriksson, P. (2008). Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. *Neurotoxicology* 29: 160-169.

Johansson, N., Eriksson, P., Viberg, H. (2009). Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. *Toxicol. Sci.* 108: 412-418.

Johnson, J. D., Gibson, S. J., Ober, R. E. (1979). Extent and route of excretion and tissue distribution of total carbon-14 in rats after a single i.v. dose of FC-95-14C. Riker Laboratories, Inc., St Paul, MN, US EPA Administrative Record, 8EHQ-1180-00374 (Cited in Lau et al., 2007).

Johnson, J.D., Gibson, S.J., Ober, R.E. (1984). Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [14C] perfluorooctanoate or potassium [14C] perfluorooctanesulfonate. *Fundam. Appl. Toxicol.* 4: 972-976.

Johnson, P.I., Sutton, P., Atchley, D.S., Koustas, E., Lam, J., Sen, S., Robinson, K.A., Axelrad, D.A., Woodruff, T.J. (2014). The Navigation Guide - evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth. *Environ. Health Perspect.* 122: 1028-39.

Kataria, A., Trachtman, H., Malaga-Diequez, L., Trasande, L. (2015). Association between perfluoroalkyl acids and kidney function in a cross-sectional study of adolescents. *Environ. Health* 14: 89.

Kato, K., Calafat, A.M., Wong, L.Y., Wanigatunga, A.A., Caudill, S.P., Needham, L.L. (2009). Polyfluoroalkyl compounds in pooled sera from children participating in the National Health and Nutrition Examination Survey 2001-2002. *Environ. Sci. Technol.* 43: 2641-2647.

Kato, K., Wong, L.Y., Jia, L.T., Kuklennyik, Z., Calafat, A.M. (2011). Trends in exposure to polyfluoroalkyl chemicals in the U.S. population: 1999-2008. *Environ. Sci. Technol.* 45: 8037-8045.

Kato, K., Ye, X., Calafat, A.M. (2015). PFASs in the general population. In: *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*. J.D. DeWitt, Editor. Humana Press. pp. 51-76.

Kawashima, Y., Kobayashi, H., Miura, H., Kozuka, H. (1995). Characterization of hepatic responses of rat to administration of perfluorooctanoic and perfluorodecanoic acids at low levels. *Toxicology* 99: 169-178.

Kemper, R.A. (2003). Perfluorooctanoic acid: Toxicokinetics in the rat. Association of Plastics Manufactures of Europe. Submitted to the U.S. Environmental Protection Agency's Administrative Record. AR226-1499. Cited in USEPA, 2005a.

Kemper, R.A., Jepson, G.W. (2003). Pharmacokinetics of PFOA in male and female rats. *Toxicologist* 72: 148.

Kennedy, G.L. (1985). Dermal toxicity of ammonium perfluorooctanoate. *Toxicol. Appl. Pharmacol.* 81: 348-355.

Kennedy, G.L. Jr., Hall, G.T., Brittelli, M.R., Barnes, J.R., Chen, H.C. (1986). Inhalation toxicity of ammonium perfluorooctanoate. *Food Chem. Toxicol.* 24: 1325-1329.

Kennedy, G.L., Butenhoff, J.L., Olsen, G.W., O'Connor, J.C., Seacat, A.M., Perkins, R.G., Biegel, L.B., Murphy, S.R., Farrar, D.G. (2004). The toxicology of perfluorooctanoate. *Crit. Rev. Toxicol.* 34: 351-384.

Kielsen, K., Shamim, Z., Ryder, L. P., Nielsen Z. F., Grandjean, P., Budtz-Jorgensen, E., Heilmann, C. (2015). Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. *J. Immunotoxicol.* 13: 270-273.

Kim, S.K., Kannan, K. (2007). Perfluorinated acids in air, rain, snow, surface runoff, and lakes: relative importance of pathways to contamination of urban lakes. *Env. Sci. Technol.* 41: 8328–8334.

Kim, S.K., Lee, K.T., Kang, C.S., Tao, L., Kannan, K., Kim, K.R., Kim, C.K., Lee, J.S., Park, P.S., Yoo, Y.W., Ha, J.Y., Shin, Y.S., Lee, J.H. (2011). Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. *Environ. Pollut.* 159: 169-174.

Kjeldsen L.S., Bonefeld-Jørgensen E.C. (2013). Perfluorinated compounds affect the function of sex hormone receptors. *Environ. Sci. Pollut. Res. Int.* 20: 8031-44.

Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A. (2003). PPAR alpha agonist-induced rodent tumors: modes of action and human relevance. *Crit. Rev. Toxicol.* 33: 655-780.

Klaunig, J. E., Hocevar, B. A., Kamendulis, L. M. (2012). Mode of Action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and human relevance. *Reprod. Toxicol.* 33: 410-418.

Kleemann, R., Gervois, P.P., Verschuren, L., Staels, B., Princen, H.M., Kooistra, T. (2003). Fibrates down-regulate IL-1-stimulated C-reactive protein gene expression in hepatocytes by reducing nuclear p50-NFkappa B-C/EBP-beta complex formation. *Blood.* 101: 545-551.

Klein, R., Klein, B. E., Cornoni, J. C., Maready, J., Cassel, J. C., Tyroler, H. A. (1973). Serum uric acid. Its relationship to coronary heart disease risk factors and cardiovascular disease, Evans County, Georgia. *Arch. Intern. Med.* 132: 401-410.

Knobeloch, L., Imm, P., Anderson, H. (2012). Perfluoroalkyl chemicals in vacuum cleaner dust from 39 Wisconsin homes. *Chemosphere* 88: 779-783.

Knox, S. S., Jackson, T., Frisbee, S. J. Javins, B., Ducatman, A. M. (2011). Perfluorocarbon exposure, gender and thyroid function in the C8 Health Project. *J. Toxicol. Sci.* 36: 403-410.

Konwick, B.J., Tomy, G.T., Ismail, N., Peterson, J.T., Fauver, R.J., Higginbotham, D., Fisk, A.T. (2008). Concentrations and patterns of perfluoroalkyl acids in Georgia, USA surface waters near and distant to a major use source. *Environ. Toxicol. Chem.* 27: 2011-2018.

Koskela, A., Finnilä, M.A., Korkalainen, M., Spulber, S., Koponen, J., Håkansson, H., Tuukkanen, J., Viluksela, M. (2016). Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. *Toxicol. Appl.*

Pharmacol. 301:14-21.

Kotthoff, M., Müller, J., Jüriling, H., Schlummer, M., Fiedler, D. (2015). Perfluoroalkyl and polyfluoroalkyl substances in consumer products. *Environ. Sci. Pollut. Res. Int.* 22: 14546-59.

Kousta E, Lam, J., Sutton, P., Johnson, P.I., Atchley, D.S., Sen, S., Robinson, K.A., Axelrad, D.A., Woodruff, T. J. (2014). The Navigation Guide - evidence-based medicine meets environmental health: systematic review of nonhuman evidence for PFOA effects on fetal growth. *Environ. Health Perspect.* 122: 1015-27.

Kudo, N., Kawashima, Y. (1997). Fish oil-feeding prevents perfluorooctanoic acid-induced fatty liver in mice. *Toxicol. Appl. Pharmacol.* 145: 285-293.

Kudo, N., Katakura, M., Sato, Y., Kawashima, Y. (2002). Sex hormone-regulated renal transport of perfluorooctanoic acid. *Chem. Biol. Interact.* 139: 301-316.

Kudo, N., Kawashima, Y. (2003). Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. *J. Toxicol, Sci.* 28: 49-57.

Kudo, N., Sakai, A., Mitsumoto, A., Hibino, Y., Tsuda, T., Kawashima, Y. (2007). Tissue distribution and hepatic subcellular distribution of perfluorooctanoic acid at low dose are different from those at high dose in rats. *Biol. Pharm. Bull.* 30: 1535-40.

Kummu, M., Sieppi, E., Koponen, J., Laatio, L., Vähäkangas, K., Kiviranta, H., Rautio, A., Myllynen, P. (2015). Organic anion transporter 4 (OAT 4) modifies placental transfer of perfluorinated alkyl acids PFOS and PFOA in human placental ex vivo perfusion system. *Placenta* 36: 1185-91.

LabDiet. Laboratory Rodent Diet 5001.

[http://www.labdiet.com/cs/groups/lolweb/@labdiet/documents/web\\_content/mdrf/mdi4/~edisp/duc m04\\_028021.pdf](http://www.labdiet.com/cs/groups/lolweb/@labdiet/documents/web_content/mdrf/mdi4/~edisp/duc m04_028021.pdf). Accessed April 10, 2016.

Lam, J., Kousta, E., Sutton, P., Johnson, P. I., Atchley, D. S., Sen, S., Robinson, K. A., Axelrad, D. A., Woodruff, T. J. (2014). The Navigation Guide - evidence-based medicine meets environmental health: integration of animal and human evidence for PFOA effects on fetal growth. *Environ. Health Perspect.* 122: 1040-1051.

Landsteiner, A., Huset, C., Johnson, J., Williams, A. (2014). Biomonitoring for perfluorochemicals in a Minnesota community with known drinking water contamination. *J. Environ. Health* 77: 14-19.

Lau, C., Strynar, M.J., Lindstrom, A.B., Hanson, R.G., Thibodeaux, J.R., Barton, H.A. (2005). Pharmacokinetic evaluation of perfluorooctanoic acid in the mouse. *Toxicologist* 84: 252.

Lau, C., Thibodeaux, J. R., Hanson, R. G., Narotsky, M. G., Rogers, J. M., Lindstrom, A. B., Strynar, M. J. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol. Sci.* 90: 510–518.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J. (2007). Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol. Sci.* 99: 366-394.

Lau, C. (2012). Perfluorinated compounds. *EXS* 101, 47–86

Lechner, M., Knapp, H. (2011). Carryover of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from soil to plant and distribution to the different plant compartments studied in cultures of carrots (*Daucus carota* ssp. *Sativus*), potatoes (*Solanum tuberosum*), and cucumbers (*Cucumis Sativus*). *J. Agric. Food Chem.* 59: 11011-11018.

Lee, S.S., Pineau, T., Drago, J., Lee, E.J., Owens, J.W., Kroetz, D.L., Fernandez-Salguero, P.M., Westphal, H., Gonzalez, F.J. (1995). Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* 15: 3012-3022.

Lee, H., D'eon, J., Mabury, S.A., (2010). Biodegradation of polyfluoroalkyl phosphates as a source of perfluorinated acids to the environment. *Env. Sci. Technol.* 44: 3305-3310.

Lee, H., Mabury, S.A. (2011). A pilot survey of legacy and current commercial fluorinated chemicals in human sera from United States donors in 2009. *Environ. Sci. Technol.* 45: 8067-8074.

Leonard, R. C. (2003). Epidemiology Surveillance Program. Cancer Incidence Report 1959-2001 and All-Case Mortality Report 1957-2000 at the Washington Works, Parkersburg, WV. DuPont. USEPA. AR226-1307-6.

Leonard, R. C., Kreckmann, K. H., Sakr, C. J., Symons, J. M. (2008). Retrospective cohort mortality study of workers in a polymer production plant including a reference population of regional workers. *Ann. Epidemiol.* 18: 15-22.

Li, Y., Ramdhan, D. H., Naito, H., Yamagishi, N., Ito, Y., Hayashi, Y., Yanagiba, Y., Okamura, A., Tamada, H., Gonzalez, F. J., Nakajima, T. (2011). Ammonium perfluorooctanoate may cause testosterone reduction by adversely affecting testis in relation to PPARalpha. *Toxicol. Lett.* 205: 265-272.

Lien, G.W., Wen, T.W., Hsieh, W.S., Wu, K.Y., Chen, C.Y., Chen, P.C. (2011). Analysis of perfluorinated chemicals in umbilical cord blood by ultra-high performance liquid chromatography/tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 879: 641-646.

Lin, C. Y., Lin, L. Y., Chiang, C. K., Wang, W. J., Su, Y. N., Hung, K. Y., Chen, P. C. (2010). Investigation of the associations between low-dose serum perfluorinated chemicals and liver enzymes in US adults. *Am. J. Gastroenterol.* 105: 1354-1363.

Lin, C. Y., Lin, L. Y., Wen, T. W., Lien, G. W., Chien, K. L., Hsu, S. H., Liao, C. C., Sung, P., Chen, C., Su, C. (2013). Association between levels of serum perfluorooctane sulfate and carotid artery intima-media thickness in adolescents and young adults. *Int. J. Cardiol.* 168: 3309-3316.

Lin, C. Y., Wen, L. L., Lin, L. Y., Wen, T. W., Lien, G. W., Chen, C. Y., Hsu, S. H., Chien, K. L., Sung, F. C., Chen, P. C., Su, T. C. (2011). Associations between levels of serum perfluorinated chemicals and adiponectin in a young hypertension cohort in Taiwan. *Environ. Sci. Technol.* 45: 10691-10698.

Lin, C. Y., Wen, L. L., Lin, L. Y., Wen, T. W., Lien, G. W., Hsu, S. H., Chien, K. L., Liao, C.C., Sung, F.C., Chen, P. C. and Su, T. C. (2013). The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults. *J. Hazard. Mater.* 244-245: 637-644.

Lindstrom, A.B., Strynar, M.J., Libelo, E.L. (2011a). Polyfluorinated compounds: Past, present, and future. *Environ. Sci. Technol.* 45: 7954-7961.

Lindstrom, A.B., Strynar, M.J., Delinsky, A.D., Nakayama, S.F., McMillan, L., Libelo, E.L., Neill, M., Thomas, L. (2011b). Application of WWTP biosolids and resulting perfluorinated compound contamination of surface and well water in Decatur, Alabama, USA. *Environ. Sci. Technol.* 45: 8015-8021.

Liu, J., Li, J. Zhao, Y., Wang, X., Zhang, L., Wu, Y. (2010). The occurrence of perfluorinated alkyl compounds in human milk from different regions of China. *Environ. Int.* 36: 433-438.

Liu, J., Li, J., Liu, Y., Chan, H.M., Zhao, Y., Cai, Z., Wu, Y. (2011). Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. *Environ Int.* 37: 1206-1212.

Liu, W, Yang, B, Wu, L, Zou, W, Pan, X, Zou, T, Liu, F, Xia, L, Wang, X, Zhang, D. (2015). Involvement of NRF2 in Perfluorooctanoic Acid-Induced Testicular Damage in Male Mice. *Biol. Reprod.* 93: 41.

Llorca, M., Farré, M., Picó, Y., Teijón, M.L., Alvarez, J.G., Barceló, D. (2010). Infant exposure of perfluorinated compounds: levels in breast milk and commercial baby food. *Environ. Int.* 36: 584-592.

Looker, C., Luster, M. I., Calafat, A. M., Johnson, V. J., Burlison, G. R., Burlison, F. G., Fletcher, T. (2014). Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. *Toxicol. Sci.* 138: 76-88.

Lopez-Espinosa, M. J., Mondal, D., Armstrong, B., Bloom, M. S., Fletcher, T. (2012). Thyroid function and perfluoroalkyl acids in children living near a chemical plant. *Environ Health Perspect* 120: 1036-1041.

Lorber, M., Egeghy, P. P. (2011). Simple intake and pharmacokinetic modeling to characterize exposure of Americans to perfluorooctanoic acid, PFOA. *Environ. Sci. Technol.* 45: 8006-8014.

Lorber, M., Eaglesham, G.E., Hobson, P., Toms, L.M., Mueller, J.F., Thompson, J.S. (2015). The effect of ongoing blood loss on human serum concentrations of perfluorinated acids. *Chemosphere* 118: 170-177.

Lou, I., Wambaugh, J.F., Lau, C., Hanson, R.G., Lindstrom, A.B., Strynar, M.J., Zehr, R.D., Setzer, R.W., Barton, H.A. (2009). Modeling single and repeated dose pharmacokinetics of PFOA in mice. *Toxicol. Sci.* 107: 331-341.

Loveless, S.E., Finlay, C., Everds, N.E., Frame, S.R., Gillies, P.J., O'Connor, J.C., Powley, C.R., Kennedy, G.L. (2006). Comparative responses of rats and mice exposed to linear/branched, linear, or branched ammonium perfluorooctanoate (APFO). *Toxicology* 220: 203-217.

Loveless, S.E., Hoban, D., Sykes, G., Frame, S.R., Everds, N.E. (2008). Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate. *Toxicol. Sci.* 105: 86-96.

Lu, Y., Luo, B., Li, J., Dai, J. (2015). Perfluorooctanoic acid disrupts the blood-testis barrier and activates the TNF $\alpha$ /p38 MAPK signaling pathway in vivo and in vitro. *Arch. Toxicol.* 90: 971-83.

Macon, M.B., Villanueva, L.R., Tatum-Gibbs, K., Zehr, R.D., Strynar, M.J., Stanko, J.P., White, S.S., Helfant, L., Fenton, S.E. (2011). Prenatal perfluorooctanoic acid exposure in CD-1 mice: low dose developmental effects and internal dosimetry. *Toxicol. Sci.* 122: 134-45.

Macon, M.B., Fenton, S.E. (2013). Endocrine disruptors and the breast: early life effects and later life disease. *J. Mammary Gland Biol. Neoplasia.* 18: 43-61.

Maher, J. M., Aleksunes, L. M., Dieter, M. Z., Tanaka, Y., Peters, J. M., Manautou, J. E., Klaassen, C. D. (2008). Nr2f2- and PPAR alpha-mediated regulation of hepatic Mrp transporters after exposure to perfluorooctanoic acid and perfluorodecanoic acid. *Toxicol. Sci.* 106: 319-328.

Maine DHHS (2014). Maine Department of Health and Human Services. Maximum Exposure Guideline for Perfluorooctanoic Acid in Drinking Water. CAS Registry Number (Free Acid): 335-67-1. March 17th, 2014. [http://www.bennington.edu/docs/default-source/docs-pfoa/me\\_pfoameg.pdf?sfvrsn=4](http://www.bennington.edu/docs/default-source/docs-pfoa/me_pfoameg.pdf?sfvrsn=4)

Maisonet, M., Terrell, M. L., McGeehin, M. A., Christensen, K. Y., Holmes, A., Calafat, A. M., Marcus, M. (2012). Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. *Environ. Health Perspect.* 120: 1432-1437.

Mak, Y.L., Taniyasu, S., Yeung, L.W.Y., Lu, G., Jin, L., Yang, Y., Lam, P.K.S., Kannan, K., Yamashita, N. (2009). Perfluorinated compounds in tap water from China and several other countries. *Environ. Sci. Technol.* 43: 4824-4829.

Maloney, E. K., Waxman, D. J. (1999). trans-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. *Toxicol. Appl. Pharmacol.* 161: 209-218.

Mann, P., Frame, S.R. (2004). FC-143: Two year oral toxicity-oncogenicity study in rats. Peer review of ovaries. Submitted by E.I du Pont de Nemours and Company. USEPA Docket AR-226-1921.

Mann, P.C., Vahle, J., Keenan, C.M., Baker, J.F., Bradley, A.E., Goodman, D.G., Harada, T., Herbert, R., Kaufmann, W., Kellner, R., Nolte, T., Rittinghausen, S., Tanaka, T. (2012). International harmonization of toxicologic pathology nomenclature: an overview and review of basic principles. *Toxicol. Pathol.* 40: 7S-13S.

Martin, J.W., Mabury, S.A., Solomon, K.R., Muir, D.C. (2003). Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* 22: 196-204.

Martin, M. T., Brennan, R. J., Hu, W., Ayanoglu, E., Lau, C., Ren, H., Wood, C. R., Corton, J. C., Kavlock, R. J., Dix, D. J. (2007). Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. *Toxicol. Sci.* 97: 595-613.

Massachusetts Department of Environmental Protection. 2018. Massachusetts Department of Environmental Protection Office of Research and Standards. Final Recommendations for Interim Toxicity and Drinking Water Guidance Values for Perfluorinated Alkyl Substances Included in the Unregulated Chemical Monitoring Rule 3. June 8, 2018. [https://www.mass.gov/files/documents/2018/06/11/pfas-ors-ucmr3-recs\\_0.pdf](https://www.mass.gov/files/documents/2018/06/11/pfas-ors-ucmr3-recs_0.pdf)

Minnesota Department of Health. Toxicological Summary for: Perfluorooctanoic Acid. May 2017. Available from: <http://www.health.state.mn.us/divs/eh/risk/guidance/gw/pfoa.pdf> Accessed June 7, 2017.

MDH (2009). Minnesota Department of Health. East Metro Perfluorochemical Biomonitoring Pilot Project. July 21, 2009. <http://www.health.state.mn.us/divs/eh/tracking/finalpfcprpt.pdf>

Melzer, D., Rice, N., Depledge, M. H., Henley, W. E., Galloway, T. S. (2010). Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. *Environ. Health Perspect.* 118: 686-692.

Michalik, L., Auwerx, J., Berger, J.P., Chatterjee, V.K., Glass, C.K., Gonzalez, F.J., Grimaldi, P.A., Kadowaki, T., Lazar, M.A., O'Rahilly, S., Palmer, C.N., Plutzky J., Reddy, J.K., Spiegelman, B.M., Staels, B., Wahli, W. (2006). International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. *Pharmacol. Rev.* 58: 726-41.

Midasch, O., Drexler, H., Hart, N., Beckmann, M.W., Angerer, J. (2007). Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. *Int. Arch. Occup. Environ. Health* 80: 643-648.

Minata, M., Harada, K.H., Kärrman, A., Hitomi, T., Hirose, M., Murata, M., Gonzalez, F.J., Koizumi, A. (2010). Role of peroxisome proliferator-activated receptor- $\alpha$  in hepatobiliary injury induced by ammonium perfluorooctanoate in mouse liver. *Ind. Health* 48: 96-107.

Mogensen, U.B., Grandjean, P., Nielsen, F., Weihe, P., Budtz-Jørgensen, E. (2015). Breastfeeding as an exposure pathway for perfluorinated alkylates. *Environ. Sci. Technol.* 49: 10466-73.

Mondal, D., Lopez-Espinosa, M-J., Armstrong, B., Stein, C.R., Fletcher, T. (2012). Relationships of perfluorooctanoate and perfluorooctane sulfonate serum concentrations between child-mother pairs in a population with perfluorooctanoate exposure from drinking water. *Environ. Health Perspect.* 120: 752-757.

Mondal, D., Weldon, R. H., Armstrong, B. G., Gibson, L. J., Lopez-Espinosa, M. J., Shin, H. M., Fletcher, T. (2014). Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. *Environ. Health Perspect.* 122: 187-192.

Monroy, R., Morrison, K., Teo, K., Atkinson, S., Kubwabo, C., Stewart, B., Foster, W.G. (2008). Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. *Environ. Res.* 108: 56-62.

Moody, C.A., Hebert, G.N., Strauss, S.H., Field, J.A. (2003). Occurrence and persistence of perfluorooctanesulfonate and other perfluorinated surfactants in groundwater at a fire-training area at Wurtsmith Air Force Base, Michigan, USA. *J. Environ. Monit.* 5: 341-345.

Morel-Symons, J., Sakr, C.J., Kreckmann, K.H., Leonard, R.C. (2007). Addendum report on confirmed and potential carcinoid tumor cases in the DuPont Cancer Registry. D. E. Program. Newark, DE. DuPont-24117-Addendum. EPA Docket AR, 226.

Morken, N.-H., Travlos, G. S., Wilson, R. E., Eggesbø, M., Longnecker, M. P. (2014). Maternal glomerular filtration rate in pregnancy and fetal size. *PLoS ONE* 9: e101897.

Murakami, M., Shinohara, H., Takada, H. (2009). Evaluation of wastewater and street runoff as sources of perfluorinated surfactants (PFSs). *Chemosphere* 74: 487-493.

Mylchreest, E. (2003). PFOA: Lactational and placental transport pharmacokinetic study in mice. Laboratory Project ID: DuPont-13309. December 19, 2003. US EPA AR226-1551.

Nakagawa, T., Ramdhan, D.H., Tanaka, N., Naito, H., Tamada, H., Ito, Y., Li, Y., Hayashi, Y., Yamagishi, N., Yanagiba, Y., Aoyama, T., Gonzalez, F.J., Nakajima, T. (2011). Modulation of ammonium perfluorooctanoate-induced hepatic damage by genetically different PPAR $\alpha$  in mice. *Arch. Toxicol.* 86: 63-74.

Nakayama, S., Strynar, M.J., Helfant, L., Egeghy, P., Ye, X., Lindstrom, A.B. (2007). Perfluorinated compounds in the Cape Fear drainage basin in North Carolina. *Environ. Sci. Technol.* 41: 5271-5276.

Nakayama, S.F., Strynar, M.J., Reiner, J.L., Delinsky, A.D., Lindstrom, A.B. (2010). Determination of perfluorinated compounds in the Upper Mississippi river basin. *Environ. Sci. Technol.* 44: 4103-4109.

Needham, L.L., Grandjean, P., Heinzow, B., Jørgensen, P.J., Nielsen, F., Patterson, D.G. Jr., Sjödin, A., Turner, W.E., Weihe, P. (2011). Partition of environmental chemicals between maternal and fetal blood and tissues. *Environ. Sci. Technol.* 45: 1121-1126.

Nelson, J.W., Hatch, E.E., Webster, T.F. (2010). Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general US population. *Environ. Health Perspect.* 118: 197-202.

Ngo, H. T., Hetland, R. B., Sabaredzovic, A, Haug, L. S., Steffensen, I. L. (2014). In utero exposure to perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) did not increase body weight or intestinal tumorigenesis in multiple intestinal neoplasia (Min/+) mice. *Environ Res* 132: 251-263.

- NH DHHS (2015). New Hampshire Department of Health & Human Services. Perfluorochemical (PFC) Testing Program: Summary of test results for children <12 y.o. September 9, 2015. <http://www.dhhs.nh.gov/dphs/documents/pediatric-results-presentation.pdf>
- Nilsson, R., Beije, B., Pr at, V., Erixon, K., Ramel, C. (1991). On the mechanism of the hepatocarcinogenicity of peroxisome proliferators. *Chem. Biol. Interact.* 78: 235-250.
- Nilsson, H., K arrman, A., Westberg, H., Rotander, A., van Bavel, B., Lindstr om, G. (2010). A time trend study of significantly elevated perfluorocarboxylate levels in humans after using fluorinated ski wax. *Environ. Sci. Technol.* 44: 2150-2155.
- NJDEP (2007a). Guidance for PFOA in drinking water at Pennsgrove Water Supply Company. Division of Science, Research, and Technology. February 13, 2007. [http://www.nj.gov/dep/watersupply/pdf/pfoa\\_dwguidance.pdf](http://www.nj.gov/dep/watersupply/pdf/pfoa_dwguidance.pdf)
- NJDEP (2007b). Determination of Perfluorooctanoic Acid (PFOA) in Aqueous Samples, Final Report, January 2007. [http://www.nj.gov/dep/watersupply/final\\_pfoa\\_report.pdf](http://www.nj.gov/dep/watersupply/final_pfoa_report.pdf)
- NJDEP (2010). New Jersey Department of Environmental Protection. Letter to Reginald C. Jordan, Ph.D., CIH. NC Division of Air Quality. June 1, 2010.
- NJDEP (2014). New Jersey Department of Environmental Protection. Occurrence of Perfluorinated Chemicals in Untreated New Jersey Drinking Water Sources Final Report. April 2014. <http://www.nj.gov/dep/watersupply/pdf/pfc-study.pdf>
- NJDHSS (2010). New Jersey Department of Health and Senior Services. Letter to Reginald C. Jordan, Ph.D., CIH. NC Division of Air Quality. June 1, 2010.
- NJDOH (2014). New Jersey Department of Health. ATSDR Technical Assistance Form. NJDOH response to NJDEP request for evaluation of showering/bathing exposure to PFNA.
- Noorlander, C.W., van Leeuwen, S.P., Te Biesebeek, J.D., Mengelers, M.J., Zeilmaker, M.J. (2011). Levels of perfluorinated compounds in food and dietary intake of PFOS and PFOA in the Netherlands. *J. Agric. Food Chem.* 59: 7496-7505.
- NRC (2006). National Research Council. Assessing the human health risk of trichloroethylene. Appendix E: Peroxisome proliferators and liver cancer. National Academies Press, Washington, DC.
- NTP (1997). National Toxicology Program. NTP Toxicology and Carcinogenesis Studies of Tetrafluoroethylene (CAS No. 116-14-3) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). Research Triangle Park, N.C., National Institute of Environmental Health Sciences.

NTP (2016). National Toxicology Program. Systematic review of immunotoxicity associated with exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS). September 2016. [https://ntp.niehs.nih.gov/ntp/ohat/pfoa\\_pfos/pfoa\\_pfosmonograph\\_508.pdf](https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf)

NYS DOH (2016). New York State Department of Health. Information Sheet. PFOA Biomonitoring Group-Level Results. June 2, 2016. <https://www.health.ny.gov/environmental/investigations/hoosick/docs/infosheetshortgroupresults.pdf>. Accessed June 9, 2016.

Ode, A., Kallen, K., Gustafsson, P., Rylander, L., Jonsson, B. A., Olofsson, P., Ivarsson, S. A., Lindh, C. H., Rignell-Hydbom, A. (2014). Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. *PLoS One* 9: e95891.

OECD (2015). Organisation for Economic Cooperation and Development. Working Towards a Global Emission Inventory of PFASs: Focus on PFCAs - Status Quo and the Way Forward. Paris, 2015. <https://www.oecd.org/chemicalsafety/risk-management/Working%20Towards%20a%20Global%20Emission%20Inventory%20of%20PFAS%20S.pdf>

Olsen, G. W., Lange, C. C., Ellefson, M. E., Mair, D. C., Church, T. R., Goldberg, C. L., Herron, R. M., Medhdizadehkashi, Z., Nobiletti, J. B., Rios, J. A., Reagen, W. K., Zobel, L. R. (2012). Temporal trends of perfluoroalkyl concentrations in American Red Cross adult blood donors, 2000-2010. *Environ. Sci. Technol.* 46: 6330-6338.

Olsen, G. W., Ehresman, D. J., Buehrer, B. D., Gibson, B. A., Butenhoff, J. L., Zobel, L. R., (2012). Longitudinal assessment of lipid and hepatic clinical parameters in workers involved with the demolition of perfluoroalkyl manufacturing facilities. *J. Occup. Environ. Med.* 54: 974-983.

Olsen, G. W., Gilliland, F. D., Burlew, M. M., Burriss, J. M., Mandel, J. S., Mandel, J. H. (1998). An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. *J. Occup. Environ. Med.* 40: 614-622.

Olsen, G. W., Burriss, J. M., Burlew, M. M., Mandel, J. H. (2000). Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. *Drug Chem. Toxicol.* 23: 603-620.

Olsen, G. W., Burriss, J. M., Burlew, M. M., Mandel, J. H. (2003). Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. *J. Occup. Environ. Med.* 45: 260-270.

Olsen, G. W., Zobel, L. R. (2007). Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. *Int Arch. Occup. Environ. Health* 81: 231-246.

Olsen, G.W. (2015). PFAS biomonitoring in higher exposed populations. In: *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*. J.D. DeWitt, Editor. Humana Press. pp. 77-126.

Omer, S.B., Phadke, V.K., Bednarczyk, R.A., Chamberlain, A.T., Brosseau, J.L., Orenstein, W.A. (2016). Impact of statins on influenza vaccine effectiveness against medically attended acute respiratory illness. *J. Infect. Dis.* 213: 1216-23.

Onishchenko, N., Fischer, C., Wan Ibrahim, W.N., Negri, S., Spulber, S., Cottica, D., Ceccatelli, S. (2010). Prenatal exposure to PFOS or PFOA alters motor function in mice in a sex-related manner. *Neurotox. Res.* 19: 452-461.

Osborne, G, Rudel, R., Schwarzman, M. (2015). Evaluating chemical effects on mammary gland development: A critical need in disease prevention. *Reprod. Toxicol.* 54: 148-55.

Palkar, P.S., Anderson, C.R., Ferry, C.H., Gonzalez, F.J., Peters, J.M. (2010). Effect of prenatal peroxisome proliferator-activated receptor alpha (PPARalpha) agonism on postnatal development. *Toxicology* 276: 79-84.

Perkins, R.G., Butenhoff, J.L., Kennedy, G.L., Palazzolo, M.J. (2004). 13-Week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. *Drug Chem. Toxicol.* 27: 361-378.

Peters, J.M., Gonzalez, F.J. (2011). Why toxic equivalency factors are not suitable for perfluoroalkyl chemicals. *Chem. Res. Toxicol.* 24: 1601-1609.

Post, G.B., Louis, J.B., Cooper, K.R., Boros-Russo, B.J., Lippincott, R.L. (2009a). Occurrence and potential significance of perfluorooctanoic acid (PFOA) detected in New Jersey public drinking water systems. *Environ. Sci. Technol.* 43: 4547–4554.

Post, G.B., Louis, J.B., Cooper, K.R., Lippincott, R.L. (2009b). Response to Comment on Occurrence and potential significance of perfluorooctanoic acid (PFOA) detected in New Jersey public drinking water systems. *Environ. Sci. Technol.* 43: 8699–8700.

Post, G.B., Cohn, P.D., Atherholt, T.A. (2011). Health and aesthetic aspects of water quality. In: J. Edzwald (Ed.), *Water Quality and Treatment: A Handbook of Community Water Supplies*, American Water Works Association, 6th edition. McGraw-Hill, New York. pp. 2.1-2.100.

Post, G.B., Cohn, P.D., Cooper, K.R. (2012). Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. *Env. Res.* 116: 93-117.

Post, G.B., Louis, J.B., Lippincott, R.L., Procopio, N.A. (2013). Occurrence of perfluorinated chemicals in raw water from New Jersey public drinking water systems. *Environ. Sci. Technol.* 47: 13266-75.

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H. (2006). Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.* 40: 32-44.

PubChem (2016). Pefluorooctanoic acid. Accessed May 15, 2016.

Qazi, M. R., Bogdanska, J., Butenhoff, J. L., Nelson, B. D., DePierre, J. W., Abedi-Valugerdi, M. (2009a). High-dose, short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory responses of macrophages to lipopolysaccharide (LPS) in a similar fashion. *Toxicology* 262: 207-214.

Qazi, M. R., Xia, Z., Bogdanska, J., Chang, S. C., Ehresman, D. J., Butenhoff, J. L., Nelson, B. D., DePierre, J. W., Abedi-Valugerdi, M. (2009b). The atrophy and changes in the cellular compositions of the thymus and spleen observed in mice subjected to short-term exposure to perfluorooctanesulfonate are high-dose phenomena mediated in part by peroxisome proliferator-activated receptor-alpha (PPARalpha). *Toxicology* 260: 68-76.

Qazi, M. R., Abedi, M. R., Nelson, B. D., DePierre, J. W., Abedi-Valugerdi, M. (2010). Dietary exposure to perfluorooctanoate or perfluorooctane sulfonate induces hypertrophy in centrilobular hepatocytes and alters the hepatic immune status in mice. *Int. Immunopharmacol.* 10: 1420-1427.

Qazi, M. R., Nelson, B. D., DePierre, J. W., Abedi-Valugerdi, M. (2012). High-dose dietary exposure of mice to perfluorooctanoate or perfluorooctane sulfonate exerts toxic effects on myeloid and B-lymphoid cells in the bone marrow and these effects are partially dependent on reduced food consumption. *Food. Chem. Toxicol.* 50: 2955-2963.

Qazi, M. R., Hassan, M., Nelson, B. D., Depierre, J. W., Abedi-Valugerdi, M. (2013). Sub-acute, moderate-dose, but not short-term, low-dose dietary pre-exposure of mice to perfluorooctanoate aggravates concanavalin A-induced hepatitis. *Toxicol. Lett.* 219: 1-7.

Quist, E.M., Filgo, A.J., Cummings, C.A., Kissling, G.E., Hoenerhoff, M.J., Fenton, S.E. (2015). Hepatic mitochondrial alteration in CD-1 mice associated with prenatal exposures to low doses of perfluorooctanoic acid (PFOA). *Toxicol. Pathol.* 43: 546-57.

Rayne, S., Forest, K. (2010). Theoretical studies on the pKa values of perfluoroalkyl carboxylic acids. *J. Mol. Struct. (Theochem)* 949: 60-69.

Rebholz S.L., Jones, T., Herrick, R.L., Xie, C., Calafat, A.M., Pinney, S.M., Woollett, L.A. (2016). Hypercholesterolemia with consumption of PFOA-laced Western diets is dependent on strain and sex of mice. *Toxicol. Rep.* 3: 46-54.

Reiner, J.L., Nakayama, S.F., Delinsky, A.D., Stanko, J.P., Fenton, S.E., Lindstrom, A.B., Strynar, M.J. (2009). Analysis of PFOA in dosed CD1 mice. Part 1. Methods development for the analysis of tissues and fluids from pregnant and lactating mice and their pups. *Reprod. Toxicol.* 27: 360-4.

Romano, M.E., Xu, Y., Calafat, A.M., Yolton, K., Chen, A., Webster, G.M., Eliot, M.N., Howard, C.R., Lanphear, B.P., Braun, J.M. (2016). Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. *Env. Research* 149: 239-46.

Roosens, L., D'Hollander, W., Bervoets, L., Reynders, H., Van Campenhout, K., Cornelis, C., Van Den Heuvel, R., Koppen, G., Covaci, A. (2010). Brominated flame retardants and perfluorinated chemicals, two groups of persistent contaminants in Belgian human blood and milk. *Environ. Pollut.* 158: 2546–2552.

Rosen, M.B., Thibodeaux, J.R., Wood, C.R., Zehr, R.D., Schmid, J.E., Lau, C. (2007). Gene expression profiling in the lung and liver of PFOA-exposed mouse fetuses. *Toxicology* 239: 15-33.

Rosen, M.B., Abbott, B.D., Wolf, D.C., Corton, J.C., Wood, C.R., Schmid, J.E., Das, K.P., Zehr, R.D., Blair, E.T., Lau, C. (2008a). Gene profiling in the livers of wild-type and PPAR alpha-null mice exposed to perfluorooctanoic acid. *Toxicol. Pathol.* 36: 592-607.

Rosen, M.B., Lee, J.S., Ren, H., Vallanat, B., Liu, J., Waalkes, M.P., Abbott, B.D., Lau, C., Corton, J.C. (2008b). Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: evidence for the involvement of nuclear receptors PPAR alpha and CAR. *Toxicol. Sci.* 103: 46-56.

Roth, M., Obaidat, A, Hagenbuch, B. (2012). OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. *Br. J. Pharmacol.* 165: 1260-87.

Rudel, R.A., Fenton, S.E., Ackerman, J.M., Euling, S.Y., Makris, S.L. (2011). Environmental exposures and mammary gland development: state of the science, public health implications, and research recommendations. *Environ. Health Perspect.* 119: 1053-1061.

Rumsby, P.C., McLaughlin, C.L., Hall, T. (2009). Perfluorooctane sulphonate and perfluorooctanoic acid in drinking and environmental waters. *Philos. Transact. A Math. Phys. Eng. Sci.* 367: 4119-4136.

Rusiecka, I., Składanowski, A.C. (2008). Induction of the multixenobiotic/multidrug

resistance system in various cell lines in response to perfluorinated carboxylic acids. *Acta Biochim. Pol.* 55: 329-37.

Russell, M.H., Berti, W.R., Szostek, B., Buck, R.C. (2008). Investigation of the biodegradation potential of a fluoroacrylate polymer product in aerobic soils. *Environ. Sci. Technol.* 42: 800-807.

Sakr, C. J., Kreckmann, K. H., Green, J. W., Gillies, P. J., Reynolds, J. L., Leonard, R. C. (2007). Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as part of a general health survey in a cohort of occupationally exposed workers. *J. Occup. Environ. Med.* 49: 1086-1096.

Sakr, C. J., Leonard, R. C., Kreckmann, K. H., Slade, M. D., Cullen, M. R. (2007). Longitudinal study of serum lipids and liver enzymes in workers with occupational exposure to ammonium perfluorooctanoate. *J. Occup. Environ. Med.* 49: 872-879.

Savitz, D. A., Stein, C. R., Elston, B., Wellenius, G. A., Bartell, S. M., Shin, H. M., Vieira, V. M. Fletcher, T. (2012). Relationship of perfluorooctanoic acid exposure to pregnancy outcome based on birth records in the mid-Ohio Valley. *Environ. Health Perspect.* 120: 1201-1207.

Schechter, A., Malik-Bass, N., Calafat, A.M., Kato, K., Colacino, J.A., Gent, T.L., Hynan, L.S., Harris, T.R., Malla, S., Birnbaum, L.S. (2011). Polyfluoroalkyl compounds in Texas children from birth through 12 years of age. *Environ. Health Perspect.* (in press).

Schlummer, M., Gruber, L., Fiedler, D., Kizlauskas, M., Muller, J. (2013). Detection of fluorotelomer alcohols in indoor environments and their relevance for human exposure. *Environ. Int.* 57-58: 42-49.

Seals, R., Bartell, S.M., Steenland, K. (2011). Accumulation and clearance of perfluorooctanoic acid (PFOA) in current and former residents of an exposed community. *Environ. Health Perspect.* 119: 119-124.

Sepulvado, J.G., Blaine, A.C., Hundal, L.S., Higgins, C.P. (2011). Occurrence and fate of perfluorochemicals in soil following the land application of municipal biosolids. *Environ. Sci. Technol.* 45: 8106-8112.

Shah, D. (2009). Healthy worker effect phenomenon. *Indian J. Occup. Environ. Med.* 13: 77-79.

Shankar, A., Xiao, J., Ducatman, A. (2011). Perfluoroalkyl chemicals and elevated serum uric acid in US adults. *Clin. Epidemiol.* 3: 251-258.

Shin, H.M., Vieira, V.M., Ryan, P.B., Steenland, K., Bartell, S.M. (2011). Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. *Environ. Health Perspect.* 119: 1760-1765.

Shrestha, S., Bloom, M. S., Yucel, R., Seegal, R. F., Wu, Q. Kannan, K., Rej, R., Fitzgerald, E. F. (2015). Perfluoroalkyl substances and thyroid function in older adults. *Environ. Int.* 75: 206-214.

Shoeib, M., Harner, T.M., Webster, G., Lee, S.C., (2011). Indoor sources of poly- and perfluorinated compounds (PFCS) in Vancouver, Canada: implications for human exposure. *Environ. Sci. Technol.* 45: 7999-8005.

Sibinski, L.J. (1987). Final report of a two year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanoate ammonium carboxylate) in rats. Vol. 1-4, 3M Company/RIKER. No.0281CR0012; 8EHQ-1087-0394, October 16, 1987.

Sinclair, E., Kannan, K. (2006). Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Env. Sci. Technol.* 40: 1408-1414.

Skutlarek, D., Exner, M., Färber, H. (2006). Perfluorinated surfactants in surface and drinking waters. *Environ. Sci. Pollut. Res. Int.* 13: 299-307.

So, M.K., Yamashita, N., Taniyasu, S., Jiang, Q., Giesy, J.P., Chen, K., Lam, P.K. (2006). Health risks in infants associated with exposure to perfluorinated compounds in human breast milk from Zhoushan, China. *Environ. Sci. Technol.* 40: 2924-2929.

Sobolewski, M., Conrad, K., Allen, J. L., Weston, H., Martin, K., Lawrence, B. P., Cory-Slechta, D. A. (2014). Sex-specific enhanced behavioral toxicity induced by maternal exposure to a mixture of low dose endocrine-disrupting chemicals. *Neurotoxicology* 45: 121-130.

Son, H.Y., Kim, S.H., Shin, H.I., Bae, H.I., Yang, J.H. (2008). Perfluorooctanoic acid-induced hepatic toxicity following 21-day oral exposure in mice. *Arch. Toxicol.* 82: 239-246.

Son, H.Y., Lee, S., Tak, E.N., Cho, H.S., Shin H.I., Kim, S.H., Yang, J.H. (2009). Perfluorooctanoic acid alters T lymphocyte phenotypes and cytokine expression in mice. *Environ. Toxicol.* 24: 580-588.

SRI. Southern Research Institute (2003). Protein binding of perfluorohexane sulfonate, perfluorooctane sulfonate and perfluorooctanoate to plasma (human, rat and monkey), and various human derived plasma protein fractions. Study ID: 9921.7. US EPA AR226-1354. Cited in USEPA (2005a).

Stahl, T., Heyn, J., Thiele, H., Hüther, J., Failing, K., Georgii, S., Brunn, H. (2009). Carryover of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from soil to plants. *Arch. Environ. Contam. Toxicol.* 57: 289-298.

Starkov, A. A., Wallace, K. B. (2002). Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol Sci* 66: 244-252.

Starling, A. P., Engel, S. M., Whitworth, K. W., Richardson, D. B., Stuebe, A. M., Daniels, J. L., Haug, L. S., Eggesbo, M., Becher, G., Sabaredzovic, A., Thomsen, C., Wilson, R. E., Travlos, G. S., Hoppin, J. A., Baird, D. D., Longnecker, M. P. (2014). Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study. *Environ. Int.* 62: 104-112.

Steenland, K., Jin, C., MacNeil, J., Lally, C., Ducatman, A., Vieira V., Fletcher, T. (2009a). Predictors of PFOA levels in a community surrounding a chemical plant. *Environ. Health Perspect.* 117: 1083-1088.

Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., Vaccarino, V. (2009b). Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. *Am. J. Epidemiol.* 170: 1268-1278.

Steenland K., Fletcher T., Savitz D.A. (2010a). Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). *Environ. Health Perspect.* 118: 1100-1108.

Steenland, K., Tinker, S., Shankar, A., Ducatman, A. (2010b). Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with uric acid among adults with elevated community exposure to PFOA. *Environ. Health Perspect.* 118: 229-233.

Steenland K., Savitz, D.A., Fletcher, T. (2014). Commentary: Class action lawsuits: can they advance epidemiologic research? *Epidemiology* 25: 167-9.

Steenland, K., Zhao, L., Winquist, A. (2015). A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). *Occup. Environ. Med.* 72: 373-380.

Stein, C. R., Savitz, D. A., Dougan, M. (2009). Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. *Am. J. Epidemiol.* 170: 837-846.

Stein, C. R., McGovern, K. J., Pajak, A. M., Maglione, P. J., Wolff, M. S. (2016). Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. *Pediatr. Res.* 79: 348-357.

Stein, C.R., Savitz, D.A., (2011). Serum perfluorinated compound concentration and Attention Deficit/Hyperactivity Disorder in children 5–18 years of age. *Environ. Health. Perspect.* 119: 1466-1471.

Stein, C. R., Wolff, M. S., Calafat, A. M., Kato, K., Engel, S. M. (2012). Comparison of polyfluoroalkyl compound concentrations in maternal serum and amniotic fluid: a pilot study. *Reprod. Toxicol.* 34: 312-316.

Suh, C.H., Cho, N.K., Lee, C.K., Lee, C.H., Kim, D.H., Kim, J.H., Son, B.C., Lee, J.T. (2011). Perfluorooctanoic acid-induced inhibition of placental prolactin-family hormone and fetal growth retardation in mice. *Mol. Cell. Endocrinol.* 337: 7-15.

Sundström, M., Ehresman, D.J., Bignert, A., Butenhoff, J.L., Olsen, G.W., Chang, S.C., Bergman, A. (2011). A temporal trend study (1972-2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden. *Environ. Int.* 37: 178-183.

Takagi, A., Sai, K., Umemura, T., Hasegawa, R., Kurokawa, Y. (1991). Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats. *Cancer Lett.* 57: 55-60.

Takagi, S., Adachi, F., Miyano, K., Koizumi, Y., Tanaka, H., Watanabe, I., Tanabe, S., Kannan, K. (2011). Fate of perfluorooctanesulfonate and perfluorooctanoate in drinking water treatment processes. *Water Res.* 45: 3925-3932

Tan, X., Xie, G., Sun, X., Li, Q., Zhong, W., et al. (2013). High Fat Diet Feeding Exaggerates Perfluorooctanoic acid-induced liver injury in mice via modulating multiple metabolic pathways. *PLoS ONE* 8: e61409.

Tao, L., Kannan, K., Wong, C.M., Arcaro, K.F., Butenhoff, J.L. (2008a). Perfluorinated compounds in human milk from Massachusetts, U.S.A. *Environ. Sci. Technol.* 42: 3096-3101.

Tao, L., Ma, J., Kunisue, T., Libelo, E.L., Tanabe, S., Kannan, K. (2008b). Perfluorinated compounds in human breast milk from several Asian countries, and in infant formula and dairy milk from the United States. *Environ. Sci. Technol.* 42: 8597-8602.

Tardiff, R.G., Carson, M.L., Sweeney, L.M., Kirman, C.R., Tan, Y.-M., Andersen, M., Bevan, C., Gargas, M.L. (2009). Derivation of a drinking water equivalent level (DWEL) related to the maximum contaminant level goal for perfluorooctanoic acid (PFOA), a persistent water-soluble compound. *Food Chem. Toxicol.* 47: 2557-2589.

Taylor, K.W., Hoffman, K., Thayer, K.A., Daniels, J.L. (2014). Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES). *Environ. Health Perspect.* 122: 145- 50.

Thomford, P.J. (2001a). 4-Week capsule toxicity study with ammonium perfluorooctanoate (APFO) in cynomolgus monkeys. Covance Laboratories Inc., Madison, WI. Covance 6329-230. December 18, 2001.

Thomford, P.J. (2001b). 26-Week capsule toxicity study with ammonium perfluorooctanoate (APFO) in cynomolgus monkeys. Covance Laboratories Inc., Madison WI. Covance 6329-231. December 18, 2001.

Thompson, J., Lorber, M., Toms, L. M. L., Kato, K.; Calafat, A. M., Mueller, J. F. (2010). Use of simple pharmacokinetic modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane sulfonic acid. *Environ. Int.* 36: 390–397.

Thompson, J., Eaglesham, G., Mueller, J., (2011). Concentrations of PFOS, PFOA and other perfluorinated alkyl acids in Australian drinking water. *Chemosphere* 83: 1320-1325.

Thomsen, C., Haug, L.S., Stigum, H., Frøshaug, M., Broadwell, S.L., Becher, G. (2010). Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation. *Environ. Sci. Technol.* 44: 9550-9556.

Tilton, S.C., Orner, G.A., Benninghoff, A.D., Carpenter, H.M., Hendricks, J.D., Pereira, C.B., Williams, D.E. (2008). Genomic profiling reveals an alternate mechanism for hepatic tumor promotion by perfluorooctanoic acid in rainbow trout. *Environ. Health Perspect.* 116: 1047-1055.

Tittlemier, S.A., Pepper, K., Seymour, C., Moisey, J., Bronson, R., Cao, X.L., Dabeka, R.W. (2007). Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. *J. Agric. Food Chem.* 18: 3203-3210.

Toms, L.M., Calafat, A.M, Kato, K., Thompson, J., Harden, F., Hobson, P., Sjödin, A., Mueller, J.F. (2009). Polyfluoroalkyl chemicals in pooled blood serum from infants, children, and adults in Australia. *Environ. Sci. Technol.* 43: 4194-4199.

Trudel, D., Horowitz, L., Wormuth, M., Scheringer, M., Cousins, I.T., Hungerbuheler, K. (2008). Estimating consumer exposure to PFOS and PFOA. *Risk Anal.* 28: 251-269.

Tucker, D. K., Macon, M. B., Strynar, M. J., Dagnino, S., Andersen, E., Fenton, S. E. (2015). The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. *Reprod. Toxicol.* 54: 26-36.

Upham, B.L., Deocampo, N.D., Wurl, B., et al. (1998). Inhibition of gap junctional intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. *Int. J. Cancer* 78: 491-495.

Upham, B.L., Park, J.S., Babica, P., Sovadinova, I., Rummel, A.M., Trosko, J.E., Hirose, A., Hasegawa, R., Kanno, J., Sai, K. (2009). Structure-activity-dependent regulation of cell communication by perfluorinated fatty acids using in vivo and in vitro model systems. *Environ. Health Perspect.* 117: 545-51.

USEPA (2000). Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. Office of Science and Technology. Office of Water. Washington, DC. EPA 822-B-00-004. October 2000.

[http://water.epa.gov/scitech/swguidance/standards/upload/2005\\_05\\_06\\_criteria\\_humanhealth\\_method\\_complete.pdf](http://water.epa.gov/scitech/swguidance/standards/upload/2005_05_06_criteria_humanhealth_method_complete.pdf)

USEPA (2005a). United States Environmental Protection Agency. Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and Its Salts. Office of Pollution Prevention and Toxics, January 4, 2005.

<http://www.epa.gov/oppt/pfoa/pubs/pfoarisk.pdf>. Accessed August 16, 2011.

USEPA (2005b). United States Environmental Protection Agency. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, USEPA, Washington, DC. EPA/630.P-03/001F, March 2005.

USEPA (2006). United States Environmental Protection Agency. Science Advisory Board Review of EPA's Draft Risk Assessment of Potential Human Health Effects Associated with PFOA and Its Salts, May 30, 2006. [http://www.epa.gov/sab/pdf/sab\\_06\\_006.pdf](http://www.epa.gov/sab/pdf/sab_06_006.pdf). Accessed August 16, 2011.

USEPA (2008a). United States Environmental Protection Agency. Child-Specific Exposure Factors Handbook. EPA/600/R-06/096F. National Center for Environmental Assessment, Washington, DC. September 2008. <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199243>. Accessed 1/17/12.

USEPA (2008b). Toxicological Review of 2,2',4,4',5-Pentabromodiphenyl Ether (BDE-99) (CAS No. 60348-60-9). June 2008. EPA/635/R-07/006F.

USEPA (2008c). Toxicological Review of 2,2',4,4'-Tetrabromodiphenyl Ether (BDE-47) (CAS No. 5436-43-1). June 2008. EPA/635/R-07/005F.

USEPA (2009a). United States Environmental Protection Agency. Long-Chain Perfluorinated Chemicals (PFCs) Action Plan. Office of Pollution Prevention and Toxics. December 30, 2009.

USEPA (2009b). Provisional Health Advisories for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS). USEPA Office of Water, Jan. 8, 2009.

[http://www.epa.gov/waterscience/criteria/drinking/pha-PFOA\\_PFOS.pdf](http://www.epa.gov/waterscience/criteria/drinking/pha-PFOA_PFOS.pdf)

USEPA (2009c). Toxicological Review of Trichloroethylene (CAS No. 79-01-6) In Support of Summary Information on the Integrated Risk Information System (IRIS), External Review Draft. October 2009. EPA/635/R-09/011A.

USEPA (2011a). United States Environmental Protection Agency. Integrated Risk Information System (IRIS) Glossary. Last updated August 21, 2011.  
[https://ofmpub.epa.gov/sor\\_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=&glossaryName=IRIS%20Glossary#formTop](https://ofmpub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=&glossaryName=IRIS%20Glossary#formTop). Accessed May 10, 2016.

USEPA (2011b). Exposure Factors Handbook 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F.

USEPA (2012a). United States Environmental Protection Agency. Benchmark Dose Technical Guidance. Risk Assessment Forum. June 2012. EPA/100/R-12/001.  
[https://www.epa.gov/sites/production/files/2015-01/documents/benchmark\\_dose\\_guidance.pdf](https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf)

USEPA (2012b). United States Environmental Protection Agency. Revisions to the unregulated contaminant monitoring regulation (UCMR 3) for public water systems, final rule. Fed. Regist. 77, 26072–26101.

USEPA (2012c). United States Environmental Protection Agency. “Preamble to IRIS Toxicological Reviews” in: Toxicological Review of Ammonia (CAS No. 7664-41-7) in Support of Summary Information on the Integrated Risk Information System (IRIS). June 2012. EPA/635/R-11/013A.

USEPA (2014). United States Environmental Protection Agency. Draft Health Effects Document for Perfluorotanoic Acid (PFOA). Office of Water. EPA Document Number: 822R14001. February 2014

USEPA (2016a). United States Environmental Protection Agency. Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA). Office of Water. EPA 822-R-16-005. May 2016.  
[https://www.epa.gov/sites/production/files/2016-05/documents/pfoa\\_health\\_advisory\\_final\\_plain.pdf](https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_health_advisory_final_plain.pdf)

USEPA (2016b). United States Environmental Protection Agency. Office of Pollution Prevention and Toxics. 2010/2015 PFOA Stewardship Program. <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/20102015-pfoa-stewardship-program>. Accessed May 15, 2016.

USEPA (2016c). United States Environmental Protection Agency. Drinking Water Health Advisory for Perfluorooctane Sulfonate (PFOS). Office of Water. EPA 822-R-16-005. May 2016.  
[https://www.epa.gov/sites/production/files/2016-05/documents/pfos\\_health\\_advisory\\_final\\_508.pdf](https://www.epa.gov/sites/production/files/2016-05/documents/pfos_health_advisory_final_508.pdf)

USEPA (2016d). United States Environmental Protection Agency. Fact Sheet: PFOA & PFOS Drinking Water Health Advisories. Office of Water. May 2016.

USEPA (2016e). United States Environmental Protection Agency. Hoosick Falls, New York. Drinking Water and Groundwater Contamination. Frequently Asked Questions.  
[https://www.epa.gov/sites/production/files/2016-01/documents/hoosickfalls\\_faqs.pdf](https://www.epa.gov/sites/production/files/2016-01/documents/hoosickfalls_faqs.pdf)

USEPA (2016f). United States Environmental Protection Agency. Occurrence Data for the Unregulated Contaminant Monitoring Rule. Data posted through January 2016.  
<https://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant-monitoring-rule>  
Accessed March 3, 2016.

USEPA (2016f). United States Environmental Protection Agency. Health Effects Support Document for Perfluorooctanoic Acid (PFOA). Office of Water. EPA 822-R-16-003. May 2016.  
[https://www.epa.gov/sites/production/files/2016-05/documents/pfoa\\_hesd\\_final\\_508.pdf](https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final_508.pdf)

Vaalgamaa, S., Vähätalo, A.V., Perkola, N., Huhtala, S. (2011). Photochemical reactivity of perfluorooctanoic acid (PFOA) in conditions representing surface water. *Sci. Total Environ.* 409: 3043-3048.

van Esterik, J.C., Sales, L.B., Dollé, M.E., Håkansson, H., Herlin, M., Legler, J., van der Ven, L.T. (2016). Programming of metabolic effects in C57BL/6JxFVB mice by in utero and lactational exposure to perfluorooctanoic acid. *Arch. Toxicol.* 90: 701-15.

Vanden Heuvel, J.P., Kuslikis, B.I., Van Rafelghem, M.J., Peterson, RE. (1991). Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. *J. Biochem. Toxicol.* 6: 83-92.

Vanden Heuvel, J. P., Thompson, J. T., Frame, S. R., Gillies, P. J. (2006). Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor-alpha, -beta, and -gamma, liver X receptor-beta, and retinoid X receptor-alpha. *Toxicol. Sci.* 92: 476-489.

Vermont Department of Health. 2018. Drinking Water Health Advisory for Five PFAS (per- and polyfluorinated alkyl substances). July 10, 2018.  
[http://www.healthvermont.gov/sites/default/files/documents/pdf/ENV\\_DW\\_PFAS\\_HealthAdvisory.pdf](http://www.healthvermont.gov/sites/default/files/documents/pdf/ENV_DW_PFAS_HealthAdvisory.pdf)

Verner, M.-A., Loccisano, A. E., Morken, N.-H., Yoon, M., Wu, H., McDougall, R., Maisonet, M., Marcus, M., Kishi, R., Miyashita, C., Chen, M.-H., Hsieh, W.-S., Andersen, M. E., Clewell, H. J., Longnecker, M. P. (2015). Associations of perfluoroalkyl substances (PFAS) with lower birth weight: an evaluation of potential confounding by glomerular filtration rate using a physiologically based pharmacokinetic model (PBPK). *Environ. Health Perspect.* 123: 1317-1324.

Verner, M.A., Ngueta, G., Jensen, E.T., Fromme, H., Völkel, W., Nygaard, U.C., Granum, B., Longnecker, M.P. (2016a). A simple pharmacokinetic model of prenatal and postnatal exposure to perfluoroalkyl substances (PFASs). *Environ. Sci. Technol.* 50: 978-86.

Verner, M.A., Ngueta, G., Jensen, E.T., Fromme, H., Völkel, W., Nygaard, U.C., Granum, B., Longnecker, M.P. (2016b). Correction to A Simple Pharmacokinetic Model of Prenatal and Postnatal Exposure to Perfluoroalkyl Substances (PFASs). *Environ. Sci. Technol.* 50:5420-1.

Vestergren, R., Cousins, I.T. (2009). Tracking the pathways of human exposure to perfluorocarboxylates. *Environ. Sci. Technol.* 43: 5565-5575.

Vesterinen, H. M., Johnson, P. I., Atchley, D. S., Sutton, P., Lam, J., Zlatnik, M. G., Sen, S., Woodruff, T. J. (2015). Fetal growth and maternal glomerular filtration rate: a systematic review. *J. Matern. Fetal Neonatal Med.* 28: 2176-2181.

Vieira, V. M., Hoffman, K., Shin, H. M., Weinberg, J. M., Webster, T. F., Fletcher, T. (2013). Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. *Environ. Health Perspect.* 121: 318-323.

von Ehrenstein, O.S., Fenton, S.E., Kato, K., Kuklennyik, Z., Calafat, A.M., Hines, E.P. (2009). Polyfluoroalkyl chemicals in the serum and milk of breastfeeding women. *Reprod. Toxicol.* 27: 239-245.

Wagner, A.M., Sánchez-Quesada, J.L., Benítez, S., Bancells, C., Ordóñez-Llanos, J., Pérez (2011). Effect of statin and fibrate treatment on inflammation in type 2 diabetes. A randomized, cross-over study. *Diabetes Res. Clin. Pract.* 93: e25-8.

Walters, M. W., Bjork, J. A., Wallace, K. B. (2009). Perfluorooctanoic acid stimulated mitochondrial biogenesis and gene transcription in rats. *Toxicology* 264: 10-15.

Wang, M., Park, J.S., Petreas, M. (2011a). Temporal changes in the levels of perfluorinated compounds in California women serum over the past 50 years. *Environ. Sci. Technol.* 45: 7510-7516.

Wang, J., Zhang, Y., Zhang, W., Jin, Y., Dai, J. (2012). Association of perfluorooctanoic acid with HDL cholesterol and circulating miR-26b and miR-199-3p in workers of a fluorochemical plant and nearby residents. *Environ. Sci. Technol.* 46: 9274-9281.

Wang, L., Wang, Y., Liang, Y., Li, J., Liu, Y., Zhang, J., Zhang, A., Fu, J., Jiang, G. (2013). Specific accumulation of lipid droplets in hepatocyte nuclei of PFOA-exposed BALB/c mice. *Sci. Rep.* 3: 2174.

Wang, Y., Rogan, P. C., Chen, G. W., Lien, H. Y., Chen, Y. C., Tseng, M. P., Longnecker, Wang, S. L. (2014). Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. *Environ. Health Perspect.* 122: 529-534.

Washburn, S.T., Bingman, T.S., Braithwaite, S.K., Buck, R.C., Buxton, L.W., Clewell, H.J., Haroun, L.A., Kester, J.E., Rickard, R.W., Shipp, A.M. (2005). Exposure assessment and risk characterization for perfluorooctanoate in selected consumer articles. *Environ. Sci. Technol.* 39: 3904-3910.

Washington, J.W., Ellington, J., Jenkins, T.M., Evans J.J., Yoo, H., Hafner, S.C. (2009). Degradability of an acrylate-linked, fluorotelomer polymer in soil. *Environ. Sci. Technol.* 43: 6617-6623.

Weaver, Y.M., Ehresman, D.J., Butenhoff, J.L., Hagenbuch, B. (2010). Roles of rat renal organic anion transporters in transporting perfluorinated carboxylates with different chain lengths. *Toxicol. Sci.* 113: 305-314.

Wang, Y., Rogan, W. J., Chen, P. C., Lien, G. W., Chen, H. Y., Tseng, Y. C., Longnecker, M. P., Wang, S. L. (2014). Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. *Environ. Health Perspect.* 122: 529-534.

Wei, Y., Dai, J., Liu, M., Wang, J., Xu, M., Zha, J., Wang, Z. (2007). Estrogen-like properties of perfluorooctanoic acid as revealed by expressing hepatic estrogen-responsive genes in rare minnows (*Gobiocypris rarus*). *Environ. Toxicol. Chem.* 26: 2440-2447.

Wen, L. L., Lin, L. Y., Su, T. C., Chen, P. C., Lin, C. Y. (2013). Association between serum perfluorinated chemicals and thyroid function in U.S. adults: the National Health and Nutrition Examination Survey 2007-2010. *J. Clin. Endocrinol. Metab.* 98: E1456-1464.

White, S.S., Calafat, A.M., Kuklennyik, Z., Villanueva, L., Zehr, R.D., Helfant, L., Strynar, M.J., Lindstrom, A.B., Thibodeaux, J.R., Wood, C., Fenton, S.E. (2007). Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. *Toxicol. Sci.* 96: 133-144.

White, S.S., Kato, K., Jia, L.T., Basden, B.J., Calafat, A.M., Hines, E.P., Stanko, J.P., Wolf, C.J., Abbott, B.D., Fenton, S.E. (2009). Effects of perfluorooctanoic acid on mouse mammary gland development and differentiation resulting from cross-foster and restricted gestational exposures. *Reprod. Toxicol.* 27: 289-298.

- White, S.S., Fenton, S.E., Hines, E.P. (2011a). Endocrine disrupting properties of perfluorooctanoic acid. *J. Steroid Biochem.* 127: 16-26.
- White, S.S., Stanko, J.P., Kato, K., Calafat, A.M., Hines, E.P., Fenton, S.E. (2011b). Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 Mice. *Environ. Health Perspect.* 119: 1070-1076.
- Winqvist, A., Steenland, K. (2014a). Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. *Environ. Health Perspect.* 122: 1299-1305.
- Winqvist, A., Steenland, K. (2014b). Perfluorooctanoic acid exposure and thyroid disease in community and worker cohorts. *Epidemiology* 25: 255-264.
- Wolf, C.J., Fenton, S.E., Schmid, J.E., Calafat, A.M., Kuklennyik, Z., Bryant, X.A., Thibodeaux, J., Das, K.P., White, S.S., Lau, C.S., Abbott, B.D. (2007). Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. *Toxicol. Sci.* 95: 462-473.
- Wolf, C.J., Zehr, R.D., Schmid, J.E., Lau, C., Abbott, B.D. (2010). Developmental effects of perfluorononanoic acid in the mouse are dependent on peroxisome proliferator-activated receptor-alpha. *PPAR Res.* 2010, pii: 282896.
- Wolf, C.J., Schmid, J.E., Lau, C., Abbott, B.D. (2012). Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPAR $\alpha$ ) by perfluoroalkyl acids (PFAAs): Further investigation of C4-C12 compounds. *Reprod Toxicol.* 33: 546-551.
- Wolf, D.C., Moore, T., Abbott, B.D., Rosen, M.B., Das, K.P., Zehr, R.D., Lindstrom, A.B., Strynar, M.J., Lau, C. (2008). Comparative hepatic effects of perfluorooctanoic acid and WY 14,643 in PPAR-alpha knockout and wild-type mice. *Toxicol. Pathol.* 36: 632-639.
- Woodruff, T.J. and Sutton, P. (2014). The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. *Environ. Health Perspect.* 122:1007–1014.
- Yahia, D., El-Nasser, M. A., Abedel-Latif, M., Tsukuba, C., Yoshida, M., Sato, I., Tsuda, S., (2010). Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. *J Toxicol. Sci.* 35: 527-533.
- Yamaguchi, M., Arisawa, K., Uemura, H., Katsuura-Kamano, S., Takami, H., Sawachika, F., Nakamoto, M., Juta, T., Toda, E., Mori, K., Hasegawa, M., Tanto, M., Shima, M., Sumiyoshi, Y., Morinaga, K., Kodama, K., Suzuki, T., Nagai, M., Satoh, H. (2013). Consumption of seafood,

serum liver enzymes, and blood levels of PFOS and PFOA in the Japanese population. *J. Occup. Health* 55: 184-194.

Yan, S., Wang, J., Zhang, W., Dai, J. (2014). Circulating microRNA profiles altered in mice after 28 d exposure to perfluorooctanoic acid. *Toxicol. Lett.* 224: 24-31.

Yang, Q., Xie, Y., Depierre, W. (2000). Effects of peroxisome proliferators in the thymus and spleen of mice. *Clin. Exp. Immunol.* 122: 219-226.

Yang, Q., Abedi-Valugerdi, M., Xie, Y., Zhao, X., Moller, G., Nelson, B.D., DePierre, J.W. (2002a). Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. *Internat. Immunopharmacol.* 2: 389-397.

Yang, Q., Xie, Y., Alexson, S. E., Nelson, B. D. DePierre, J. W. (2002b). Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. *Biochem. Pharmacol.* 63: 1893-1900.

Yang, Q., Kurotani, R., Yamada, A., Kimura, S., Gonzalez, F.J. (2006). Peroxisome proliferator-activated receptor alpha activation during pregnancy severely impairs mammary lobuloalveolar development in mice. *Endocrinology* 147: 4772-4780.

Yang, C., Tan, Y.S., Harkema, J.R., Haslam, S.Z. (2009a). Differential effects of peripubertal exposure to perfluorooctanoic acid on mammary gland development in C57Bl/6 and Balb/c mouse strains. *Reprod. Toxicol.* 27: 299-306.

Yang, C. H., Glover, K. P., Han, X. (2009b). Organic anion transporting polypeptide (Oatp) 1a1-mediated perfluorooctanoate transport and evidence for a renal reabsorption mechanism of Oatp1a1 in renal elimination of perfluorocarboxylates in rats. *Toxicol. Lett.* 190: 163-171.

Yang, C. H., Glover, K. P., Han, X. (2010). Characterization of cellular uptake of perfluorooctanoate via organic anion-transporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for their potential roles in mediating human renal reabsorption of perfluorocarboxylates. *Toxicol. Sci.* 117: 294-302.

Yang, B., Zou, W., Hu, Z., Liu, F., Zhou, L., Yang, S., Kuang, H., Wu, L., Wei, J. Wang, J., Zou, T., Zhang, D. (2014). Involvement of oxidative stress and inflammation in liver injury caused by perfluorooctanoic acid exposure in mice. *Biomed. Res. Int.* 2014: 409837.

Yao, X., Zhong, L. (2005). Genotoxic risk and oxidative DNA damage in HepG2 cells exposed to perfluorooctanoic acid. *Mutat. Res.* 587: 38-44.

Yao, P. L., Ehresman, D. J., Rae, J. M., Chang, S. C., Frame, S. R., Butenhoff, J. L., Kennedy, G. L., Peters, J. M. (2014). Comparative in vivo and in vitro analysis of possible estrogenic effects of perfluorooctanoic acid. *Toxicology* 326: 62-73.

Ylinen, M., Kojo, A. Hanhijdrvi, H., Peura, P. (1990). Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. *Bull. Environ. Contam. Toxicol.* 44: 46-53.

Yoo, H., Washington, J.W., Jenkins, T.M., Ellington, J.J. (2011). Quantitative determination of perfluorochemicals and fluorotelomer alcohols in plants from biosolid-amended fields using LC/MS/MS and GC/MS. *Environ. Sci. Technol.* 45: 7985–7990.

York, R.G. (2002). Oral (gavage) two-generation (one litter per generation) reproduction study of ammonium perfluorooctanoic (APFO) in rats. Argus Research Laboratories, Inc. Protocol Number: 418-020, Sponsor Study Number: T-6889.6, March 26, 2002. USEPA AR226-1092.

Zeng, X. W., Qian, Z., Emo, B., Vaughn, M., Bao, J., Qin, X. D., Zhu, Y., Li, J., Lee, Y. L., Dong, G. H. (2015). Association of polyfluoroalkyl chemical exposure with serum lipids in children. *Sci. Total Environ.* 512-513: 364-370.

Zhang, T., Sun, H., Lin, Y., Wang, L., Zhang, X., Liu, Y., Geng, X., Zhao, L., Li, F., Kannan, K. (2011). Perfluorinated compounds in human blood, water, edible freshwater fish, and seafood in China: daily intake and regional differences in human exposures. *J. Agric. Food Chem.* 59: 11168-11176.

Zhang, Y., Beesoon, S., Zhu, L., Martin, J.W. (2013). Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. *Environ. Sci. Technol.* 47: 10619-27.

Zhang, H., Lu, Y., Luo, B., Yan, S., Guo, X., Dai, J. (2014). Proteomic analysis of mouse testis reveals perfluorooctanoic acid-induced reproductive dysfunction via direct disturbance of testicular steroidogenic machinery. *J. Proteome Res.* 13: 3370-85.

Zhao, Y., Tan, Y.S., Haslam, S.Z., Yang, C. (2010). Perfluorooctanoic acid effects on steroid hormone and growth factor levels mediate stimulation of peripubertal mammary gland development in C57BL/6 mice. *Toxicol. Sci.* 115: 214-224.

Zhao, Y., Tan, Y. S., Strynar, M. J., Perez, G., Haslam, S. Z., Yang, C. (2012). Perfluorooctanoic acid effects on ovaries mediate its inhibition of peripubertal mammary gland development in Balb/c and C57Bl/6 mice. *Reprod. Toxicol.* 33: 563-576.

