

**Expert Report of
Barbara D. Beck, Ph.D., DABT, ATS, ERT
in the Matter of
State of Minnesota vs. 3M Company**

Prepared by



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Abbreviations

3M	3M Company
ACGIH	American Conference of Governmental Industrial Hygienists
Acox1	Acyl Coenzyme A Oxidase
ADHD	Attention Deficit/Hyperactivity Disorder
ADME	Absorption, Distribution, Metabolism, and Excretion
ALP	Alkaline Phosphatase
ALSPAC	Avon Longitudinal Study of Parents and Children
ALT	Alanine Aminotransferase
AOR	Adjusted Odds Ratio
APFO	Ammonium Perfluorooctanoate
ASD	Autism Spectrum Disorder
AST	Aspartate Aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area Under the Curve
BMCL ₅	Benchmark Concentration for a 5% Response
BMDL ₁₀	Benchmark Dose Level for a 10% Response
BMI	Body Mass Index
C8 Cohort	C8 Health Project Cohort
CCK	Cholecystokinin
CDC	Centers for Disease Control and Prevention
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CHEF	Children's Health and Environment in the Faroe Islands
CHiRP	Chemicals, Health, and Pregnancy
CI	Confidence Interval
CIMT	Carotid Intima Media Thickness
CNS	Central Nervous System
ConA	Concanavalin A
COPC	Constituent of Potential Concern
CSF	Cancer Slope Factor
CVD	Cardiovascular Disease
DA	Dermal Absorption Fraction
Danish EPA	Danish Environmental Protection Agency
DCH	Diet, Cancer, and Health
DWEL	Drinking-water-equivalent Level
DWI	Drinking Water Intake
ECF	Electrochemical Fluorination
ED-RIA	Equilibrium Dialysis Followed by Radioimmunoassay
EFSA	European Food Safety Authority
eGFR	Estimated Glomerular Filtration Rate
Ehhadh	Enoyl Coenzyme A Hydratase
ELCR	Excess Lifetime Cancer Risk
EPC	Exposure Point Concentration
F0	Parental Generation

F1	First Generation
F2	Second Generation
FA	Fraction Absorbed
FAI	Free Androgen Index
FSH	Follicle-stimulating Hormone
GAC	Granular Activated Carbon
GACA	Genetics and Biomarkers Study for Childhood Asthma
GD	Gestational Day
GFR	Glomerular Filtration Rate
GGT	Gamma-glutamyl Transferase
GWSS	Ground Water Supply Survey
HA	Health Advisory
HBV	Health-based Value
HDL	High-density Lipoprotein
HED	Human Equivalent Dose
HI	Hazard Index
hPPAR α	Humanized Peroxisome Proliferator-activated Receptor α
HQ	Hazard Quotient
HR	Hazard Ratio
HRI	Health Risk Index
HRL	Health Risk Limit
IARC	International Agency for Research on Cancer
IFN- γ	Interferon Gamma
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHD	Ischemic Heart Disease
IL-2	Interleuken 2
IL-4	Interleuken 4
IL-5	Interleuken 5
INUENDO	Biopersistent Organochlorines in Diet and Human Fertility
IQR	Interquartile Range
IRIS	Integrated Risk Information System
IRR	Incidence Rate Ratio
IUR	Inhalation Unit Risk
iv	Intravenous
K _{ow}	Octanol-Water Partition Coefficient
LD ₅₀	Median Lethal Dose
LDL	Low-density Lipoprotein
LH	Luteinizing Hormone
LOAEL	Lowest Observed Adverse Effect Level
LOEL	Lowest Observed Effect Level
LOQ	Limit of Quantitation
MCL	Maximum Contaminant Level
MDH	Minnesota Department of Health
Minnesota	State of Minnesota

MIRC	Maternal-Infant Research on Environmental Chemicals
MMR	Measles, Mumps, and Rubella
MoA	Mode of Action
MOE	Margin of Exposure
MPCA	Minnesota Pollution Control Agency
N-EtFOSE	N-ethyl Perfluorooctanesulfonamido Ethanol
NAS	National Academy of Sciences
NHANES	National Health and Nutrition Examination Survey
NK	Natural Killer
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NPL	National Priorities List
NRC	National Research Council
NTP	National Toxicology Program
OAT	Organic Anion Transporter
Oatp	Organic Anion Transporting Polypeptide
OECD	Organisation for Economic Co-operation and Development
OR	Odds Ratio
PBPK	Physiologically Based Pharmacokinetic
PFBA	Perfluorobutanoic Acid
PFBS	Perfluorobutane Sulfonate
PFC	Perfluorinated Chemical
PFHxA	Perfluorohexanoic Acid
PFHxS	Perfluorohexane Sulfonate
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
PFPeA	Perfluoropentanoic Acid
PND	Post-natal Day
POD	Point of Departure
PPAR α	Peroxisome Proliferator-activated Receptor α
ppb	Parts Per Billion
ppm	Parts Per Million
PXR	Pregnane X Receptor
QC	Quality Control
RAGS	Risk Assessment Guidance for Superfund
RCRA	Resource Conservation and Recovery Act
RfC	Reference Concentration
RfD	Reference Dose
RME	Reasonable Maximum Exposure
RSC	Relative Source Contribution
RSL	Regional Screening Level
SARA	Superfund Amendments and Reauthorization Act
SDWA	Safe Drinking Water Act
SGA	Small for Gestational Age
SHBG	Sex-hormone-binding Globulin
SIR	Standardized Incidence Ratio
siRNA	Small Interference RNA
SMR	Standard Mortality Ratio

SRBC	Sheep Red Blood Cell
SRV	Soil Reference Value
T3	Triiodothyronine
T4	Thyroxine
TDAR	T-cell-dependent Antibody Response
TGAb	Thyroglobulin Antibody
TIAR	T-cell-independent Antibody Response
TMAb	Thyroid Microsomal Antibody
TNP	Trinitrophenyl
TPOAb	Thyroid Peroxidase Antibody
TRH	Thyrotropin-releasing Hormone
TSCA	Toxic Substances Control Act
TSH	Thyroid-stimulating Hormone
TTP	Time to Pregnancy
TWA	Time-weighted Average
UCLM	Upper Confidence Limit on the Mean
UF	Uncertainty Factor
UK FSA	United Kingdom Food Standards Authority
UK	United Kingdom
UN	Minnesota Unique Well Number
US	United States
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
V _d	Volume of Distribution
VLDL	Very Low Density Lipoprotein
VOC	Volatile Organic Compound

1 Introduction

1.1 Case Overview

The State of Minnesota (herein, Minnesota) claims that starting in the 1950s, 3M Company (3M) disposed of waste and discharged wastewater containing certain perfluorinated chemicals (PFCs) from their industrial facility located in the City of Cottage Grove, Minnesota, as well as at a disposal site located in the City of Oakdale, Minnesota; a disposal site located on the border of the cities of Cottage Grove and Woodbury, Minnesota; and the Washington County Landfill, located in the City of Lake Elmo, Minnesota (Minnesota, Attorney General, 2011). Minnesota further claims that 3M discharged PFC-containing wastewater into surface water that flows directly into the Mississippi River. Minnesota claims that 3M's discharge of PFCs caused over 100 square miles of groundwater and various surface water bodies to be contaminated, thereby causing injury to and destruction and loss of natural resources of Minnesota (Minnesota, Attorney General, 2011). Minnesota further claims that, during the period that 3M manufactured and disposed of such PFCs and PFC-containing wastes, 3M knew or should have known that its activities would have adverse impacts on the natural resources of the state, including groundwater, surface water, sediments, and aquatic life, such as fish (Minnesota, Attorney General, 2011).

I was retained by Brewer, Attorneys and Counselors on behalf of 3M in this matter to evaluate, to a reasonable degree of scientific probability, the potential for human health effects from certain PFCs, specifically perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorobutanoic acid (PFBA), perfluorobutane sulfonate (PFBS), and perfluorohexane sulfonate (PFHxS) in the environment as a result of discharges from 3M facilities in Hennepin County, Minnesota, and to evaluate the development of scientific understanding by 3M and the general medical and scientific community over time of the toxicology of PFCs in the environment. My *curriculum vitae* is attached in Appendix A. It reflects each of my publications over the last 10 years. Gradient is compensated for my time at the rate of \$575/hour.

1.2 Qualifications of Dr. Barbara D. Beck

I received an A.B. degree (*cum laude*) in biology from Bryn Mawr College and a Ph.D. in molecular biology and microbiology from Tufts University. I am an expert in toxicology, with a specialty in human health risk assessment. I hold several certifications in toxicology, listed as follows. I am a diplomate of the American Board of Toxicology and a Fellow and Past President of the Academy of Toxicological Sciences. Both of these certifications are internationally recognized. The membership of each includes individuals from many countries, including countries in Europe, Asia, and Africa, as well as North America. I am also certified as a European Registered Toxicologist, through the United Kingdom (UK) Registry of Toxicologists. All of these certifications are current.

I am a Visiting Scientist in the Department of Environmental Health at the Harvard T.H. Chan School of Public Health and a Principal at Gradient, an environmental consulting company that specializes in the fate and transport of chemicals in the environment and human health risk assessment.

I was a research associate and a fellow in the Interdisciplinary Program in Health at the Harvard T.H. Chan School of Public Health, where I developed a short-term bioassay to predict the toxicity of particulate matter and gases for the lungs. I was also an editor and author of a monograph on variations in susceptibility to inhaled pollutants.

I was Regional Expert in Toxicology and chief of the Air Toxics Staff at Region I of the United States Environmental Protection Agency (US EPA), which includes the New England states. In this capacity, I provided advice on matters of toxicology, particularly as related to air toxics.

I joined Gradient in 1987. My consulting practice consists of health risk assessments for cancer and non-cancer endpoints, review of animal toxicology and human epidemiology studies, multi-media assessment of exposure to environmental chemicals, and evaluation of the historical development of toxicology.

I have served in an advisory capacity to a wide range of governmental and non-profit institutions on issues relating to toxicology, risk assessment, and public health. I have been active in the Society of Toxicology for many years, in both elected and appointed roles.

I have published articles on toxicology and risk assessment in peer-reviewed journals, books, and meeting proceedings. These publications have addressed a range of topics, such as the use of toxicology in the regulatory process and the toxicology of specific chemicals, including arsenic and manganese. I have been a peer reviewer, which includes editorial positions, for multiple journals.

I have evaluated epidemiological, animal toxicological, and mechanistic studies for purposes of drawing conclusions regarding the risk to humans from exposure to specific chemicals. These analyses, which have been conducted using appropriate scientific principles and various analytical frameworks, have addressed a wide range of chemicals, including metals, chlorinated solvents, and aromatic compounds.

I co-authored two studies of PFCs that were presented at the 47th and the 55th Annual Society of Toxicology meetings (Lewis *et al.*, 2008; Lynch *et al.*, 2016) and have been designated as an expert on the toxicology of PFCs (including, but not limited to, PFOS) in several litigation cases in the United States (US).

I have been a designated expert and given deposition testimony for 3M on two occasions: once in January 2007 (Felicia Palmer *et al.* vs. 3M Company; State of Minnesota, Tenth Judicial District, County of Washington, Case C2-04-6309) and once in August 2008 (Gary A. Paulson, Karen Paulson, William Henry, and Bradley Krank vs. 3M Company; State of Minnesota, County of Washington, Case C2-04-6309). I was also a designated expert for 3M, but never testified, in the Matter of 3M United Kingdom PLC vs. The States of Guernsey vs. 3M Company, High Court of Justice, Queen's Bench Division, Commercial Court, Claim No. 2013 Folio 1044. In addition, at the request of 3M, I submitted comments on the US EPA draft Health Effect Documents for PFOA and PFOS in 2014, and on the National Toxicology Program (NTP) draft systematic review of immunotoxicity associated with PFOA and PFOS in 2016.

2 Summary of Opinions

2.1 Summary of Primary Opinions

As a toxicologist and human health risk assessor, I have been asked to provide an expert opinion in the matter of State of Minnesota vs. 3M Company, considering the following questions:

- What are the potential human health effects of PFOA, PFOS, PFBA, and PFBS, considering studies in animals and humans and mechanistic studies?
- How has Minnesota evaluated the health effects of PFOA, PFOS, PFBA, and PFBS in the environment and what are the strengths and limitations of the methodology it employed to do so?
- Based on an independent evaluation, what is the toxicological significance to humans of the PFOA, PFOS, PFBA, and PFBS found in groundwater, surface water, sediment, and aquatic life, such as fish?
- How did 3M's scientific knowledge of the health effects of PFOA and PFOS and the potential for their presence in the environment develop over time, and did 3M's development and communication of such knowledge to the public proceed at a reasonable rate?

It is my overall opinion that:

- MDH's approach to evaluating exposures to PFOA, PFOS, PFBA, and PFBS overestimates risk (*i.e.*, the potential for adverse health effects in humans), and agency guidelines could be higher and still be health-protective.
- While some environmental concentrations of PFOA, PFOS, and PFBA exceed health-based criteria for drinking water, such exposures are below those concentrations at which health effects could potentially occur.¹ Similarly, hypothetical exposures to PFOA, PFOS, PFBA, and PFBS in surface water, groundwater, sediment, and fish are below those at which health effects could potentially occur.
- Serum concentrations of PFOA and PFOS in individuals residing in southern Washington County are well below serum concentrations measured in studies of 3M workers, which found no reliable and consistent evidence of health effects from exposure to these PFCs, and below serum concentrations in animal studies at which no adverse effects have been consistently and reliably observed.
- Thus, environmental concentrations of PFOA, PFOS, PFBA, and PFBS do not present a toxicological concern. There is no reliable evidence that people residing in southern Washington County are being harmed by the presence of these chemicals in the surrounding environment.
- 3M developed scientific knowledge of the health effects of PFOA and PFOS and the potential for these PFCs to be present in the environment at an appropriate rate. The development of such understanding required technical advances (*e.g.*, in analytical chemistry), and particular attention was reasonably directed towards more highly exposed populations. 3M shared the information

¹ I have not identified any drinking water exceedances for PFBS.

about PFOA and PFOS they gleaned from their investigations with the broader scientific community through presentations, publications, and other approaches.

My specific opinions are summarized in the following subsections. To the extent that I receive additional materials, I reserve the right to update my conclusions as appropriate.

2.2 Methodology

It is my opinion that the development of reliable conclusions regarding the health effects of chemicals entails considering multiple sources of evidence and appropriately integrating such findings.

For my analysis here, I have applied the principles of toxicology, epidemiology, and regulatory risk assessment for the purposes of evaluating causal relationships between the PFCs at issue and health effects. Examples of the key principles of toxicology, epidemiology, and regulatory risk assessment include evaluation of dose-response and exposure. For my comprehensive and independent analysis, I evaluated the evidence summarized in toxicological reviews and evidence presented in primary scientific literature.

2.3 PFC Chemistry and Monitoring

Generally, PFCs refer to a class of fully fluorinated compounds that have carbon chain lengths consisting of 4-16 carbons ("C4" to "C16") with a functional end group attached with a double bond. This chemical structure results in multiple useful properties, which vary depending on the particular chemical formula of a compound. PFCs are man-made and do not occur naturally in the environment. They have been found to persist in the environment and to bioaccumulate in certain organisms. Nonetheless, such findings do not indicate that the presence of PFCs in the environment or in living organisms means that adverse effects are occurring or are likely to occur. Such a determination must be made in the context of other relevant information, as discussed below.

It is my opinion that not only has 3M been active in commercial production of certain PFCs, but also 3M has been active in monitoring the presence of certain PFCs in the environment and, when appropriate, undertaking remediation of PFC-contaminated sites.

3M began commercial production of PFCs in the early 1950s and produced PFCs at its Cottage Grove facility from the late 1940s until 2002. In 2000, 3M began to phase out all production of PFOA- and PFOS-based products, and phase-out of these products in the US was complete by 2002. Since the early 2000s, 3M has been monitoring PFC levels and remediating waste sites in the areas surrounding the Minnesota production and disposal sites.

2.4 ADME/PBPK and Modes of Action

It is my opinion that an understanding of the processes of absorption, distribution, metabolism, and excretion (also referred to as ADME) of PFCs is important for appropriately interpreting findings of potential health effects of PFCs in human studies and animal studies, in particular for extrapolating exposures across and within species and for developing health-based toxicity limits.

Collectively, the processes of ADME determine the concentration of a chemical in target tissues where health effects occur. Because ADME can differ both across and within species, understanding ADME is

important for extrapolating health effects between and within species. This is because, given the same administered dose, differences in ADME can result in differences in target tissue chemical concentrations. Understanding ADME can also aid interpretation of study findings and determination of whether observed associations are causal. To this end, physiologically based pharmacokinetic (PBPK) modeling approaches are useful for incorporating ADME factors and for estimating exposures across species and life stages for the modeled substances.

PFOA, PFOS, PFBA, and PFBS are all well-absorbed following oral exposure, with PFOA and PFOS preferentially distributing to the liver and PFBA preferentially distributing to serum.² Unlike some chemicals, which are metabolized and for which metabolism is an important determinant of toxicity, none of the PFCs discussed herein appear to undergo metabolism. PFOA and PFOS both transfer across the placenta and into breast milk, such that the developing fetus and newborn can be exposed to PFCs both *in utero* and post-natally *via* lactation. With respect to elimination, there are important differences between the PFCs, across species, and between sexes. Compared to PFOA and PFOS, PFBA and PFBS are eliminated more rapidly, which likely contributes to these compounds' lower toxicity. For rodents, PFBA and PFOA are eliminated more rapidly in females than in males. Across species, elimination is considerably slower in humans than in either monkeys or rodents, resulting in higher serum PFC concentrations in humans given the same dose. Because of these differences in elimination, serum concentration is a better metric than dose for extrapolating effect levels across species.

PBPK models, which incorporate the factors governing ADME processes, are useful for estimating exposures across species and life stages for the modeled substances. PBPK models are useful when there are differences in physiological processes across species, sexes, or age groups, as are observed for PFOA and PFOS. For example, PBPK models provide evidence that the associations between PFOS exposure and late puberty onset and early menopause are likely the result of reverse causation, meaning menstruation/menopause affects serum PFOS concentrations, rather than PFOS affecting the timing of menstruation/menopause. Other models estimating PFOA and PFOS concentrations in maternal and cord plasma determined that glomerular filtration rate (GFR) during pregnancy confounds the association between low birth weight and PFOA or PFOS exposure observed in certain epidemiology studies. PBPK models can also be used for establishing regulatory criteria, as was done by the Minnesota Department of Health (MDH) for developing their most recent Health-based Values (HBVs) for PFOA and PFOS (discussed further below).

It is my opinion that understanding the mode of action (MoA, a description of the key events involved in mediating the specific health effects of a chemical) is important for understanding the human relevance of PFCs findings in animals. As with ADME, MoAs can also differ across species, such that certain health effects may occur in some species, while in other species, the effects may not occur or may occur only at higher exposure concentrations. For the PFCs at issue in this report, the most well-studied MoA involves activation of peroxisome proliferator-activated receptor α (PPAR α), which controls the expression of enzymes involved in lipid metabolism. PPAR α activation can cause liver effects, such as increased liver weight, and is likely to play a role in mediating other effects, such as certain immunological and developmental endpoints, with rodents being more sensitive than humans to effects mediated by PPAR α activation. I identified studies that evaluated whether PPAR α mediates the liver, immunological, or developmental effects of PFOA or PFOS. Collectively, these studies indicate that PPAR α is likely involved in mediating at least some of the liver, immunological, and developmental effects of PFOA but not PFOS. As such, humans would be expected to be less susceptible than rodents to the liver and developmental effects of PFOA.

² I did not identify any studies that evaluated the distribution of PFBS to the liver.

2.5 Animal and Human Studies

It is my opinion that studies of PFCs, particularly PFOA and PFOS, in animals and in humans (*i.e.*, epidemiology studies in humans) provide a robust basis for understanding the potential health effects of PFCs in humans under environmental exposure conditions. Overall, the most consistent and reliable finding in animal studies is liver effects, although such findings are not necessarily adverse. Other findings (*e.g.*, developmental and reproductive effects) are inconsistent and inconclusive. There is very little evidence of associations between PFOS exposure and health effects in humans, even at the relatively high exposure levels seen in worker studies.

Certain PFCs, especially PFOA and PFOS, have been studied in animals and humans. Such studies have evaluated multiple endpoints (*e.g.*, changes in liver weight or alterations in levels of thyroid hormone in blood). These studies have been conducted with exposure durations ranging from days to an entire lifespan in animals and up to decades in humans. Relevant findings include the following:

Animal Studies. I note that while some agencies have recently focused on reproductive/developmental and immune endpoints as being of particular relevance to PFOA and PFOS, I do not consider these findings to be conclusive. The most-sensitive endpoints for PFOA, PFOS, PFBA, and PFBS vary among animal species. In general, effects on the liver, decreases in serum lipids, and changes in serum thyroid hormones are sensitive endpoints in monkeys; liver effects are the most sensitive endpoint in rats; and immune effects are the most sensitive endpoint in mice. It is important to note that some of these changes, such as liver weight changes, are sufficiently small that they may reflect non-adverse, adaptive changes. The immune findings in mice are inconsistent across and within studies and not as reliable as findings noted for monkeys and rats.

Human Studies. Studies in workers exposed to PFCs have not found any consistent adverse effects as a result of PFC exposure. Workers typically had PFOA or PFOS serum concentrations much greater than the concentrations found in the general population. Such studies have evaluated a range of health endpoints, such as altered liver enzymes, cardiovascular disease (CVD), and cancer. While some increases in serum lipid levels have been associated with exposure to PFCs, the results have been inconsistent across studies and these increases have not been associated with CVD.

Studies of PFCs in community populations, like those in occupational populations, have inconsistent results for certain endpoints, such as reproductive and developmental effects. A number of these studies have important methodological limitations, such as one-time, concurrent measurements of exposure and effect. Overall, the studies in community populations do not provide sufficient evidence of adverse health effects from PFC exposure. For example, changes in thyroid hormones have been associated with PFOS exposure, but the direction of these changes has been discordant, and changes among males and females are inconsistent. In some cases (for example, the observed changes in birth weight associated with PFOA or PFOS exposure), the results may be explained by the endpoint affecting the pharmacokinetics of PFOA or PFOS (*i.e.*, reverse causality).

2.6 Evaluation of US EPA and Minnesota Toxicity Criteria and Environmental Guideline Levels

It is my overall opinion that the toxicity and drinking water criteria developed by US EPA and MDH could be higher and still be health-protective.

US EPA and MDH have developed criteria concerning acceptable human exposures to specific PFCs. Agency exposure limits for chemicals represent levels below which no health effects are anticipated. These values tend to overestimate risk by design, and exceedance of a health-based guideline indicates that further investigation may be warranted but does not imply that any exposures above the recommended level will lead to health effects.

It is also important to note that the US EPA and MDH guidelines for PFOA and PFOS are not based on new findings from recent animal or human studies. All of the studies that provided the health effects upon which the guidelines are based were conducted in 2005 and 2006. These studies were also included in prior US EPA and MDH guideline evaluations, but were either not chosen as critical studies or were chosen for different health effect outcomes in the earlier assessments. It is not clear why US EPA and MDH changed course in their selection of key studies and health effects for evaluating PFOA and PFOS. It is also noteworthy that none of the US EPA or MDH PFC guidelines are based on findings of human health effects. All of the health effects chosen as guideline bases were from animal study results, and these effects have not been confirmed in humans.

US EPA's and MDH's choices of endpoints for developing PFOA, PFOS, PFBA, and PFBS drinking water guidelines are highly uncertain, and other choices are better supported by the underlying science. US EPA's Lifetime Health Advisories (HAs) and MDH's HBVs for PFOA and PFOS are based on developmental endpoints that I do not consider to be conclusive. The MDH HBV for PFBA is based on a short-term animal study, even though there is an appropriate longer-term animal study available that would eliminate some of the uncertainty in the guideline derivation. For PFBS, the MDH HBV is based on endpoints that are of uncertain biological or toxicological significance.

My specific opinions regarding the guidelines for PFOA are as follows:

- US EPA and MDH both chose the lowest observed effect level (LOEL) from a mouse developmental study and calculated a human equivalent dose (HED) based on differences in PFOA half-life between mice and humans. The LOEL chosen by US EPA and MDH was 1 mg/kg-day. The HED was 0.0053 mg/kg-day. This was the basis of the reference dose (RfD) of 0.00002 mg/kg-day derived by US EPA and the RfD of 0.000018 derived by MDH.
- The developmental endpoints that were chosen as the basis of RfDs by both US EPA and MDH (*i.e.*, delayed ossification and accelerated male puberty in mice) are not supported by science. Neither endpoint exhibits a regular dose-response; in fact, the accelerated puberty endpoint shows the greatest effects at the lowest dose. The ossification effects are not necessarily adverse and are reported in an irregular manner that is not consistent with accepted methodology.
- An endpoint in the underlying study that is better supported by the science is reduced pup body weight; the no observed effect level (NOEL) for reduced pup body weight is 1 mg/kg-day, and the use of this value would eliminate the need for a 10-fold uncertainty factor (UF) in US EPA's RfD derivation and for a 3-fold UF in MDH's RfD derivation.
- A UF of 10 that was applied by MDH to account for intraspecies differences could be reduced to 3 when setting the HBV. The reason for this is that the component that accounts for sensitive subpopulation pharmacokinetics is already built into the model used to derive the HBV.
- An additional UF factor of 3 for database uncertainty that was applied by MDH is unnecessary, because there is a sufficient quantity of high-quality studies of PFOA toxicity from which to derive the RfD.
- Thus, the RfD derived by MDH for PFOA could be 30 times higher, or 0.00054 mg/kg-day, in the context of setting the HBV and still be health-protective.

My specific opinions regarding the guidelines for PFOS are as follows:

- US EPA and MDH both chose a LOEL from a rat developmental study and calculated an HED based on differences in PFOS half-life between rats and humans. The NOEL chosen by US EPA and MDH was 0.1 mg/kg-day. The HED was 0.00051 mg/kg-day. This was the basis of the RfD of 0.00002 mg/kg-day derived by US EPA and the RfD of 0.0000051 mg/kg-day derived by MDH.
- The developmental endpoint of reduced pup body weight in rats that both US EPA and MDH chose as the basis of deriving an RfD for PFOS is not supported by the science. This endpoint was not adverse, was transient, and was not considered to be toxicologically significant by the study authors.
- A NOEL for this study that is better supported by the science is 0.4 mg/kg-day, and the use of this value would increase both the US EPA and MDH RfDs 4-fold.
- A UF of 10 that was applied by MDH to account for intraspecies differences could be reduced to 3 when setting the HBV. The reason for this is that the component that accounts for sensitive subpopulation pharmacokinetics is already built into the model used to derive the HBV.
- An additional UF of 3 for database uncertainty (to account for possible immune effects at PFOS doses lower than 0.1 mg/kg-day) that was applied by MDH is unnecessary, because the science does not support there being immune effects at such low doses of PFOS.
- Thus, the RfD derived by MDH could be 40 times higher, or 0.00020 mg/kg-day, in the context of setting the HBV and still be health-protective.

My specific opinions regarding the guidelines for PFBA are as follows:

- MDH chose a NOEL from a rat study and calculated a benchmark dose level for a 10% response (BMDL₁₀) in rats of 3.01 mg/kg-day. MDH then calculated an HED based on differences in PFBA half-life between rats and humans. The NOEL chosen by MDH was 6 mg/kg-day. The HED was 0.38 mg/kg-day. This was the basis of the RfD of 0.0038 mg/kg-day and the Health Risk Limit (HRL) of 7 µg/L derived by MDH.
- MDH based its guidance on a short-term animal study, because its calculation based on a long-term study gave higher allowable drinking water values. It is not plausible that a short-term exposure would be associated with higher risk than a longer-term exposure to the same dose of PFBA.
- The fact that the derivation of a short-term guidance value for PFBA yielded a more conservative (*i.e.*, lower) allowable intake than the derivation of a chronic guidance value underscores the uncertainty involved in these types of calculations.

My specific opinions regarding the guidelines for PFBS are as follows:

- MDH chose a NOEL from a rat study and calculated an HED based on differences in PFBS half-life between rats and humans. The NOEL chosen by MDH was 60 mg/kg-day. The HED was 0.42 mg/kg-day. This was the basis of the RfD of 0.0014 mg/kg-day and the HRL of 7 µg/L derived by MDH.
- MDH guidance for PFBS is based on endpoints that are not supported by the science. MDH chose small changes in blood (hemoglobin and hematocrit) in rats as a critical effect, even though

these effects were not considered to be biologically significant by the study authors or by US EPA. MDH also chose histological changes in the kidney as a co-critical effect, even though there was no report of such an effect at the dose that MDH chose as the LOEL (200 mg/kg-day).

It is my opinion that alternative, higher permissible levels for drinking water would be scientifically supported and still health-protective. To put the health-protectiveness of the MDH guidelines for PFOA and PFOS in drinking water into perspective, I compared the guideline values with the HEDs at the NOEL and LOEL doses from the animal studies that provided the bases for the guidelines (Table 2.1).³ In order to reach the HEDs of PFOA or PFOS that were associated with health effects in the animal studies, an adult would have to drink thousands of glasses of water at the guideline concentrations.

Table 2.1 Comparative Doses from Animal Studies: Number of 8 oz. Glasses of Water at the HBV an Adult Would Need to Drink to Reach the Serum Concentrations Used as the Basis for the HBVs^a

PFC	Agency Guidance (mg/L)	Study/Species	Effects	LOEL/NOEL	Dose (mg/kg-day)	HED (mg/kg-day)	Intake of Water at Guideline Value Needed for a 70 kg Adult to Reach the HED	
							L/Day	8 oz. Glasses/Day
PFOA	MDH HBV = 0.000035	Lau <i>et al.</i> (2006)/ Mouse	Delayed skeletal ossification, accelerated male puberty	LOEL	1	0.0053	11,000	45,000
PFOS	MDH HBV = 0.000027	Luebker <i>et al.</i> (2005a)/ Rat	Reduced weight gain in F2 pups	NOEL	0.1	0.00051	1,300	5,600

Notes:

F2 = Second Generation; HBV = Health-based Value; HED = Human Equivalent Dose; LOEL = Lowest Observed Effect Level; MDH = Minnesota Department of Health; NOEL = No Observed Effect Level; PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

(a) Water intake is rounded to two significant digits.

Moreover, MDH has sometimes not followed its own guidelines. Its calculation of Health Risk Indices (HRIs), for example, leads to conclusions regarding risk from PFC exposure that are not supported, based on my analysis using the MDH guidelines. In addition, Minnesota has not followed its own regulation concerning the definition of hazardous waste with regard to PFOA, PFOS, PFBA, and PFBS.

2.7 Evaluation of Data in Specific Locations at Issue

It is my opinion that, while some environmental concentrations of PFOA, PFOS, and PFBA⁴ in groundwater exceed drinking water guidelines, the significance of such concentrations must be based on a comprehensive analysis of the available data, the underlying health-based criteria, and, for PFOA and PFOS, the serum concentrations of these chemicals in individuals residing in the southern Washington County area. A similar analysis must be performed for evaluating the significance of PFOA, PFOS, PFBA, and PFBS concentrations in surface water, sediment, and fish. Using this approach, I conclude

³ Note that the PFOA value presented by US EPA and MDH as a LOEL is more correctly interpreted as a NOEL, and the PFOS NOEL value used by US EPA and MDH more correctly should be 4-fold higher (*i.e.*, 4-fold less conservative).

⁴ I have found no exceedances of PFBS drinking water guidelines.

that hypothetical exposure levels of PFOA, PFOS, PFBA, and PFBS among residents of southern Washington County from groundwater, surface water, sediment, and fish are below those at which health effects could potentially occur. Serum concentrations of PFOA and PFOS are below those found in worker populations and below those in animals at which there is no consistent and reliable evidence of health effects. Thus, the presence of PFOA, PFOS, PFBA, and PFBS in southern Washington County does not present a toxicological concern, and residents of southern Washington County are not being harmed by the presence of these chemicals in the surrounding environment.

For this assessment, I conducted a scientifically appropriate analysis of human exposures to PFOS, PFOA, PFBA, and PFBS in southern Washington County. I used information developed from MDH and 3M data, regulatory agency methodology, and the general scientific literature in the areas of exposure assessment, risk assessment, toxicology, and epidemiology. I considered the circumstances under which people may plausibly come into contact with either groundwater or sediment, surface water, and fish at the water bodies within southern Washington County that were named in the Interrogatories. Based on MDH's use of developmental endpoints in setting its regulatory guidance levels for PFOA and PFOS, I identified the most-appropriate target receptors to be an adult female resident who uses public or private drinking water in southern Washington County and an adult female recreational user who fishes in the area. These receptors would potentially be exposed to PFCs through groundwater (ingestion exposure), surface water (ingestion exposure), sediment (ingestion and dermal exposures), and fish (ingestion exposure). I conducted this in-depth assessment of PFCs exposures using accepted US EPA risk assessment guidelines and methodology. Other individuals, including child residents or child recreational users, may be present in these areas, but I selected the adult female for the main exposure analysis because, as noted above, developmental effects are the endpoints used by MDH for non-cancer health effects of PFOA and PFOS.

I reviewed the data and the available sample locations for inclusion in the named water bodies or groundwater within southern Washington County as described in the Responses to the Interrogatories (Minnesota, Attorney General, 2012). For the adult female resident's exposure to groundwater, I evaluated past exposures and more-recent exposures to private and public drinking water wells. To evaluate past exposures, I calculated a 95% upper confidence limit on the mean (UCLM) based on the highest 12-month moving average for each well or group of wells. To evaluate recent exposures, I used the two most-recent rounds of groundwater sampling, as recommended by US EPA exposure assessment methodology (US EPA, 2014a). For the adult female recreational user, I evaluated surface water, sediment, and fish data from the named water bodies. I limited my sediment dataset within the named water bodies to shallow sediment (approximately 6-in depth) and within areas of the water bodies that are accessible to humans. I used a fish dataset from the named water bodies that would most closely model human consumption practices (*i.e.*, I excluded whole organism fish samples and used fillet samples).

My exposure analysis used the most-current US EPA methodology and assumptions specific to the geographic area and population of interest. The calculated exposures are high-end hypothetical values, which overestimate exposures for most residents of southern Washington County. I compared the exposures with point of departure (POD) values chosen by US EPA and MDH to develop their RfDs and drinking water guidelines.⁵ The resulting comparison yielded margin of exposure (MOE) values; MOE values greater than 1 indicate that hypothetical exposures are below those associated with possible health effects in animals.

Table 2.2 presents the lowest MOE values for all the drinking water wells tested and the MOE values for the adult female recreational user exposure pathway. Note that I present two sets of MOE values for

⁵ As discussed earlier in this section, the POD values are likely to overestimate risk, based on full consideration of the underlying toxicological and epidemiological evidence.

PFOS: one calculated using the POD chosen by US EPA and MDH and one calculated using the more scientifically supported POD discussed in Section 2.6. The remaining MOE values (for PFOA, PFBA, and PFBS) were calculated using only the PODs chosen by US EPA and MDH.

Table 2.2 Lowest MOE Values^a for Specific Pathways

PFC	POD (mg/kg-day)	Past Hypothetical Exposure, Private Well with Highest Concentration	Past Hypothetical Exposure, City Well with Highest Concentration	Current Hypothetical Exposure, Private Well with Highest Concentration	Current Hypothetical Exposure, City Well with Highest Concentration	Hypothetical Exposure, Adult Female Recreational User
PFOA	0.0053	50	190	170	1,000	3,200
PFOS ^b	0.00051	4.3	12	31	83	4.6
PFOS ^c	0.002	17	47	120	330	18
PFBA	0.86	1,100	10,000	5,000	21,000	2,400,000
PFBS	0.42	39,000	39,000	170,000	220,000	1,500,000

Notes:

PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate; POD = Point of Departure.

(a) MOEs rounded to two significant digits.

(b) POD selected by US EPA and MDH.

(c) Alternative and better-supported POD (see Section 2.6).

This analysis demonstrates that hypothetical PFC exposures for the adult female recreational user are below levels that have been associated with possible health effects in toxicological studies. The lowest MOE values range from 50-3,200 for PFOA; from 4.3-83, using the US EPA/MDH POD for PFOS, and from 17-330 using the more scientifically supported POD for PFOS; from 1,100-2,400,000 for PFBA; and from 39,000-1,500,000 for PFBS.⁶ It should be noted that, in comparison with levels that have been associated with possible health effects in toxicological studies, hypothetical exposures to PFCs in sediment and surface water for the adult female recreational user are especially negligible. These MOE comparisons indicate that health effects are not expected at the environmental concentrations of these PFCs found in the southern Washington County area, particularly when considering the underlying basis for the selected POD values.

I also considered serum concentrations of PFOA and PFOS in residents in the southern Washington County area. Serum concentrations reflect exposures to PFOA and PFOS in media such as food, dust, and air, as well as exposures considered in this report (*i.e.*, PFCs in groundwater, surface water, sediment, and fish). Serum concentration comparisons provide a more realistic, but still conservative, evaluation of exposures to PFOA and PFOS in southern Washington County (by virtue of including other sources) than the hypothetical exposure estimates used in the MOE analysis.

I determined that, based on the highest average measurements from 2008, southern Washington County residents had serum concentrations of PFOA and PFOS 220 and 68 times, respectively, lower than serum concentrations of PFOA and PFOS measured in PFC workers. As discussed in this report, the PFC worker studies did not demonstrate any clear and consistent adverse health effects from these exposures. In addition, I determined that the highest average measurements for PFOA in the southern Washington County residents, sampled in 2008, are 2,600 times lower than the animal serum concentrations for PFOA at the dose chosen by US EPA and MDH as the basis for their RfDs. The highest average measurements for PFOS in the southern Washington County residents, sampled in 2008, are 180 times lower than the

⁶ While I could have derived better scientifically supported PODs for PFOA, PFBS, and PFBA, the exposure estimates using the current PODs are so far below levels of toxicological concern that it would not affect my conclusions.

animal serum concentrations for PFOS at the dose chosen by US EPA and MDH as the basis for their RfDs. Using more recent measurements from 2010 and 2014 would yield even larger margins between the residents' serum concentrations and the comparison values.

I also performed an MOE analysis that is conceptually similar to the hypothetical dose-based MOE analysis, but instead using serum concentrations of PFOA and PFOS. In this analysis, I compared southern Washington County resident serum concentrations of these PFCs to serum concentrations associated with the PODs chosen by MDH to represent doses at (for PFOA)⁷ or above (for PFOS) which health effects were observed in animal studies. I also performed this analysis using a more scientifically supported POD for PFOS.⁸

Table 2.3 presents the lowest MOEs using 95th percentile PFOA and PFOS serum concentrations in southern Washington County residents and POD serum concentrations from the rodent studies used by US EPA and MDH to develop their RfDs for these PFCs, as well as a more scientifically supported POD serum concentration for PFOS. It should be noted that the lowest serum-based MOEs are well above the lowest MOEs based on the hypothetical dose estimates. This observation provides evidence that my dose-based MOE analysis overestimates plausible exposures for many residents.

Table 2.3 MOEs Based on 95th Percentile Serum Concentrations in Southern Washington County Residents

PFC	Animal Serum POD (µg/L)	95 th Percentile Serum Concentrations in Residents (µg/L)		MOE ^a
		Year		
PFOA	38,000 (LOEL) ^{b,c}	2008	60	630
		2010	49	780
		2014	26	1,500
PFOS	6,260 (NOEL) ^b	2008	100	63
		2010	70	90
		2014	70	89
PFOS	25,000 (NOEL) ^d	2008	100	250
		2010	70	360
		2014	70	360

Notes:

LOEL = Lowest Observed Effect Level; MOE = Margin of Exposure; NOEL = No Observed Effect Level; PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate; POD = Point of Departure.

(a) MOE = POD / Serum Concentration. MOEs were rounded to two significant digits.

(b) Serum concentrations at the US EPA/MDH POD.

(c) Using the more scientifically supported POD would change the PFOA LOEL to a NOEL.

(d) Based on a more scientifically supported NOEL of 0.4 mg/kg-day (see Section 8.2).

The results of my dose-based MOE analysis and my serum-based MOE analysis, as well as consideration of the underlying toxicology and epidemiology of the PFCs at issue, demonstrate that exposure to PFCs in the southern Washington County area are below levels of toxicological concern and that health effects from PFC exposure are not expected in southern Washington County residents.

⁷ As discussed in Section 8.1, the POD for PFOA is more appropriately described as a NOEL.

⁸ As discussed in Section 8.2, a more scientifically supported POD for PFOS would be 4-fold higher than the one chosen by MDH in developing its RfD.

2.8 State of Knowledge

It is my opinion that the development of 3M's knowledge about the properties of PFCs proceeded at an appropriate pace, consistent with that of a reasonably prudent manufacturer, and resulted in a coherent, generally reliable body of information about these compounds. Much of today's understanding of the potential health risks of PFCs derives from research conducted by or sponsored by 3M.

Understanding of the state of knowledge about PFCs' toxicity with respect to human health risk must be developed within the context of all the appropriate data. The development of scientific knowledge about PFCs, much of which was conducted by 3M or by academic or contract laboratories that 3M funded, proceeded on at least three interdependent paths: analytical chemistry, animal studies, and human studies. Developments in one area informed the others, and in some cases, developments in one area were necessary to spur developments in another. For example, it was not until the mid-to-late 1990s that PFOS and PFOA could be reliably measured in environmental or biological matrices at trace levels. This development resulted in new lines of inquiry, including measurements of PFOS in the environment and wildlife, additional worker surveillance studies, longer-term studies in monkeys and rats, and studies aimed at understanding how PFOS interacts with cells and molecules. 3M's choice of studies to conduct and the information developed from such studies were reasonable.

By the 1970s, the presence of the element fluorine in human blood had been recognized for decades. In the late 1960s and 1970s, however, the presence of organic fluorine was found in pooled human blood samples. The identity of specific organic fluorine entities was not known, nor was the source. Starting in the late 1970s, 3M and others worked on improving the specificity and sensitivity of organic fluorine measurement, which proved to be challenging. At this time, the detection limit of total organic fluorine was high (in the parts per million [ppm] range), and the method of detection was laborious and not specific to individual PFCs, such as PFOS and PFOA. Likely motivated by the finding of organic fluorine in human blood, 3M began studying the toxicity of PFCs in animals in the late 1970s. Of particular relevance were repeated-dose studies in rats and monkeys conducted with PFOS and PFOA, which identified significant toxicity at the doses administered. Around the same time, 3M started monitoring the health of its workers. This population was reasonably assumed to have the highest levels of human exposure to PFCs. The findings in the animal studies informed 3M of what to look for, so they measured certain parameters that indicated whether the findings in animals were also occurring in workers. Despite finding total organic fluorine in workers, no indications of adverse effects were observed. Most likely, the doses used in the animal studies were much higher than what the workers were being exposed to. Thus, there was no basis to conclude that the findings in the animal studies were an indication that humans, and in particular the general population, would be at risk from exposure to PFCs.

Throughout the 1980s and 1990s, 3M continued to monitor its workers for PFCs in blood and study their health, and found no evidence of disease or patterns of adverse effects. 3M continued to initiate new and refined toxicology studies and advance analytical capabilities for measuring PFCs in a variety of media. By the mid-1990s, it became possible to reliably measure PFOS and PFOA specifically with low sensitivity in a variety of matrices (in the low parts per billion [ppb] range in blood, for example). Not long after, PFOS and PFOA were discovered to be distributed widely in human serum and wildlife globally. These later developments were a major reason why 3M phased out its manufacture of PFOS and PFOA.

Historically, scientists' and state officials' lack of understanding of human health risks from and exposure to industrial wastes influenced how they viewed disposal practices. When 3M began commercial production of PFCs in the early 1950s, and for decades thereafter, there was little understanding by public health professionals, chemical manufacturers, or the general public that, from a public health perspective,

land disposal of industrial chemicals could reach and potentially impact groundwater. Moreover, even when such recognition began, it was in the context of specific organic chemicals (*e.g.*, volatile organic compounds [VOCs], such as trichloroethylene and perchloroethylene) for which measurement methods existed and that were used in many different industries and applications throughout the US (USGS, 2006).

2.9 Comments on Plaintiff's Expert Reports

I have reviewed the expert reports of Dr. Jamie DeWitt, Dr. Phillippe Grandjean, Dr. David Sunding, and Ms. Jessica Schmor. My overall opinions regarding the reports of Drs. DeWitt, Grandjean, and Sunding are summarized here. Because Ms. Schmor did not present any conclusions regarding the effects of PFCs on the population of southern Washington County, I have no specific response to her report.

Dr. DeWitt's overall conclusion is that PFCs pose a substantial present and potential hazard to human health, based on what she identified as particularly strong evidence for associations between PFCs exposure and cancer, developmental toxicity, and immunotoxicity. It is my opinion that Dr. DeWitt did not perform an appropriate weight-of-evidence analysis. Specifically, Dr. DeWitt overstated the evidence for PFC association with health effects, did not conduct an independent analysis of the studies that are the bases of the MDH HBVs and HRLs, did not critically evaluate the conclusions of various health agencies that made toxicity determinations regarding PFCs, and did not compare the exposure of the residents of southern Washington County to serum concentrations at which health effects occurred in animal studies. Dr. DeWitt's conclusion as to the present and potential hazard posed by PFCs to residents of southern Washington County is not supported by a comprehensive and balanced weight-of-evidence analysis.

Dr. Grandjean's overall conclusion is that PFCs pose a substantial present and potential hazard to human health, stating that there are "convincing associations" between PFOA and PFOS and multiple health outcomes and that, although less studied, the human and animal evidence of PFBA suggests it is also a substantial present and potential hazard to human health. Dr. Grandjean indicated that more protective drinking water exposure limits for these PFCs than those currently in place are justified. In my opinion, Dr. Grandjean overstated the strength and consistency of the evidence for an association between PFCs and adverse health outcomes. He selectively chose positive results in some cases and, in general, downplayed null associations. Furthermore, Dr. Grandjean often did not discuss the clinical relevance of findings, relied heavily on other agency and panel reviews of the toxicity of PFCs without performing a critical analysis of these materials, and drew very broad conclusions about the toxicity of numerous PFCs without providing supporting evidence. As with my opinion regarding Dr. DeWitt's analysis, Dr. Grandjean's conclusion as to the present and potential hazard of PFCs to residents of southern Washington County is not supported by a comprehensive and balanced weight-of-evidence analysis.

Dr. Sunding evaluated associations between PFC exposure and adverse birth outcomes, population fertility rates, and cancer incidences in certain Minnesota communities. Dr. Sunding's analyses, with the exception of those on adverse birth outcomes (based on birth certificates), do not contain information on disease outcome at the individual level. Furthermore, none of Dr. Sunding's analyses contain information regarding exposure to PFCs at the individual level. These studies are of ecological or cross-sectional design and, while useful for hypothesis generation, cannot be used to reliably draw causal inferences regarding southern Washington County residents' exposure to PFCs and health effects. In addition, Dr. Sunding's analyses do not adequately consider some key confounders, such as smoking. Dr. Sunding's claims regarding population disease burden are not appropriate, because he has not established a causal link between PFCs and disease outcomes, and thus his analysis does not constitute an appropriate basis for calculating damages resulting from health impacts.

3 Methodology

3.1 Information Used and Analyses Performed

My opinions are based on my training and experience in toxicology and risk assessment and a review of the documents available as of the date of this report. Specific documents I have cited are presented in the References section. Other materials considered but not cited are listed in Appendix E. In addition, my opinions are informed by my extensive professional history and experience in toxicology and risk assessment. The types of information I relied upon for my analyses in this matter include the following.

- Case-specific documents, such as complaints, depositions, expert reports, and environmental sampling data from various environmental firms.
- General guidance documents in the fields of toxicology and risk assessment by agencies such as US EPA and the Agency for Toxic Substances and Disease Registry (ATSDR).
- Publicly available environmental and regulatory documents that are not case-specific but provide data and information that are relevant to my analyses. Such documents include toxicity criteria and secondary toxicological reviews.
- Scientific literature, such as peer-reviewed journal articles and scientific textbooks that review toxicology and epidemiology principles, and toxicological and epidemiology studies relating to PFOA, PFOS, PFBA, and PFBS.

The specific analyses I performed include the following:

- I identified the key scientific principles relevant to evaluating the potential health risks of chemicals in the environment, specifically focusing on Minnesota's claims in this matter.
- I conducted a search of the scientific literature using the PubMed search engine. PubMed is a widely used resource in the scientific community, maintained by the National Center for Biotechnology Information at the National Library of Medicine, that accesses a database of references for publications on life sciences and biomedical topics. Search terms included PFOA, PFOS, PFBA, PFBS, and their full chemical names, in combination with any of the following: mouse, rat, monkey, humans, liver, reproductive, developmental, thyroid, immune, cancer, serum, gastrointestinal, ulcerative colitis, PPAR, absorption, distribution, metabolism, excretion, or transport. Search dates were from January 1, 2009 (for ADME studies) or January 1, 2014 (for human and animal studies) until August 11, 2017. I relied on the ATSDR Toxicological Profile of PFCs (ATSDR, 2015) for identifying literature published before those dates.
- I analyzed the scientific literature to evaluate the possible relationships between exposure to PFOA, PFOS, PFBA, or PFBS and health effects.
- I evaluated the scientific basis of health-based agency guidelines developed by MDH and US EPA for PFOA, PFOS, PFBA, and PFBS.
- I evaluated the relevant Minnesota environmental data and compared that information to conservative screening guidelines and to health effect levels.

- I evaluated how the scientific understanding of the toxicity of PFCs has developed over time. I similarly evaluated how the awareness of potential health risks from waste disposal has developed over time.
- I analyzed the scientific basis of the opinions expressed by Plaintiff's experts, Dr. DeWitt, Dr. Grandjean, Dr. Sunding, and Ms. Schmor.

3.2 Evaluating Risks from Chemical Exposures

3.2.1 Introduction to Toxicology

An understanding of the scientific principles in the fields of toxicology and risk assessment is necessary for evaluating the potential for a relationship between exposure to chemicals and health effects. One of the most fundamental concepts in the field of toxicology is the dose-response relationship. This concept is commonly summarized as "the dose makes the poison," which was first attributed to the famous Swiss-German physician-physicist Paracelsus in the sixteenth century (Eaton and Gilbert, 2013). Virtually all substances exhibit a dose-response relationship, which is characterized by response levels that increase as dose increases. However, for most chemicals, biological effects occur only when the dose exceeds a threshold level for a certain period of time. At doses ranging between zero and the threshold, biochemical or physiological mechanisms can negate a chemical's effects, thereby preventing any adverse effects. As the magnitude and duration of exposure begin to exceed the threshold, these protective mechanisms can become less effective. Consequently, the effect begins to appear in a manner that corresponds to the increase in dose.

The specific effect will vary from chemical to chemical. For example, small amounts of salt may be consumed without adverse effects, because the body is able to adequately maintain proper salinity levels in its fluids and tissues. The adequate intake of salt needed to sustain health ranges from 3-3.8 g/day, depending on age, sex, and lifestyle (active or sedentary) (IOM, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, 2005). However, ingestion of much larger quantities of salt can override these homeostatic mechanisms and lead to adverse effects, such as hypertension (abnormally high blood pressure), in some individuals (IOM, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, 2005). Similarly, aspirin also shows a dose-response relationship. At the recommended dose of two tablets, aspirin provides pain relief from headaches or other minor aches. Even lower doses can be preventative with respect to CVD. Taking more than the recommended dose, however, may eventually lead to toxicity (Roberts and Morrow, 2001; Burke *et al.*, 2006). Ingestion of 10 aspirin tablets is associated with nausea, 30 tablets with acidosis (excess acidity in the blood) and hyperventilation, and 65 tablets with brain damage. Hemorrhage and death occur with ingestion of 100 tablets (Roberts and Morrow, 2001; IOM, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, 2005; Burke *et al.*, 2006). Thus, because the appearance of biological effects is directly related to the magnitude and duration of an individual's exposure, estimation of dose is a critical element in determining potential human health risks from chemical exposures.

In some cases, an increased theoretical risk can be calculated based on a certain chemical dose level even though the actual risk associated with that level of chemical exposure could be nonexistent or very small, as is the case with certain calculated risk levels compared to the risk of cancer from background environmental levels of a substance. For example, in studies investigating chronic (long-term) exposure to arsenic in soil, it is not until concentrations of arsenic in soil are approximately 100 mg/kg or higher that a consistent impact on body burden can be detected (Wong *et al.*, 1992; Hewitt *et al.*, 1995; Valberg

et al., 1997; Gebel *et al.*, 1998; Tollestrup *et al.*, 2003; Tsuji *et al.*, 2005), even though a hypothetical excess risk can be calculated at low concentrations of arsenic in soil.

Another important factor considered in toxicology is the frequency and duration of a chemical exposure. The period over which the dose of a chemical is received may be critically important in determining the resulting health effects (see, for example, US EPA, 1989; Paustenbach and Madl, 2014). For example, ingestion of sufficient quantities of ethanol (alcohol) in a single event may lead to central nervous system (CNS) depression, coma, and death. However, lower doses, if repeated over years, may lead to liver and cardiovascular damage, effects not observed in acute alcohol intoxication (Fleming *et al.*, 2006). At even lower doses, adverse effects will not occur. In fact, smaller doses (for example, a maximum of one to two glasses of red wine per day) may be beneficial, decreasing the risk of cardiovascular mortality (Goldberg *et al.*, 2001; Harvard T.H. Chan School of Public Health, 2017).

The duration of exposure is also important to consider when evaluating risk. The severity of health effects could be significantly different if the chemical exposure is experienced acutely in a single dose than if the chemical exposure is experienced chronically, with the same cumulative dose spread out over time. This is because during chronic exposure, the body has time to eliminate the dose *via* excretion, to repair any damage that may have occurred, or to adapt and find other means of accommodating the chemical dose (Eaton and Gilbert, 2013). Thus, even if two individuals are receiving similar daily doses, their risk can differ depending on their respective exposure durations.

It should also be appreciated that not all of the changes observed in studies (in humans or animals) are adverse. Rather, the body is able to adapt to various chemical and physical stresses to maintain homeostasis. Only when the body's natural homeostatic and defense mechanisms become overwhelmed will the stress or toxic insult lead to an adverse toxicological outcome. Multiple authors have published guidelines for distinguishing an adverse effect from an effect with limited toxicological significance (Hall *et al.*, 2012; Kerlin *et al.*, 2015; Lewis *et al.*, 2002; Keller *et al.*, 2012; Frame *et al.*, 2014). In Hayes' *Principles and Methods of Toxicology* (Hayes and Kruger, 2014), Frame *et al.* (2014) discussed several publications that address the adverseness of effects (*e.g.*, Keller *et al.* [2012], also discussed by Dr. DeWitt [2017a] in her expert report). As outlined by Frame *et al.* (2014), in a list adapted from Lewis *et al.* (2002), effects are less likely to be adverse if:

- There is no functional impairment,
- The response is adaptive,
- The response is transient (*i.e.*, reversible),
- The effect is of low severity,
- The response is isolated or independent,
- It is not a known precursor to a known adverse effect,
- It is secondary to another effect, or
- The effect is a consequence of the study methodology (for example, stress due to the method of dose delivery).

Reversibility is a concept that is important to consider when evaluating potential health effects (in particular for short-term exposures). For many exposures, when exposure to a chemical ceases, the health effect or symptom also ceases, with no long-term adverse consequences. For example, in animal experiments with PFCs, reversibility has been demonstrated for a variety of effects, including changes in

liver effects, serum cholesterol, and thyroid hormone levels (see for example Perkins *et al.*, 2004; Butenhoff *et al.*, 2012a).

3.2.2 Introduction to Epidemiology

Epidemiology has been defined as the study of the occurrence of disease or other health-related characteristics in human populations (Driscoll and Winder, 2004). Epidemiology studies measure actual disease outcomes in a population and examine associations that may exist between chemicals and adverse health effects. It is common for data from epidemiological investigations to be used in risk assessment, a tool to predict adverse health effects based on knowledge of the effects of chemicals and exposures (US EPA, 1989; Faustman and Omenn, 2013). A major strength of epidemiology studies is that they deal with humans and real exposures (Olsen *et al.*, 2014). Unlike scientists involved in laboratory investigations, however, epidemiologists are rarely able to exert control over the parameters of their studies and must grapple with a variety of technical biases (Driscoll and Winder, 2004; Olsen *et al.*, 2014). As a result, robust exposure estimates are often difficult to obtain from epidemiology studies, because they are frequently done retrospectively (*e.g.*, through employment records). Another challenge of interpreting epidemiology studies is that subjects are often exposed to multiple chemicals, especially when a lifetime exposure period is considered (Faustman and Omenn, 2013).

There are different types of epidemiology studies, each with different strengths and weaknesses. Two major design types are cohort and case-control studies (Faustman and Omenn, 2013). In a cohort study, subjects are selected based on their exposure status (exposed *versus* non-exposed), then the proportion of each group that gets the disease of interest is evaluated. Cohort studies are useful when the exposure of interest is rare (Hennekens *et al.*, 1987). In case-control studies, the case group consists of subjects who have the disease of interest, while the control group consists of those who do not. The groups are then compared with respect to the proportion of each that has a history of the exposure of interest. Case-control studies are commonly used for studying rare diseases or diseases with a long latency (Hennekens *et al.*, 1987).

3.2.3 Extrapolation from Animals to Humans

For many chemicals, government agencies use animal studies, typically rodent bioassays, for the purposes of risk assessment and regulatory decision making.⁹ Animal studies are used to make up for the often limited information available from human studies regarding the toxicity potential of chemicals (NRC, 2004). When animal studies are used to evaluate toxicity potential, the results are extrapolated across species and from the relatively high doses used in animal studies to the much lower doses associated with human exposures (US EPA, 2005). In the case of carcinogens, this extrapolation typically assumes that carcinogenic potential in animals is comparable to carcinogenic potential in humans, and that carcinogenic potential at high doses (without causing mortality in animals) over shorter periods of time is comparable to carcinogenic potential at low doses over a lifetime (US EPA, 2005). Typically, regulatory scientists assume that humans are as sensitive as the most sensitive animal species and that effects in animals may occur in humans (US EPA, 2005). However, there are a number of examples of animal-specific findings that involve modes of action that are not relevant for humans or of animals having a greater sensitivity to an agent than humans (US EPA, 2005; Holsapple *et al.*, 2006; Aschner *et al.*, 2016; Boobis *et al.*, 2016; Cohen, 2017). For example, the drug Tamoxifen is biotransformed similarly in both

⁹ Such decisions might include, for example, setting permissible limits of chemicals in food or in environmental media, such as air.

humans and rats, but the amount of reactive metabolites and DNA adducts¹⁰ that form in the liver (and, thus, are able to cause toxic effects) as a result of Tamoxifen exposure is much lower in humans than in rats (Hengstler *et al.*, 1999, as cited in NRC, 2004).

3.2.4 Toxicity Criteria

For chronic (*i.e.*, long-term exposure) non-cancer effects, toxicity is typically characterized using a chemical-specific RfD for ingested chemicals or reference concentration (RfC) for inhaled chemicals. For cancer, toxicity is typically characterized using a cancer slope factor (CSF) or inhalation unit risk (IUR).

The RfD or RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure or continuous inhalation exposure, respectively, of a human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (Barnes and Dourson, 1988; US EPA, 2011a). RfDs and RfCs are derived from a no observed adverse effect level (NOAEL) or a lowest observed adverse effect level (LOAEL). NOAELs and/or LOAELs are typically identified from studies in laboratory animals, most often rats or mice, in which animals are exposed *via* ingestion or gavage (*i.e.*, oral exposure). To account for potential uncertainties and variability (differences between laboratory animals and humans, variation in sensitivity among individuals, use of a LOAEL instead of a NOAEL, insufficient study duration, missing key toxicological studies, *etc.*), the NOAEL is divided by UFs, typically factors of 10 each, to account for inter- and intraspecies variability and, if necessary, other sources of variability (US EPA, 2002a). The application of UFs helps ensure that exposures below the RfD or RfC are sufficiently health-protective. However, adverse health effects will not necessarily occur even at exposures greater than the RfD or RfC.

As described by US EPA (2000a):

It should be noted that exposures above an RfD or RfC do not necessarily imply unacceptable risk or that adverse health effects are expected. Because of the inherent conservatism of the RfC/RfD methodology, the significance of exceedances must be evaluated on a case by case basis, considering such factors as the confidence level of the assessment, the size of UFs used, the slope of the dose-response curve, the magnitude of the exceedance, and the number or types of people exposed at various levels above the RfD or RfC.

In 2011, US EPA reiterated how values in its Integrated Risk Information System (IRIS), including RfDs and RfCs, "cannot be validly used to accurately predict the incidence of human disease or the type of effects that chemical exposures have on humans. This is due to the numerous uncertainties involved in risk assessment, including those associated with extrapolations from animal data to humans and from high experimental doses to lower environmental exposures" (US EPA, 2014b).

For cancer, US EPA frequently uses mathematical models to extrapolate risks observed at high doses or concentrations (either in humans or animals) down to low doses/concentrations typical of actual exposure levels, often based on the assumption that, for many carcinogens, there is no threshold for cancer.¹¹ Using these models, either a CSF or IUR is identified, which represents the incremental (*i.e.*, above background)

¹⁰ A DNA adduct is a DNA molecule to which a foreign chemical is covalently bound (*i.e.*, the chemical and the DNA molecule share electrons) (Klaunig, 2013).

¹¹ It should be noted that this is a regulatory construct and not a reflection of "true" risks. That is, regulatory risk estimates are protective, but not predictive, and the true risk is likely to be well below the hypothetical regulatory risk. See for example, James *et al.* (2015).

upper-bound risk of an additional cancer per dose (in mg/kg-day) or concentration (in $\mu\text{g}/\text{m}^3$) of the chemical, assuming low-dose linearity (US EPA, 2005). US EPA defines the IUR as the upper-bound excess lifetime cancer risk (ELCR) estimated to result from continuous exposure to an agent at a concentration of $1 \mu\text{g}/\text{m}^3$ in air (US EPA, 2005).

Because the CSF or IUR is a plausible upper-bound value, US EPA recognizes that actual (or "true") cancer risks could be lower than those calculated using the CSF/IUR (US EPA, 2005). US EPA indicates that the "use of upper bounds generally is considered to be a health-protective approach for covering the risk to susceptible individuals" (US EPA, 2005).

Assuming linearity at low doses is a conservative assumption that is used as a default when chemical-specific information regarding the shape of the dose-response curve at low doses is uncertain. Based on current understanding of the carcinogenic process, many scientists today have concluded that this model is likely to overestimate risks for many chemicals (see, for example, US EPA, 2005; Cohen and Arnold, 2011; Aschner *et al.*, 2016; Boobis *et al.*, 2016; Cohen, 2017).

3.2.5 Risk Assessment Methodology

Risk assessment is defined as "the characterization of the potential adverse health effects of human exposures to environmental hazards" (NRC, 1983). Risk assessments form the scientific basis for many regulations pertaining to environmental contaminants. These include Maximum Contaminant Levels (MCLs) for contaminants in drinking water, National Ambient Air Quality Standards for criteria air pollutants, Maximum Achievable Control Technology standards for hazardous pollutants, emissions standards for hazardous mobile source pollutants, and limits on the use of pesticides (NRC, 2009).

Risk assessment involves the following four steps, which were first presented as a framework by the National Academy of Sciences (NAS) in 1983 (NRC, 1983).

1. **Hazard Identification:** The potential hazard is identified; this involves determining whether a particular chemical is causally linked to health effects.
2. **Dose-Response Assessment:** A dose-response assessment is performed to determine the relationship between the magnitude of exposure to the hazard and the probability of the occurrence of a health effect.
3. **Exposure Assessment:** The level of human exposure to the hazard is estimated.
4. **Risk Characterization:** The estimated exposure level is compared with the value obtained from the dose-response assessment and characterized in a risk estimate, with an assessment of the magnitude of uncertainty.

Toxicologists like myself frequently rely on this four-step methodology to guide our analyses of potential human health risks in a reliable manner. For non-carcinogens, risk is presented as a hazard quotient (HQ) (for a single chemical and pathway) or hazard index (HI) (for multiple chemicals and exposure pathways). The HQ compares the estimated exposure concentration (determined as part of the exposure assessment) to the RfC (identified as part of the dose-response assessment). The HQ is calculated from the RfC using the following equation (US EPA, 1989):

$$\text{Hazard Quotient} = \frac{\text{Exposure Concentration } (\mu\text{g}/\text{m}^3)}{\text{Reference Concentration } (\mu\text{g}/\text{m}^3)}$$

The HI is calculated as the sum of the HQs across chemicals and exposure pathways. If an HI is less than 1, adverse health effects are not expected, and there is no need for further evaluation. If an HI is greater than 1, there may be the potential for non-cancer health effects to occur, and further evaluation is warranted. However, it must be emphasized that exceeding a health-protective RfC does not mean that adverse health effects will occur or are even likely to occur.

Cancer risks are typically characterized as the incremental probability that an individual will develop cancer during his or her lifetime due to chemical exposure under the specific exposure scenarios evaluated. The term "incremental" refers to risk above the background cancer risk experienced by all individuals during daily life. According to Greenlee *et al.* (2001), the lifetime probability of developing cancer (*i.e.*, background cancer risk) is approximately 0.435 (435 cases in a population of 1,000) in men, and 0.383 in women (383 cases in a population of 1,000). Cancer risks are expressed as a unitless probability (*e.g.*, 1 in 1,000,000 or 1×10^{-6}) of an individual developing cancer over a lifetime, above the background probability of developing cancer, due to exposure to a chemical or chemicals. It should be noted that calculations of cancer risks are conservative, theoretical estimates and may not represent actual risks (US EPA, 2004a). In fact, in many cases, if exposure to a certain chemical is low, the risk may be zero.

Cancer risks are calculated using lifetime average daily exposure doses (calculated as part of the exposure assessment) and toxicity factors (*i.e.*, the CSF) that quantify cancer potency. CSFs (identified above as part of the dose-response assessment step of the four-step risk assessment methodology) are used to calculate cancer risk as follows (US EPA, 1989):

$$\text{Cancer Risk} = \text{Exposure Dose} \left(\frac{\text{mg}}{\text{kg} - \text{day}} \right) \times \text{CSF} \left(\frac{\text{mg}}{\text{kg} - \text{day}} \right)^{-1}$$

Cancer risks that are considered acceptable by the federal government and by state agencies are typically not defined as a single precise value, but rather as a range of values that allows for selection of an acceptable risk within that range, based on a number of considerations. For carcinogens, regulatory agencies generally seek to limit exposures to avoid future incremental lifetime risks greater than somewhere between 1 in 1,000,000 and 1 in 10,000, although somewhat larger risks have been tolerated in certain situations (see, for example, Rodricks and Rieth, 1998).

In many situations, a complete risk assessment is not necessary, and a screening risk assessment is employed instead. US EPA has published a set of risk-based media concentrations known as Regional Screening Levels (RSLs) (US EPA, 2017a) for use in these types of evaluations. Prior to 2008, US EPA Regions III, VI, and IX each published their own set of risk-based screening levels. In 2008, these three US EPA Regions combined their screening levels into one set of values, now called "Regional Screening Levels (RSL) for Chemical Contaminants at Superfund Sites" (US EPA, 2017a). RSLs present risk-based screening values for individual compounds in residential and industrial soil, air, and drinking water. US EPA derives RSLs by combining generic conservative exposure assumptions with US EPA toxicity criteria. These levels are considered to be protective for humans (including sensitive subgroups) over a lifetime (US EPA, 2017a). RSLs are based on a cancer risk of 1×10^{-6} (1 in 1,000,000) and a non-cancer HQ of 1 or 0.1.

RSLs are protective of human health in hypothetical high-end (*e.g.*, intentionally using assumptions that overestimate chemical intake), chronic exposure scenarios. RSLs are intentionally conservative to help identify sites that do not warrant further investigation (*i.e.*, when all maximum detected concentrations are

less than corresponding RSLs). As described under US EPA guidelines, screening values are used to identify chemicals of interest at a site for the purposes of further investigation and decision making (US EPA, 2017b). RSLs are meant to be used as comparison values for screening site concentrations, not as final cleanup standards; the goal of screening is to determine areas and contaminants that require further evaluation. Exceedance of a screening level does not mean that an exposure presents an unacceptable health risk, only that further evaluation of potential risks is warranted (US EPA, 2017b).

3.2.6 Regulatory Toxicology vs. Causation Analysis

There are substantial differences in how toxicological data are used in a regulatory framework to protect public health *versus* how those data are used to make determinations regarding causation vis-à-vis individuals claiming various health symptoms from chemical exposure (Eaton and Gilbert, 2013). The approach to regulatory decision making is, in part, directed by policy. As practitioners of public health, regulatory toxicologists are concerned more with avoiding adverse health effects than with estimating the likelihood of health effects actually occurring in a population or an individual (US EPA, 2004a; ATSDR, 2017). This difference in perspective is important, because regulators often use high-end estimates of exposure and toxicity (which can result in overprediction of potential health risks) to be protective of human health. The aim of US EPA and other public health agencies is not to precisely define which effects are expected to occur, but to define the level at which health effects are *unlikely* to occur (US EPA, 1993; ATSDR, 2017). Thus, regulatory criteria are designed to "protect the health of everyone in general and no one in particular" (Rodricks and Rieth, 1998). Indeed, US EPA guidelines for developing regulatory criteria state that such criteria are applicable to "susceptible groups" or sensitive subpopulations, which include life stages (such as developing individuals [embryo, fetus]) and other factors that may predispose individuals to greater response to an exposure (US EPA, 2002a; CalOEHHA, 2008). In some cases, regulatory agencies are required by statute to apply these safety factors; for instance, the Food Quality Protection Act requires that an additional 10-fold safety factor be accounted for when evaluating pre- and post-natal pesticide exposures (US Congress, 2002, Section 408(b)(2)(C)).

In contrast to evaluations performed for regulatory or guidance purposes, assessing the risk of disease in an individual from a specific chemical exposure (*i.e.*, a toxicological causation analysis), which I have undertaken here, requires an estimate of *actual* risk, based on an individual exposure assessment, dose characterization, and an understanding of the demonstrated health effects that the chemicals of interest have on humans (Olsen *et al.*, 2014).

In this type of analysis, exceedance of a risk-based screening level can be used as an exclusionary tool (*i.e.*, to remove a chemical or pathway from further consideration). And, for the reasons stated above, it is scientifically inappropriate to interpret such an exceedance as indicative of a causal link between the chemical and the observed health effect.

3.2.7 Evaluating the Weight of Evidence

To establish a reasonable link between exposure to a chemical or chemicals and disease in any one individual, a scientifically credible methodology is required.

First, there should be reliable evidence demonstrating that the chemical(s) in question can be causally associated with a specific illness in humans. This is referred to as "general causation": does Chemical X cause Disease Y?

An appropriate evaluation of the evidence requires not just citing individual study results, but examining the body of relevant available studies to determine whether findings are reliable, repeatable, and

biologically plausible. It is necessary to evaluate multiple studies, both positive and negative, considering the strengths and weaknesses of each and weighing their points of agreement and contradiction, to arrive at an overall scientific assessment of the evidence (Weed, 2005; US EPA Region III, 2007; Wickwire and Menzie, 2010; Linder *et al.*, 2010).

Postulates for establishing whether an association between an environmental exposure and a specific disease outcome is causal or simply due to chance were formally presented by Sir Bradford Hill in 1965 to the British Royal Society of Medicine's Section of Occupational Medicine (Hill, 1965). The "Hill Postulates," which are still used today to establish causality, include:

1. **Strength of the Association:** Is the magnitude of the association between the exposure and disease strong enough that it is not likely due to chance?
2. **Consistency:** Is the association consistently observed under different circumstances of exposure and in different study populations?
3. **Specificity:** Is the association specific to a certain disease?
4. **Temporality:** Does the exposure precede the disease, and with sufficient time for the disease to manifest after the exposure?
5. **Biological Gradient:** Does the magnitude of the association increase with the magnitude of the exposure (*i.e.*, is there a dose-response relationship)?
6. **Plausibility:** Is it biologically plausible that the suspected cause leads to the effect?
7. **Coherence:** Is the association consistent with what is known about the etiology¹² of the disease?
8. **Experiment:** If preventative action is taken (*i.e.*, the source of suspected exposure removed), is the frequency of effects altered?
9. **Analogy:** In the absence of data, are there similar chemicals and/or exposures that compare?

¹² Etiology is the cause or causes of a disease

4 Overview of PFC Chemistry, Properties, Production, Disposal, and Monitoring

4.1 The Chemistry of PFCs

PFCs are a distinct family of chemicals containing a chain of between 4 and 16 carbons that are fully bonded to fluorine atoms and another chemical group, such as a carboxylate or sulfonate group, that varies with different PFCs. In addition to fluorine, PFCs have functional groups that determine to which classes of PFCs they belong (ATSDR, 2015). For example, most PFCs that are of interest to this case have either a sulfonate group or a carboxylate group attached to a terminal carbon. The structures of PFOA, PFOS, PFBA, and PFBS are depicted in Figure 4.1 (Danish EPA, 2015a).

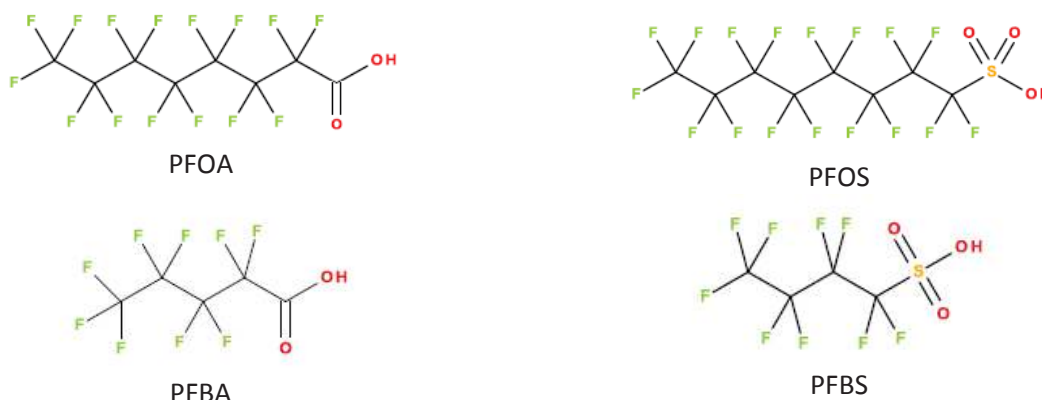


Figure 4.1 Chemical Structures of PFOA, PFOS, PFBA, and PFBS.¹³ PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

PFCs do not occur naturally and are the result of anthropogenic activities (ATSDR, 2015). Historically, manufacturers used PFOA as a processing aid in the creation of fluoropolymers that have the ability to repel oil, stains, grease, and water under high temperatures, making these chemicals ideal for use in protective coatings for non-stick cookware and clothing (Lau, 2015). PFOS possesses surfactant properties (the ability to reduce the surface tension between liquids or between a liquid and solid). Because of these properties, PFOS was used in numerous chemical applications, such as fire-fighting foams, hydraulic fluids, carpet cleaners, and oil well surfactants. Tables 4.1 and 4.2 present sulfonated and carboxylated PFCs, respectively, described in ATSDR (2015) and/or Dagnino *et al.* (2015).

¹³ This Copyrighted Material Is Being Used for Governmental Regulatory/Judicial Purposes. No Further Reproduction Is Permitted Without Permission from the Copyright Holder.

Table 4.1 PFCs with a Sulfonate Functional Group

Compound	Abbreviation	Carbon Length
Perfluorobutane Sulfonate	PFBS	4
Perfluorohexane Sulfonate	PFHxS	6
Perfluorooctane Sulfonate	PFOS	8
Perfluorodecane Sulfonate	PFDS	10

Table 4.2 PFCs with a Carboxylate Functional Group

Compound	Abbreviation	Carbon Length
Perfluorobutanoic Acid	PFBA	4
Perfluoropentanoic Acid	PFPeA	5
Perfluorohexanoic Acid	PFHxA	6
Perfluoroheptanoic Acid	PFHpA	7
Perfluorooctanoic Acid	PFOA	8
Perfluorononanoic Acid	PFNA	9
Perfluorodecanoic Acid	PFDA	10
Perfluoroundecanoic Acid	PFUnA	11
Perfluorododecanoic Acid	PFDoA	12
Perfluorotetradecanoic Acid	PFTA	14

4.2 Environmental and Biological Properties of PFCs

PFCs are resistant to degradation by water, sunlight, microbes, and animal metabolism. This resistance is attributable to the strong carbon-fluorine bond present in PFCs. Chain length does not affect these compounds' persistence in the environment; shorter PFCs are comparable to longer-chain PFCs in terms of their resistance to environmental degradation (Buck, 2015; ATSDR, 2015). In addition, it has been shown that in certain circumstances, PFCs have the potential to enter the food chain, bioaccumulate in certain organisms, and undergo long-range transport away from the original source. One study detected PFOS in the tissues of fish, birds, and marine mammals in both urban and non-urban areas, with higher concentrations in predatory animals, suggesting that both transport of PFOS far from its anthropogenic sources and biomagnification of PFOS up the food chain are possible (Giesy and Kannan, 2001). In the same study, all concentrations of PFCs were less than the limit of quantitation (LOQ), and only a few samples contained PFOA at levels greater than the LOQ. In general, PFOS is the most commonly detected perfluorinated sulfonate, and PFC, in wildlife (Conder *et al.*, 2008; ATSDR, 2015).

The biological properties of PFCs depend on both carbon chain length and functional groups. In general, PFCs with longer chain lengths, up to eight carbons, are more persistent in the body and therefore have a greater tendency for bioconcentration and bioaccumulation (Fujii *et al.*, 2015; Lau, 2015; Buck, 2015; ATSDR, 2015). Bioaccumulation potential decreases again at chain lengths greater than eight (ATSDR, 2015), likely due to a decreased capacity for absorption. In addition, sulfonate PFCs generally have longer half-lives than carboxylic acid PFCs of the same chain length (Lau, 2015; ATSDR, 2015). Increasing chain length also correlates, in general, to increasing health effects in experimental animals, due to the increased half-lives of the longer chain PFCs in the body (ATSDR, 2015).

4.3 The Synthesis of PFCs

PFCs are synthesized using two distinct industrial processes. 3M has used the electrochemical fluorination (ECF) process to synthesize PFCs. The ECF process replaces the normal carbon-hydrogen

bonds with carbon-fluorine bonds on a given organic molecule (3M Co., 1999a). Perfluorination is complete when all the carbon-hydrogen bonds are replaced with carbon-fluorine bonds. The ECF process yields a mixture of branched and straight-chain isomers of the final product, as well as a mixture of other fluorinated organic molecules of various carbon-chain lengths (ATSDR, 2015; Lau, 2015). Other companies have used a different process for the production of PFCs called the telomerization process, which was developed by DuPont (ATSDR, 2015). The telomerization process causes tetrafluoroethylene to react with other fluorine-bearing chemicals to yield fluorinated intermediates. This process yields predominantly straight-chain acids with an even number of carbon atoms that can then be converted into the final product (US EPA, 2003a; Lau, 2015).

4.4 3M Production of PFOA and PFOS

3M produced PFCs at its Cottage Grove facility starting in the late 1940s. PFOA was the main type of PFC made at this site (MDH, 2016a). 3M began commercial production of PFCs in the early 1950s (ATSDR and MDH, 2012). PFOS precursors and PFOA have been the most widely synthesized of the PFCs. Production of PFCs at 3M plants in the US occurred at two plants: one in Cottage Grove, Minnesota, and one in Decatur, Alabama. Another major manufacturing site for PFOA and PFOS was at a 3M plant in Antwerp, Belgium (Olsen *et al.*, 2003a).

3M decided to phase out all production of PFOA- and PFOS-based products in 2000. By 2002, 3M's phase-out of these products in the US was complete (3M, 2006a). Several US companies continued to produce or import PFCs in the US beyond 2002 (*e.g.*, E.I. DuPont de Nemours and Company; Arkema, Inc.) (ATSDR, 2015).

The 3M plant in Cottage Grove previously served as a major production site for PFOA. Research and development of PFCs at this plant began in the late 1940s. Commercial production of PFOA at the Cottage Grove plant began in the early 1950s and continued through 2002 (ATSDR and MDH, 2012). 3M has not used or processed PFOA for commercial purposes at the plant since 2002 (ATSDR, 2015). PFBA was also manufactured at the Cottage Grove plant, but production of this PFC was discontinued in 1998 due to decreased demand (3M, 2007). 3M currently produces four-carbon PFBS-based products at the Cottage Grove plant, which are substitutes for the earlier eight-carbon PFCs (ATSDR and MDH, 2012). In addition, 3M continues to use and/or produce one- to three-carbon perfluoroalkyl substances at the Cottage Grove plant (ATSDR and MDH, 2012).

4.5 PFC Waste Disposal

For a time, production wastes from the Cottage Grove plant were disposed of on-site and PFCs were not removed in water-treatment processes. Thus, PFCs were in the wastewater from the plant that went into the Mississippi River (MDH, 2016a). The groundwater, which is not used for public drinking water consumption, beneath the 3M Cottage Grove plant, which discharges to the Mississippi River, contains PFOA, PFOS, and PFBA at levels that exceed MDH drinking water criteria in some areas (MDH, 2016a). Currently, an extensive system of wells contains and collects much of the contaminated groundwater from under the site so that it can be treated. A large granular activated carbon (GAC) filter system was added in 2010 to remove PFCs from the groundwater before it goes into the river. From 2009-2011, 3M excavated the former waste disposal areas and the sediments in a cove next to the river, through which PFC-contaminated wastewater flowed in the past. The soil and sediments were disposed of off-site in lined containment facilities (MDH, 2016a).

In addition to storing PFC waste on-site, 3M used some off-site landfills for the disposal of plant waste. Several locations in Minnesota were used, including the Oakdale and Woodbury Disposal Sites and the Washington County Landfill in Lake Elmo. 3M has monitored areas in the vicinity of the Cottage Grove plant and off-site disposal sites for the presence of PFCs, and has implemented corrective actions. The Oakdale Disposal Site is listed as a Superfund site. It consists of three old chemical waste dump sites that were used during the late 1940s-1950s for waste burial, drum reclamation, and open burning of combustible materials (MDH, 2016b). The Oakdale Disposal Site received liquid and solid industrial waste from 3M from approximately 1956-1960 (ATSDR and MDH, 2012). Groundwater and surface water near the area is contaminated with a wide variety of organic chemicals. Soil contamination also occurred at the site. The Minnesota Pollution Control Agency (MPCA) first investigated the site in 1980 and found a variety of hazardous substances, particularly VOCs, which are chemically distinct from PFCs in their lack of fluorine atoms. 3M completed several remedial actions in the 1980s to address soil and groundwater VOC contamination at this site. More recently, PFCs have also been detected in the monitoring wells at the 3M Oakdale Disposal Site and in several Oakdale municipal wells (MDH, 2016b). In 2006, 3M funded the construction of a GAC treatment plant to treat water from Oakdale's primary municipal water wells and also funded the installation of a new city well outside the area of PFC contamination. In 2008-2011, 3M completed additional cleanup actions to further reduce both VOC and PFC contamination (MDH, 2016b).

In 2004, MDH and MPCA learned that PFC wastes from 3M's Cottage Grove manufacturing plant were also disposed of in the former Washington County Landfill from 1969-1975 (MDH, 2016c). PFC-containing wastes disposed of in the Oakdale Disposal Site and the Washington County Landfill seeped into groundwater. They also entered Raleigh Creek, which flows from the Oakdale Disposal Site eastward into the city of Lake Elmo, where it discharges to Eagle Point Lake in the Lake Elmo Park Reserve. PFCs from both sites have created groundwater plumes extending to the south-southwest of the disposal areas, while PFCs traveling in Raleigh Creek have also entered the groundwater, so that in Lake Elmo, the groundwater, and some lakes contain PFCs from both sites (MDH, 2016b).

The Woodbury Disposal Site was also used as a disposal site for liquid and solid industrial waste from 3M from approximately 1960-1966 (ATSDR and MDH, 2012). In 2005, low levels of PFOA and PFOS were detected in the groundwater pump-out system, which prompted MDH and MPCA to conduct an investigation of nearby residential wells to determine whether PFCs were migrating from the dump into groundwater. PFOS and PFOA were not detected in any of the wells (MDH, 2016d). In late 2006/early 2007, additional sampling of monitoring wells at the 3M Woodbury Disposal Site and private drinking water wells near the site detected PFBA (MDH, 2016d).

4.6 PFC Monitoring Programs

By 2006, MDH was sampling drinking water supplies for PFOS, PFOA, PFBA, PFBS, perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and PFHxS (ATSDR and MDH, 2012). PFCs have been detected in most of the Oakdale municipal wells (MDH, 2016b), in private wells located in Lake Elmo (MDH, 2016c), and, as noted above, in private drinking water wells near the Woodbury Disposal Site (MDH, 2016d).¹⁴

Initial sampling for PFOA and PFOS in drinking water from private wells in Lake Elmo that were near the former landfill found only low levels of PFOA in several wells south of the landfill (MDH, 2016c). In 2005, however, PFOS and PFOA were detected in most Oakdale city wells, including one near the border

¹⁴ As discussed in subsequent sections, it should be noted that the presence of PFCs in such wells is not necessarily indicative of a toxicological hazard.

of the landfill with Lake Elmo, which led to additional sampling that detected both PFOS and PFOA in more wells in the Tablyn Park and Lake Elmo Heights neighborhoods (MDH, 2016c). In 2006, with an expanded list of PFCs that could be measured, five additional PFCs were detected in the landfill. Widespread detection of PFBA led to a much larger well sampling effort throughout southwest Lake Elmo, including over 400 private wells. PFCs have been detected in over 300 private wells (MDH, 2016c). Environmental sampling determined that PFCs in the groundwater in Lake Elmo came from both the former Washington County Landfill and the 3M Oakdale Disposal Site. MDH has also tested for PFCs in groundwater, surface water, soil, sediment, and fish in southern Washington County.

In homes in which drinking water levels exceeded the applicable criteria, residents were provided with bottled water and then carbon treatment systems were installed to remove the PFOS and PFOA in drinking water. 3M donated funds and land to Lake Elmo and neighboring areas to expand the Lake Elmo municipal water supply (in which PFOA and PFOS have not been detected) to those who were obtaining drinking water from private wells. This extension project was completed in 2007. 3M also agreed to pay for the first 2 years of water bills for households connected to municipal water when their well was closed (3M, 2006b; MDH, 2016c).

In 2008, 2010, and 2014, MDH completed biomonitoring studies of selected residents in Cottage Grove, Lake Elmo, and Oakdale who were known to have been exposed to PFOA and/or PFOS in their drinking water. The average concentrations of PFOA and PFOS in participants' serum samples were approximately 3-4 times higher than those of the general population in each of the years they were measured, but over time are decreasing in parallel with the US population serum concentrations as the participants drink treated water (MDH, 2016d). Biomonitoring results are discussed in more detail in Section 10.5.

5 Understanding Mechanisms of Toxicity

In this section, I discuss: (1) how the processes of ADME aid in interpreting findings from studies in animals and humans, and how to extrapolate animal doses to human doses, considering the differences in these processes; (2) PBPK models developed for PFOA and PFOS and their use both for interpreting specific findings from studies in humans and in developing drinking water guidelines for PFOA and PFOS; and (3) potential species specificity with respect to the underlying mechanisms by which PFOA causes toxicity.

The discussion below is based on my own review of studies discussed by the ATSDR (2015) in its toxicological review for PFCs and the US EPA (2016a,b) in its reviews of PFOA and PFOS. I also identified additional literature based on a search of the PubMed database, starting in 2013 for PFOA and PFOS and starting in 2009 for PFBA and PFBS.¹⁵

5.1 Absorption, Distribution, Metabolism, and Excretion

The concentration of a chemical and its metabolites in the body and importantly, at target sites where key critical effects occur, is governed by the ADME processes. Thus, it is important to understand ADME and how these processes may differ between laboratory animals, in which health effects of chemicals are frequently evaluated, and in humans. Understanding species differences in ADME is of particular importance for PFCs, because there are marked differences in these processes between animals and humans. These interspecies differences indicate that exposure should be compared based on serum concentration rather than exposure dose. Understanding ADME factors thus allows for appropriate interpretation of studies showing associations between serum concentrations and biological effects. In this case, an understanding of ADME can help evaluate whether observed associations are caused by a chemical exposure or can be explained by reverse causation.¹⁶ This is relevant to evaluating associations between certain health effects and PFC exposures.

5.1.1 Absorption

Absorption describes the movement of a substance into the bloodstream, with the speed and extent to which a substance is absorbed depending on several factors, including the particular route of exposure and the physicochemical properties of the substance (Lehman-McKeeman, 2013). Insight as to how and to what extent a substance is absorbed affects other parameters, such as bioavailability and tissue dose, that will ultimately impact potential toxicity and overall body burden.

¹⁵ In addition to the particular PFC (*e.g.*, PFOA, PFOS), PubMed search terms included absorption, metabolism, excretion, ADME, elimination, penetration.

¹⁶ Reverse causation refers to a situation in which an association between a health effect and a chemical occurs because the health condition causes an increased body burden of the chemical (*e.g.*, in blood), rather than the chemical causing the health effect.

5.1.1.1 Rats and Mice

PFOA and PFOS are well absorbed following oral exposure, with estimated absorption fractions in rats of >90% (Chang *et al.*, 2008a; ATSDR, 2015; US EPA, 2016a,b). Findings from a study by Hinderliter *et al.* (2006a), in which PFOA plasma concentrations were higher in fasted *vs.* non-fasted rats, suggests that absorption is greater for fasted rats. There is also qualitative evidence that PFOA is absorbed by rats following both inhalation and dermal exposure (Kennedy, 1985; Kennedy *et al.*, 1986). The observation that the peak serum PFOA concentration was approximately 30-fold lower following inhalation *vs.* oral exposure to a comparable PFOA dose suggests that PFOA may be less well absorbed following inhalation exposure than following oral exposure (Kennedy *et al.*, 2004).¹⁷ The observation that PFOA serum concentration following cumulative dermal exposure to 200 mg/kg ammonium PFOA¹⁸ was comparable to that following a single oral exposure to 25 mg/kg PFOA similarly suggests that PFOA is less well absorbed following dermal exposure than following oral exposure (Kennedy, 1985; Kennedy *et al.*, 2004).

Absorption data are limited for PFBA and PFBS. PFBA oral absorption appears to be nearly complete in rats, based on comparable values for peak serum concentration following either oral or intravenous (iv) exposure to the same dose (Chang *et al.*, 2008a). According to Chang *et al.* (2008a), PFBA is also well absorbed by mice, although absorption is less than in rats. Data from a study by Olsen *et al.* (2009) similarly indicate that PFBS oral absorption is nearly complete in rats, based on comparable values for peak serum concentration and the amount excreted in the urine following either oral or iv exposure to the same dose.

Based on my analysis of the literature, I did not identify any data for mice regarding absorption of PFOA, PFOS, or PFBS. I did not identify any data for rats regarding absorption of PFOS, PFBA, or PFBS following either inhalation or dermal exposure.

5.1.1.2 Monkeys and Humans

I did not identify quantitative data regarding absorption of PFOA in monkeys. Based in part on the observation of a lower steady-state serum¹⁹ PFOA concentration following oral exposure than what would be predicted following iv exposure, Butenhoff *et al.* (2004a) hypothesize that absorption in monkeys may not be complete. This is in contrast to rats, as discussed above.

As discussed by ATSDR (2015), observations that PFOA concentrations in serum or plasma are related to PFOA exposure *via* drinking water, provide evidence of oral absorption of PFOA in humans (*e.g.*, Hoffman *et al.*, 2011; Seals *et al.*, 2011; Bartell *et al.*, 2010; Holzer *et al.*, 2008; Wilhlem *et al.*, 2008). Fasano *et al.* (2005) calculated a human *in vitro* dermal permeability coefficient for PFOA of 9.49×10^{-7} cm/hour, with approximately 0.05% of administered PFOA penetrating through the skin, which is on the same order of magnitude as sucrose²⁰ (US EPA, 2004b). In comparison, the dermal permeability coefficient for nicotine is approximately 1×10^{-3} (US EPA, 2004b) or approximately 4 orders of magnitude greater than that for PFOA.

¹⁷ Based on a comparison of serum concentration following acute oral exposure to 25 mg/kg PFOA with serum concentration following 6-hour inhalation exposure to 0.1 mg/m³ ammonium PFOA, assuming a minute ventilation for rats of 0.681 L/min-kg, as recommended by Bide *et al.* (1997).

¹⁸ Rats were exposed *via* dermal application to 20 mg/kg PFOA, 5 days per week, for 2 weeks (Kennedy, 1985).

¹⁹ Steady-state occurs when the rate at which a chemical is cleared from the blood is the same as the rate at which a chemical is absorbed into the blood.

²⁰ Based on predicted and measured permeability for sucrose of 6.0×10^{-7} and 5.2×10^{-6} , respectively (US EPA, 2004b).

I did not identify any data regarding absorption of PFOS, PFBA, or PFBS for either monkeys or humans.

5.1.2 Distribution

Distribution of a chemical in the body describes the transfer of a chemical throughout the body, across different compartments (*e.g.*, blood, specific tissues). Distribution processes are typically reversible and ultimately determine the free concentration of the chemical in the blood or tissues (Lehman-McKeeman, 2013). The circulating or tissue concentration dictates the onset and intensity of any potential toxicity observed after exposure.

5.1.2.1 Volume of Distribution

Substance distribution can be characterized by its apparent volume of distribution (V_d), a theoretical term that indicates how widely a chemical is distributed in the body. Mathematically, the V_d is the ratio of the total amount of chemical in the body divided by the amount in blood plasma (Shen, 2013). Chemicals that are found primarily in physiological fluid spaces (*e.g.*, the blood or extracellular fluid)²¹ have low V_d s, of less than 1 L/kg (Shen, 2013).²² Chemicals that are highly fat soluble or extensively bound to proteins in cells, such that the bulk of the chemical in the body is not found in blood plasma, have V_d s much greater than 1 L/kg (Shen, 2013). Thus, aspirin, which is primarily found dissolved in the blood, has a V_d of approximately 0.2 L/kg, whereas the antipsychotic drug chlorpromazine, which is highly fat soluble, has a reported V_d greater than 40 L/kg (Snodgrass, 1996).

The V_d is typically an important determinant of metabolism, because only chemicals that are present in blood have the ability to be metabolized and excreted by the liver and kidneys. Table 5.1 presents V_d values for the PFCs at issue, for humans, monkeys, rats, and mice. These values indicate that the PFCs at issue distribute primarily to extracellular fluid and not other body compartments, such as fat or intracellular proteins (Butenhoff *et al.*, 2004a; Chang *et al.*, 2008a, 2012; Olsen *et al.*, 2009).

²¹ Extracellular fluids consist of interstitial fluid (*i.e.*, surrounding tissue cells), blood plasma, and lymph (*i.e.*, fluid inside blood and lymphatic vessels).

²² Note that V_d is sometimes expressed in units of liters without being divided by body weight. For a typical adult weighing 70 kg, a V_d of 1 L/kg is the same as a V_d of 70 L.

Table 5.1 PFC Volume of Distribution Values in Humans, Monkeys, Rats, and Mice (L/kg)

Species	Sex	Exposure Route	PFBA	PFBS	PFOA	PFOS
Humans ^a		N/A	N/A	N/A	0.170	0.230
Cynomolgus Monkeys ^b	Females	iv	0.443	0.255	0.198	0.274
	Males		0.526	0.254	0.181	0.202
Sprague-Dawley Rats ^c	Females	iv	0.187	0.351	N/A	0.586
	Males		0.253	0.330	N/A	0.649
Sprague-Dawley Rats ^d	Females	iv	N/A	N/A	0.171	0.352
	Males		N/A	N/A	0.112	0.383
Sprague-Dawley Rats ^c	Females	Oral	0.173	0.391	N/A	0.521
	Males		0.209	0.676	N/A	0.765
Sprague-Dawley Rats ^d	Females	Oral	N/A	N/A	0.154	0.289
	Males		N/A	N/A	0.106	0.280
Wistar Rats ^e	Females	iv	N/A	N/A	0.211	N/A
	Males		N/A	N/A	0.339	N/A
CD-1 Mice ^f	Females	Oral	0.134	N/A	N/A	0.261
	Males		0.296	N/A	N/A	0.263

Notes:

iv = Intravenous; N/A = Not Available; PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

Volume of distribution (V_d) values for animals are all based on a single dose.

(a) Data from Thompson *et al.* (2010); calculated assuming a first-order, one-compartment model.

(b) Data from Chang *et al.* (2008a) for PFBA (10 mg/kg); Olsen *et al.* (2009) for PFBS (10 mg/kg); Butenhoff *et al.* (2004a) for PFOA (10 mg/kg); and Chang *et al.* (2012) for PFOS (2 mg/kg).

(c) Data from Chang *et al.* (2008a) for PFBA (30 mg/kg); Olsen *et al.* (2009) for PFBS (30 mg/kg); and Chang *et al.* (2012) for PFOS (2 mg/kg).

(d) Data from Kim *et al.* (2016) for PFOA (1 mg/kg) and PFOS (2 mg/kg).

(e) Data from Ohmori *et al.* (2003) for PFOA (~20 or 50 mg/kg; estimated based on administered dose reported by Ohmori *et al.* as being, alternatively, 48.64 μ mol/kg or 48.64 mmol/[2.5 mL/kg]; conversion based on molecular weight of 414.069 [ATSDR, 2015]).

(f) Data from Chang *et al.* (2008a) for PFBA (30 mg/kg) and from Chang *et al.* (2012) for PFOS (20 mg/kg).

Once absorbed, both PFOA and PFOS bind with proteins in the blood, primarily albumin, and do not appear to either bind to or be taken up into red blood cells (Kerstner-Wood *et al.*, 2003; Ehresman *et al.*, 2007). *In vitro*, > 90% of PFOA binds to albumin, with comparable binding affinity in rats and humans, and comparable binding in male and female rats (Han *et al.*, 2003). Ohmori *et al.* (2003) found that more than 98% of PFOA was bound to plasma proteins in rats. Similarly, an *in vitro* binding study showed that 99-100% of PFOS is bound to plasma proteins, such as albumin and low-density lipoproteins (LDLs), in rats, monkeys, and humans (Kerstner-Wood *et al.*, 2003).

5.1.2.2 Tissue Distribution

In addition to understanding the apparent V_d , evaluating specific tissue distribution is important for understanding the amount of a substance reaching specific tissues (*i.e.*, tissue dose) and, subsequently, potential target organ toxicity. As with other pharmacokinetic parameters, a better understanding of distribution patterns may help elucidate observed differences in toxicity, either among or within species, and may also help predict whether such differences might exist, in the absence of information to that effect. Tissue distribution is largely controlled by lipid solubility, with more lipid-soluble substances preferentially distributing to hydrophobic areas, such as lipid bilayers of tissues, and more water-soluble substances preferentially distributing to aqueous media, such as blood plasma or serum (Lehman-McKeeman, 2013). Specific tissue distribution is evaluated by comparing concentrations in the specific

tissue to those in serum or plasma; ratios greater than 1 indicate preferential partitioning into the specific tissue relative to the serum or plasma.

Table 5.2 presents the predominant tissues in which the PFCs distribute. As shown in Table 5.2, while there are similarities in overall PFC tissue distribution, there are also notable differences that appear to vary by species and, to a lesser extent, by sex. Overall, the evidence suggests that PFOA, PFOS, and PFBS preferentially distribute to the liver in most species and do not readily cross the mature blood-brain barrier. The limited data in humans indicate that, while PFOA preferentially distributes to the liver, PFOS, PFBS, and PFBA concentrations found in the kidney are higher than those found in the liver. Data from Harada *et al.* (2007), in which PFOA and PFOS cerebral spinal fluid concentrations in adult humans were more than 500-fold lower than serum concentrations, provide additional evidence that these compounds do not readily cross the blood-brain barrier in humans. PFBA appears to preferentially distribute to the serum and, to a much lesser extent, to the liver. It is important to note that data for all species aside from rats, and for PFBA and PFBS, are limited, and thus, it is not possible to draw strong conclusions regarding the observed differences in specific tissue distribution for species other than rats, or for PFBA and PFBS for any species, based on these data.

Table 5.2 Tissue Distribution of PFCs By Species and Sex

Species	Sex	PFBA	PFBS	PFOA	PFOS
Rats	Males	Serum >> Liver ^a	N/A	≤ 5 mg/kg Liver >> Serum > Kidney >> Spleen >> Brain ^b ≥ 10 mg/kg Serum $\geq$ Liver $\geq$ Kidney >> Spleen >> Brain ^b	≤ 0.3 mg/kg Liver >>> Serum $\approx$ Kidney >> Spleen >> Brain ^c 2 mg/kg Liver >> Serum >> Kidney >> Spleen ^d
	Females	N/A	N/A	≤ 5 mg/kg Serum $\approx$ Liver $\geq$ Kidney >> Spleen ^b ≥ 10 mg/kg Serum > Kidney > Liver >> Spleen >>> Brain ^b	2 mg/kg Liver >> Serum >> Kidney >> Spleen ^d
Mice	Males	Serum >> Liver ^a	Liver > Kidney >> Spleen >> Brain ^e	Liver > Serum ^f	Liver >> Kidney >> Spleen >> Brain ^g
	Females	N/A	N/A	Liver >> Serum ^f	N/A
Monkeys	Males	N/A	N/A	Serum >> Liver ^h	Liver > Serum ⁱ
Humans	N/A	Kidney >>> Liver >> Brain ^j	Kidney >> Liver (ND in brain) ^j	Liver >> Kidney (ND in brain) ^j	Kidney $\geq$ Liver >>> Brain ^j

Notes:

CSF = Cerebral-spinal Fluid; N/A = Not Available; ND = Not Detected; PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

$\geq$ indicates tissue concentration is comparable or slightly greater than tissue concentration in comparison tissue.

> indicates there is a less than 2-fold difference in relative tissue concentration.

>> indicates there is a 2- to 10-fold difference in relative tissue concentration.

>>> indicates there is a greater than 10-fold difference in relative tissue concentration.

(a) Chang *et al.* (2008a).

(b) Data from multiple studies (summarized in Appendix Table B.1); data for PFOA levels in female rat brain at low doses are not available.

(c) Iwabuchi *et al.* (2017).

(d) Kim *et al.* (2016).

(e) Bogdanska *et al.* (2014).

(f) Data from one male and one female mouse (Hundley *et al.*, 2006).

(g) Bogdanska *et al.* (2011).

(h) Butenhoff *et al.* (2004a); evaluated only serum and liver concentrations.

(i) Seacat *et al.* (2002); evaluated only serum and liver concentrations.

(j) Data from 20 human cadavers (Perez *et al.*, 2013).

Table 5.3 presents the ratios of different PFCs in the liver, a predominant tissue for PFC distribution, to serum or plasma in various species. As shown in Table 5.3, liver concentrations (relative to serum or plasma) decrease in the order of PFOS > PFOA > PFBA. I did not identify information regarding liver-to-serum ratios for PFBS.

Table 5.3 PFC Liver to Serum Ratios^a

Species	PFBA	PFOA	PFOS
Rats	<u>Males</u> 0.24 (0.22-0.27) ^b	<u>Females (≤ 0.1 mg/kg)^c</u> N/A	<u>Females^c</u> 2.9 (1.9-47)
		<u>Males (≤ 0.1 mg/kg)^c</u> 2.4 (1.7-3.7)	<u>Males^c</u> 8.8 (2.6-51)
		<u>Females (≥ 1 mg/kg)^c</u> 0.64 (0.28-0.81)	
		<u>Males (≥ 1 mg/kg)^c</u> 0.9 (0.59-2.3)	
Mice	<u>Females</u> 0.17 (0.15-0.17) ^d	<u>Females (10 mg/kg)^e</u> 2.4	N/A
	<u>Males</u> 0.23 (0.21-0.28) ^d	<u>Males (10 mg/kg)^e</u> 1.6	
Monkeys	N/A	0.18 (3 mg/kg) ^f 0.13 (10 mg/kg) ^f	1.8 (0.9-2.2) ^g
Humans	N/A	1.2, 3.2 ^h	1.3 ⁱ

Notes:

N/A = Not Available; PFBA = Perfluorobutanoic Acid; PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

(a) Values represent median and range; studies report concentrations in either serum or plasma, with values in either serum or plasma considered equivalent. Unless otherwise specified, there was no clear dose-dependent or sex-dependent differences in liver and serum concentrations.

(b) Data for males only (Chang *et al.*, 2008a).

(c) Ratios based on data from multiple studies (summarized in Appendix Table B.2).

(d) Data for doses of 10-100 mg/kg (Chang *et al.*, 2008a).

(e) Serum PFOA concentrations were estimated from whole-blood concentrations from Hundley *et al.* (2006) using a factor of 1.9, as estimated from data reported by Iwabuchi *et al.* (2017) and Kudo *et al.* (2007).

(f) Data from Butenhoff *et al.* (2004a) for males only; values represent median for n = 4 monkeys/dose.

(g) Data from Seacat *et al.* (2002); no apparent difference between females and males, or among doses (from 0.03-0.75 mg/kg-day).

(h) Data available for only two cadavers (Olsen *et al.*, 2001; also discussed in Olsen *et al.*, 2003b).

(i) Data as reported by Olsen *et al.* (2003b).

The relationships shown in the Table 5.3 apply to animals studied post-natally, but may not apply to the developing fetus. Studies in rats and mice indicate that PFOS can pass through the immature blood-brain barrier. In rats, PFOS levels in fetal and pup brains are greater than in maternal brains, with levels in rat pups observed to decrease after birth (Chang *et al.*, 2009; Ishida *et al.*, 2017). Zeng *et al.* (2011) also observed that PFOS concentrations in the rat pup hippocampus and cortex decreased between post-natal day (PND) 0 and PND 21, consistent with the fetal blood-brain barrier not being fully developed. Similarly, PFOS levels in fetal and pup mouse brains are greater than in maternal mouse brains (Borg *et al.*, 2010).^{23,24}

Table 5.4 highlights the differences in liver-to-serum ratios across species, sexes, and doses (for the values presented in Table 5.3). As shown in Tables 5.3 and 5.4, the exact distribution patterns differ

²³ PFOS levels were not measured in mouse pups.

²⁴ Note that observations of higher PFOS levels in fetal and pup brains compared to maternal brains do not, by themselves, provide evidence that PFOS can cause neurodevelopmental effects. The potential for PFOS to cause such effects is discussed further in Section 6.2.1.5.

across species and sexes for the different PFCs. The species differences are more pronounced for PFOA and PFOS than for PFBA. As discussed further in Section 5.1.4, the sex differences in PFOA distribution for rats (but not for PFOS in rats) are consistent with differences in excretion rates. The reasons for such species differences in liver tissue distribution is unclear. The information in Tables 5.3 and 5.4 also indicates that PFOS distributes to the liver to a lesser extent in monkeys and humans than in rats. This suggests that, given the same serum or plasma concentration, monkeys and humans may be less susceptible to liver effects of PFOS than rats.

Table 5.4 Liver Tissue Distribution Across Species, Sex, and Dose

Comparison Across:	PFBA	PFOA	PFOS
Species	Rats ≈ Mice	Mice ≈ Humans > Rats > Monkeys	Rats > Monkeys ≈ Humans
Sexes	Males ≈ Females (Mice)	Males > Females (Rats) Females ≈ Males (Mice) N/A (Monkeys and Humans)	Males > Females (Rats) N/A (Mice, Monkeys, and Humans)
Doses	N/A	Low Dose > High Dose (Rats) N/A (Mice, Monkeys, and Humans)	N/A

Notes:

N/A = Not Available; PFBA = Perfluorobutanoic Acid; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

5.1.2.3 Placental and Lactational Transfer

Studies of humans and laboratory animals demonstrate that both PFOA and PFOS can cross the placenta, referred to as placental transfer, and into breast milk, referred to as lactational transfer. Thus, developing fetuses can be exposed to PFOA and PFOS *in utero*, and newborns can be exposed to PFOA and PFOS *via* lactation.

As shown in Table 5.5, although placental and lactational transfer of PFOA are comparable in rats and humans, PFOA blood concentrations in offspring relative to maternal blood concentrations are greater in humans than in rats. Similarly, for PFOS, blood concentrations in offspring relative to maternal blood concentrations are somewhat greater in humans compared to rats, despite the fact that placental and lactational transfer of PFOS is considerably greater in rats compared to humans. The basis for higher post-natal offspring/maternal blood ratio in humans compared to rats is not clear, but could be related to differences in biological half-lives for PFOA and PFOS, which are considerably longer in humans compared to rats (as discussed further in Section 5.1.4). The differences in placental and lactational transfer between rats and humans highlight the importance of considering pharmacokinetic differences between species. Given the same exposure level to the mother or dams, the differences shown in Table 5.5 suggest that the developing rat fetus would be exposed to higher serum PFOA concentrations than the developing human fetus, and the newborn human would be exposed to higher serum PFOA concentrations than the newborn rat. As I discuss below, there are other important differences in elimination kinetics that result in higher PFOA (and PFOS) serum concentrations in humans compared to rats, at similar exposure levels. In Section 8, I discuss the significance of these differences with respect to setting health-based criteria for PFOA and PFOS.

Table 5.5 Placental and Lactational Transfer

PFC	Placental Transfer ^a		Lactational Transfer ^b		Offspring/Maternal Ratio ^c	
	Humans	Rats	Humans	Rats	Humans	Rats
PFOA	0.79 (0.62-1.5)	0.42	0.04 (0.03-0.12)	0.10	3.0 (1.8-4.6)	0.26
PFOS	0.37 (0.29-0.56)	2.3	0.01 (0.01-0.03)	0.31	1.1 (0.93-1.4)	0.68

Notes:

PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

Underlying data included in Appendix Table B.3.

(a) Presented as fetal (cord blood)/maternal (serum or plasma) ratio. Values for humans represent median and range. Value for rats selected as lowest dose (as being most comparable to exposure and sample collection in humans).

(b) Presented as breast milk/serum (or plasma) ratio. Values for humans represent median and range. Values for rats selected as lowest dose, and at earliest time-point (as being most comparable to exposure and sample collection in humans).

(c) Values for humans represent average of three studies, using earliest post-natal time point, based on PFC concentrations quantified in serum or plasma. Values for rats selected as lowest dose, at earliest post-natal time point; based on PFC concentrations quantified in either serum or plasma.

5.1.3 Metabolism

Neither PFOA nor PFOS appear to undergo metabolism in the liver or other tissues (ATSDR, 2009; US EPA, 2016a,b). PFOA and PFOS are rather unusual in this regard, because liver metabolism is common for many chemicals. Potential detoxification or bioactivation²⁵ in the liver complicates the extrapolation of health effects across species, because different species sometimes show differences in metabolism. There is also the potential for differences in metabolism among members of the same species (*e.g.*, ethnic differences in the ability to metabolize ethanol among humans). Because PFOA and PFOS do not undergo metabolism in humans and the various species studied, these potential complications are not present, which facilitates comparing exposures across species. Although I did not find any discussions regarding the metabolism of PFBA or PFBS, the observations that female rats excrete 100% of PFBA within 24 hours following oral administration of PFBA (Chang *et al.*, 2008a) and that combined 24-hour urinary/fecal excretion of PFBS is 100% in mice (Olsen *et al.*, 2009) provide evidence that these PFCs do not appear to undergo metabolism.

5.1.4 Excretion

Elimination rate is characterized by an elimination half-life, which is the time required for half of the total amount of a chemical to be eliminated from the body, assuming there is no ongoing exposure. Approximately 97% of the total amount of a chemical in the body will be eliminated in five half-lives (*i.e.*, $1-0.5^5$). A related term is clearance. Clearance refers to the rate at which a chemical can be removed from blood plasma (*e.g.*, by metabolism or excretion). Clearance is typically measured in units of liters of blood plasma cleared of the substance per hour (Shen, 2013). Because the body has a fixed amount of blood plasma (about 3 L in an adult human), knowing the clearance allows one to estimate how long a chemical will remain in the body.

²⁵ Bioactivation involves enzymatic alteration of a parent compound to a more biologically active and potentially more toxic compound.

Clearance (which can be abbreviated as CL), elimination half-life, and V_d are connected *via* the following equation (Shen, 2013):

$$t_{1/2} = \frac{\ln 2 \times V_d}{CL}$$

Consistent with this equation, the faster a chemical is cleared from the plasma (*i.e.*, larger clearance), the shorter its elimination half-life (*i.e.*, smaller $t_{1/2}$). Clearance is expressed either in units of volume per time (*e.g.*, L/hour) or in units that account for body weight (*e.g.*, L/kg-hour).

In humans, biliary clearance²⁶ of PFOA and PFOS exceeds urinary clearance (Harada *et al.*, 2007), although results from a study by Zhang *et al.* (2015) indicate that urine is also an important elimination pathway for PFOA and PFOS in humans. In contrast, urinary elimination of PFOA exceeds fecal elimination in monkeys and rats (Butenhoff *et al.*, 2004a; Kemper, 2003). Urine is also a primary elimination pathway for PFOS in rats (Chang *et al.*, 2012),²⁷ and for PFBA and PFBS in monkeys and rats (Chang *et al.*, 2008a; Chengelis *et al.*, 2009; Olsen *et al.*, 2009).

As shown in Table 5.6, half-lives are longer for the 8-carbon *vs.* the 4-carbon PFCs and are also longer for the sulfonates *vs.* the carboxylates. There are substantial differences in PFC elimination rates between humans, monkeys, and rats, with much longer half-lives found in humans. As discussed by Harada *et al.* (2007), the long half-lives for PFOA and PFOS in humans may be due to low levels of urinary excretion coupled with a high rate of biliary reabsorption (0.89 and 0.97 for PFOA and PFOS, respectively). Reabsorption from kidney tubules by organic anion transporter (OAT) 4 and urate transporter 1 may also contribute to the long biological half-life of PFOA in humans (Nakagawa *et al.*, 2009; Yang *et al.*, 2010). In contrast to humans, in which the half-lives of PFOA and PFOS are comparable, the half-life of PFOA in monkeys and rats is considerably shorter than the half-life of PFOS. In male rats, Kemper (2003) found that PFOA plasma elimination half-life was independent of dose (ranging from approximately 138-202 hours), whereas in female rats, elimination rates ranged from approximately 2.8 hours at a dose of 0.1 mg/kg to 16 hours at a dose of 25 mg/kg.

²⁶ Biliary clearance describes the process by which substances are excreted into bile in the liver, removing the substance and its metabolites from the body prior to entering general circulation. Depending on the physicochemical characteristics of the substance, substances excreted into bile may be transported to the intestine and excreted *via* feces, or circulate between the liver and the intestines, via a process referred to as enterohepatic circulation (Lehman-McKeeman, 2013).

²⁷ I did not locate any studies that provided information on urinary *vs.* fecal elimination of PFOS in monkeys.

Table 5.6 PFC Elimination Half-lives^a

Species	Sex	PFBA	PFBS	PFOA	PFOS
Humans ^b	Females/ Males	2.9 days	25.8 days	2.3-8.5 years	3.3-5.4 years
Monkeys	Females	1.7 days	3.5 days ^c	32.6 days	110 to ~200 days
	Males	1.7 days	4.0 days ^c	~20 days	132 to ~200 days
Rats	Females	1.0 hours (iv exposure)	0.64-7.4 hours	1.9-4.6 hours (<25 mg/kg)	24-83 days
		1.8 hours (oral exposure)		16.2 hours (25 mg/kg)	
	Males	6.4 hours (iv exposure) 9.2 hours (oral exposure)	2.1-4.7 hours	24 hours (50 mg/kg) 1.6-15 days (<25 mg/kg) ^d 6.5 days (25 mg/kg) 4.4 days (50 mg/kg)	26-82 days ^e
Mice	Females	2.9 hours (10 mg/kg) 3.1 hours (30 mg/kg) 2.8 hours (100 mg/kg)			38 days (1 mg/kg-day) 30 days (20 mg/kg-day)
	Males	13.3 hours (10 mg/kg) 16.3 hours (30 mg/kg) 5.2 hours (100 mg/kg)			43 days (1 mg/kg-day) 36 days (20 mg/kg-day)

Notes:

iv = Intravenous; PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

Data for individual studies is included in Appendix Table B.4.

(a) Unless otherwise specified, data represent both oral and iv exposures, both acute and chronic exposures, and a range of doses.
 (b) PFBA data from Chang *et al.* (2008a); represents geometric mean for three employees from the Cottage Grove, Minnesota, facility and nine employees from the Cordova, Illinois, facility. PFBS data from Olsen *et al.* (2009). Data for PFOA and PFOS are as summarized in Appendix Table B.4..

(c) Mean terminal serum elimination half-life ($T_{0.5}$) for PFBS is from Olsen *et al.* (2009), estimated using a three-compartment model. Note that Chengelis *et al.* (2009) reported elimination half-lives of 8.1 and 15 hours, respectively, for males and females. However, they monitored serum PFBS concentrations for only 7 days vs. 31 days in Olsen *et al.* (2009). Hence, Chengelis *et al.* (2009) might not have captured the third phase of elimination observed by Olsen *et al.* (2009), reflected in the much longer half-lives estimated by Olsen *et al.* (2009).

(d) Excludes data from Johnson and Ober (1980). Although the elimination half-life as reported by ATSDR (2015) was 4.8 days, the basis for estimating the half-life was not clear from the underlying data provided in Johnson and Ober (1980).

(e) Excludes data from Johnson and Ober (1979a). Although the elimination half-life as reported by ATSDR (2015) was 7.5 days, the basis for estimating the half-life was not clear from the underlying data provided in Johnson and Ober (1979a).

5.1.4.1 Sex Differences in Excretion

Urinary excretion of PFOA is greater for female rats than male rats, with >70% eliminated in urine within 24 hours in females *vs.* <10% in males (Vanden Heuvel *et al.*, 1991; Hanhijarvi *et al.*, 1982; Kudo *et al.*, 2001; Kemper, 2003; Ohmori *et al.*, 2003; Lau *et al.*, 2006). The increased urinary excretion by female rats results in shorter elimination half-lives, as shown in Table 5.6. Sex differences with respect to elimination are also observed following inhalation exposure (Hinderliter *et al.*, 2006b). Findings by Hinderliter *et al.* (2006b) that sex differences with respect to PFOA elimination are similar for oral *vs.* inhalation exposure indicate that plasma PFOA is a suitable dose metric for route-to-route extrapolation for rats.

Increased excretion of PFOA by female relative to male rats is likely due to differential activity of kidney OATs and organic anion transporting polypeptides (Oatps)²⁸. Hanhijarvi *et al.* (1982) found that that PFOA clearance in female rats is several-fold greater than clearance of inulin²⁹ (which, as discussed by Hanhijarvi *et al.*, 1982, is excreted exclusively by glomerular filtration)³⁰ and is substantially inhibited by probenecid³¹, which inhibits renal organic acid transport. In male rats, PFOA clearance was a fraction of inulin clearance and was not affected by probenecid. Although the specific OATs and Oatps involved in the sex-specific excretion of PFOA have not been definitively identified, Oatp1/Oatp1a1, which may be involved in reabsorption of organic anions, is expressed at higher levels in male *vs.* female rat kidneys (Kato *et al.*, 2002; Kudo *et al.*, 2002; Yang *et al.*, 2009; Weaver *et al.*, 2010).³² On the other hand, OAT2 is expressed at higher levels in female *vs.* male rat kidneys and may be involved in secretion of organic anions, such as PFOA (Kudo *et al.*, 2002; Ljubojevic *et al.*, 2007). As discussed by ATSDR (2015), saturation of kidney transporters may contribute to the longer elimination rates observed in female rats exposed to higher PFOA doses, as shown in Table 5.6.

In contrast to PFOA, for which the elimination half-life is less in female than in male rats, Chang *et al.* (2012) observed longer PFOS elimination half-lives for females (62 and 71 days, at doses of 2 mg/kg-day and 15 mg/kg-day, respectively) than for males (38 and 41 days, at doses of 2 and 15 mg/kg-day, respectively). Consistent with the longer elimination half-lives observed in female rats, Butenhoff *et al.* (2012a) found that PFOS serum concentrations in female rats were approximately 3-fold higher than in male rats. Sex differences with respect to PFOS elimination have not been observed for mice (Hundley *et al.*, 2006; Lau *et al.*, 2006; Chang *et al.*, 2012), rabbits (Hundley *et al.*, 2006), monkeys (Chang *et al.*, 2012), or humans (Harada *et al.*, 2005; Zhang *et al.*, 2015).

As with PFOA, urinary excretion of PFBA is greater for female than for male rats, with 24-hour post-dosing excretion of approximately 100% *vs.* 60%, respectively. Urinary excretion of PFBA is also greater for female than for male mice, with 24-hour post-dosing excretion of approximately 70% *vs.* 35%, respectively (Chang *et al.*, 2008a).³³ As shown in Table 5.6, the increased urinary excretion of PFBA by female rats and monkeys results in shorter half-lives. Although the basis for the sex differences in PFBA

²⁸ OATs and Oatps are kidney proteins involved in both the excretion of chemicals into the urine, and reabsorption of chemicals back into the blood (Weaver *et al.*, 2010).

²⁹ Inulin is a carbohydrate that is resistant to digestion in the stomach, used in tests to diagnose kidney function (Merriam-Webster, Inc., 2016).

³⁰ Compounds that are excreted in the urine can either be filtered from the blood capillaries in the glomerulus, which is a part of the kidney, or they can be actively transported from the blood into the urine forming in the proximal tubule, which is a different part of the kidney.

³¹ Probenecid is a drug that inhibits excretion by the kidneys of certain other drugs (such as penicillin) to increase their concentration in the blood, and that also increases excretion of uric acid, for treating gout (Merriam-Webster, Inc., 2016).

³² According to Kuo *et al.* (2012), Oatp1a1 was previously referred to as Oatp1.

³³ 24-hour excretion as presented in Table 1 in Chang *et al.* (2008a) for rats, for doses of 3, 10 and 10 mg/kg, and as presented in Table 3 in Chang *et al.* (2008a) for mice, for doses of 10 and 30 mg/kg.

elimination have not been evaluated, Chang *et al.* (2008a) suggest that, as with PFOA, the differences may relate to sex-specific reabsorption kinetics in kidney tubules. As discussed by Chang *et al.* (2008a), their observation that the 24-hour urinary excretion rate of PFBA in male rats at 300 mg/kg was substantially greater than that at doses of 100 mg/kg or less (90% vs. 50-60%) indicates the presence of saturable reabsorption process in kidney tubules. In monkeys, urinary excretion in females is slightly less than in males, with 24-hour post dosing urinary excretion of 36% vs. 42% in females vs. males (Chang *et al.*, 2008a).

5.1.4.2 Excretion *via* Menstruation and Lactation

In addition to urine and feces, menstruation and lactation can be important elimination routes in women. Using a PBPK model, Wong *et al.* (2014) estimated that the PFOS serum elimination half-life for women increased from 3.7 to 4.0 years by including loss of PFOS *via* menstruation in their model. Compared to an estimated PFOS serum elimination half-life in men of 4.7 years, the increase of 0.3 years represents 30% of the difference between half-lives in men and women when loss of blood to menstruation is not accounted for. Kang *et al.* (2016) observed an inverse correlation between the PFOA breastmilk concentration and length of lactation and suggested that lactation may be an important excretion route for lactating women. Other studies show that serum concentrations of PFOA and PFOS are lower in women who breastfeed compared to women who do not breastfeed, with maternal serum decreasing approximately 2-3% per month of breastfeeding (Brantsaeter *et al.*, 2013; Mondal *et al.*, 2014).

5.1.4.3 Accounting for Interspecies Differences in Elimination Half-lives

Because of the substantial difference in elimination half-lives between humans and animals, serum concentrations of PFOA and PFOS in humans following repeated exposure to a given dose (in mg/kg-day) will be greater than that in laboratory animals exposed to the same dose. Because serum concentrations govern concentrations in tissues in which biological effects occur, serum concentrations provide a better metric than exposure doses for comparing effect levels between humans and laboratory animals. An HED for the doses used in the animal studies (*i.e.*, a dose associated with comparable serum concentrations in humans and animals) can be calculated as follows (per US EPA, 2016a):

HED (mg/kg-day) = Average Serum Concentration in Animals (mg/L) × Clearance in Humans (L/kg-day)

The average serum concentration can be calculated from the area under the curve (AUC) as predicted from PBPK model (discussed in Section 5.1.5), according to the following equation (US EPA, 2016a):

$$\text{Average Serum Concentration (mg/L)} = [\text{AUC (mg/L-hour)} \times 1 \text{ day}/24 \text{ hours}] / [\text{Exposure Duration}]$$

Using an elimination half-life ($t_{1/2}$) in humans of 2.3 years (839.5 days) for PFOA (Bartell *et al.*, 2010) and 5.4 years (1,971 days) for PFOS (as estimated by Olsen *et al.*, 2007), and V_d s as calculated by Thompson *et al.* (2010) of 0.17 and 0.23 L/kg for PFOA and PFOS, respectively, US EPA (2016a,b) calculated clearance rates in humans at steady-state of 0.00014 and 0.000081 L/kg-day for PFOA and PFOS, respectively, according to the following equation:

$$CL = V_d \times \left(\frac{\ln 2}{t_{1/2}} \right)$$

US EPA uses these clearance rates to calculate the HED values associated with effect levels (*i.e.*, doses) from the animal studies; I used these HED values in my calculations to estimate exposure to PFOA and PFOS (see Section 10.4).

5.2 Physiologically Based Pharmacokinetic (PBPK) Models

PBPK modeling involves using a computer program to describe the ADME of a chemical (or chemicals) in the body (Krishnan and Andersen, 2001). The computer program contains a series of equations that describe the behavior of the chemical in different compartments. In a PBPK model, the different compartments correspond to actual tissues (*i.e.*, the liver, brain, kidney) and are defined using actual physiological data (*e.g.*, data on tissue volume or blood flow). The equations typically have a form that looks like the following:

$$\text{change in tissue concentration} = \text{amount coming into the tissue via blood flow} - \\ \text{amount leaving the tissue via blood flow} - \\ \text{amount leaving due to metabolism}$$

A set of these equations is created for each tissue in the body (some tissues may be grouped together in order to simplify calculations), and the computer program solves this set of equations repeatedly (*e.g.*, each second) over the timeframe of interest (ranging from days to years). After each round of calculation, the program takes note of the amount of the chemical in each tissue, which then becomes the inputs for the next round of calculations. The result is a table or plot of chemical concentrations in each tissue over the course of the simulation. More complex models are also possible, such as those that account for the interconnections and rapid changes in maternal and fetal tissues during pregnancy (*e.g.*, Luecke *et al.*, 1997; Loccisano *et al.*, 2012a).

A key advantage of PBPK modeling is that it provides a more accurate basis for extrapolating doses and tissue concentrations of specific chemicals across species, sexes, or age groups (Thompson *et al.*, 2008; US EPA, 2006) than other methods, such as the use of body weight to the $\frac{3}{4}$ power (Rhomborg and Lewandowski, 2006; US EPA, 2011b). For example, it is possible to build a PBPK model using physiological data for a rat and then replacing the inputs with physiological data for humans, often referred to as "scaling up." Assuming the metabolic processes are similar for both species, the human model should predict chemical concentrations relatively accurately.

One key area in which PBPK modeling is used is in those cases in which there are important differences in metabolic processes across species, sexes, or age groups (Wang *et al.*, 2000; Clewell *et al.*, 2000), such as those observed for PFOA and PFOS. In these cases, models can be revised to account for differences in biology (*e.g.*, higher levels of fat in women *vs.* men, differences in metabolic enzymes in rats *vs.* humans, significant differences in metabolic capacity in the developing fetus *vs.* adults). The predictions of the revised models can then be compared to tissue data collected in exposed animals or humans to see whether the revised model provides a better match. In this way, PBPK models provide valuable information that can be used in relating effect levels in animals to effect levels in humans and in interpreting the results of human studies. As with all models, there is some uncertainty associated with PBPK models and how well they describe underlying physiological parameters. Uncertainty arises from the assumptions and choice of inputs applied to the model, such as specific characterization and number of body compartments or rate constants describing distribution or excretion.

There are several available models of PFOA and PFOS in the published scientific literature (I did not identify any PBPK models of PFBA or PFBS in any species). These PFOA and PFOS models have contributed significantly to the current understanding of sex- and species-specific differences in PFOA and PFOS pharmacokinetics, such as in half-life and clearance mechanisms that ultimately have bearing on the degree of internal levels of these substances in exposed individuals. For example, PBPK models developed for PFOA and PFOS have been useful for corroborating findings from experimental studies regarding the involvement of renal transporters in the elimination of these PFCs (discussed in Section

5.1.4.1). Andersen *et al.* (2006) developed a PBPK model that incorporated reabsorption of PFOA and PFOS in the kidney by saturable, high-efficiency transporters, which accurately described serum concentrations of these PFCs in monkeys. In particular, modeled plasma concentrations reflected the biphasic elimination curve observed in monkeys, characterized by initial rapid clearance at high plasma concentrations, followed by a slower secondary elimination phase at lower plasma concentrations. Building on the model developed by Andersen *et al.* (2006) for monkeys, as well as a rat PBPK model developed by Loccisano *et al.* (2012b), Worley and Fisher (2015) developed a model in rats incorporating saturable renal reabsorption of PFOA by the OATs. The Worley and Fisher (2015) model accurately predicted sex-specific and dose-dependent elimination of PFOA in rats, with saturation of the OATs at high doses, and thus supports a role for the OATs in the sex-specific differences in PFOA elimination observed in rats (discussed in Section 5.1.4.1).

PBPK models of PFOA and PFOS developed for humans are useful for considering the importance of specific elimination pathways for these substances from the body (*e.g.*, in breast milk and menstrual blood). For example, PBPK models of menstrual blood elimination of PFOS suggest that the associations between PFOS and late puberty onset and early menopause are likely the result of reverse causation, *i.e.*, menstruation/menopause affects serum PFOS concentrations, rather than PFOS affecting the timing of menstruation/menopause (Wong *et al.*, 2014; Wu *et al.*, 2015). Similarly, Verner *et al.* (2015) used a PBPK model to estimate PFOA and PFOS concentration in maternal and cord plasma and determined that GFR during pregnancy likely confounds the association between low birth weight and PFOA and PFOS exposure observed in certain epidemiologic studies. I discuss this further in Section 7.

PBPK models that evaluate the elimination of PFOA and PFOS through lactation in humans were developed to explore the ways in which physiological changes associated with development affect the pharmacokinetics of these compounds in the mother, fetus, and infant (Verner *et al.*, 2016; Loccisano *et al.*, 2013). Both models incorporate elements of placental and lactational transfer of PFOA and PFOS to the developing fetus and infant. Loccisano *et al.* (2013) predicted PFOA and PFOS concentrations in maternal plasma throughout pregnancy and lactation (up to 6 months), fetal plasma throughout gestation, and infants during lactation up to 6 months of age. Verner *et al.* (2016) developed a PBPK model of prenatal and post-natal PFOA and PFOS exposure to predict concentrations of these substances in children from birth to 3 years of age. While the models differed somewhat in their construction and data sources, they both predicted approximately 3- to 4-times higher PFOA plasma concentrations in breastfeeding infants as compared to the mothers at 6 months post-birth. In contrast to predictions with PFOA, mean or median PFOS plasma concentrations in breastfeeding infants at 6 months (Loccisano *et al.*, 2013; Verner *et al.*, 2016) or children at 3 years (Verner *et al.*, 2016) were predicted to be similar or only slightly increased as compared to concentrations in lactating mothers at the same time-point.

The results of the models by Loccisano *et al.* (2013) and Verner *et al.* (2016) have implications for setting regulatory limits for PFOA and PFOS in drinking water for the general population. Because these models predict an increased body burden of PFOA and PFOS in breastfed infants and children as compared to mothers, regulatory agencies may decide to use infant PFOA or PFOS serum concentrations (as opposed to maternal concentrations) as the basis for health-based drinking water criteria for these substances. This ultimately means that the levels of PFOA or PFOS considered "safe" in drinking water will be lower if they are set based on infant serum concentrations than if they are set based on maternal serum concentrations, in order to ensure that nursing infants are not accumulating internal levels of these substances that exceed serum concentrations associated with the critical effects chosen for the POD for the given regulatory criteria value. MDH recently incorporated such pharmacokinetic concepts in the development of their HBVs for PFOA and PFOS in drinking water, using a simplified pharmacokinetic

model for PFOA and PFOS in humans that incorporated placental and lactational transfer to infants (MDH, 2017a).³⁴ This model is discussed further in Section 8 of this report.^{35,36}

5.3 PPAR α Mode of Action Associated with a Species-specific Response for PFOA

Chemicals can cause adverse health effects *via* a variety of processes, for example, by inhibiting specific enzymes, damaging DNA, competing for absorption of a critical substance, or binding to a specific receptor and altering gene expression. A MoA describes the underlying processes by which a chemical causes toxicity, for example, by inhibiting a specific enzyme, competing for absorption of a critical substrate, or binding to a specific receptor and causing changes in gene expression. Understanding a chemical's MoA, and potential differences in MoA among species, can inform whether, and to what extent, effects observed in animals would occur in humans. Depending on the MoA, effects may be expected to occur only above a critical threshold dose or the nature of the effect may differ at low vs. high doses (for example, if effects at low doses depend on saturable processes, such as those mediated by enzymes or receptors). For the PFCs, the most well-studied MoA involves activation of PPAR α . Although other MoAs have been proposed for PFCs (*e.g.*, activation of the constitutive androstane receptor and the pregnane x receptor [PXR]), I am not addressing them because there is insufficient information regarding their involvement in mediating PFC toxicity.

PPAR α is a ligand-activated nuclear hormone receptor that controls expression of enzymes involved in lipid metabolism, such as acyl coenzyme A oxidase (Acox1), catalase, cytochrome P450s, enoyl coenzyme A hydratase (Ehhadh), and fatty acid binding protein. Natural ligands for PPAR α include eicosanoids³⁷ and fatty acids. PPAR α can also be activated by structurally diverse synthetic ligands, including fibrates, phthalates, chlorinated solvents, and PFCs. PPAR α activators target the liver, where, in addition to enzyme induction, they can cause a substantial increase in the number and volume of peroxisomes³⁸ (*i.e.*, peroxisome proliferation) and hepatocellular hypertrophy and proliferation, which can result in increased liver weight (Kroetz *et al.*, 1998; Berger and Moller, 2002; Klaunig *et al.*, 2003; Lee *et al.*, 2003; Gonzalez and Shah, 2008; Rosen *et al.*, 2008a). Although a role for PPAR α activation in developmental processes is less well studied and less certain than for liver effects, their expression in certain tissues during mouse embryonic development and their ability to induce differentiation of certain cell types provides some support for their involvement in developmental processes (Keller *et al.*, 2000; Peraza *et al.*, 2006). Thus, understanding whether PPAR α activation is involved in specific toxicological effects of a chemical is relevant to understanding species differences in toxicological response to that chemical.

There are important species differences in terms of levels of PPAR α expression, sensitivity to PPAR α -mediated effects and specific effects mediated by PPAR α activation. As discussed by Klaunig *et al.*

³⁴ It should be noted that, because such models address pharmacokinetic variability within species, the use of a PBPK model in this circumstance eliminates the need for an intraspecies uncertainty factor of 3, as used in setting an RfD. This is further described in Section 8.

³⁵ It should also be noted, as discussed in Section 8, that the use of PBPK models in this circumstance reduces the need for an intraspecies pharmacokinetic uncertainty. Otherwise, the proposed criteria would overestimate risk. Section 8 also presents other examples of the overestimation of risk in the drinking water criteria.

³⁶ Also, as discussed in more detail in Sections 6, 7, and 8, the developmental endpoints in animals chosen by US EPA for setting the RfDs for PFOA and PFOS and employed by MDH in setting the HBVs are not robust and not corroborated by epidemiological evidence.

³⁷ Eicosanoids are biologically active lipids derived from arachidonic acid and other polyunsaturated fatty acids that promote inflammation as part of the immune system's response to infection and injury (Dennis and Norris, 2015).

³⁸ Peroxisomes are membrane-bound cellular organelles that contain enzymes involved in lipid metabolism and in the generation and destruction of hydrogen peroxide (Keller *et al.*, 2000).

(2003), peroxisome proliferation in humans occurs at much higher doses of fibrates and is less robust than in rats and mice. The higher doses required to elicit peroxisome proliferation in humans could be related to PPAR α expression levels, which are about 10-fold lower than those in mice (Palmer *et al.*, 1998). Using cells transfected with chimeric proteins containing either the mouse or human PPAR α ligand-binding domain,³⁹ Bility *et al.* (2004) found that activation of human PPAR α generally required higher concentrations of phthalate ligands and elicited weaker responses than mouse PPAR α . Transgenic mice, in which the mouse gene for PPAR α is either deleted (referred to as either PPAR α -knockout or PPAR α -null mice)⁴⁰ or replaced with a gene for the humanized peroxisome proliferator-activated receptor α (hPPAR α) (*i.e.*, hPPAR α mice), are useful for elucidating whether PPAR α mediates a specific effect (*i.e.*, does the effect occur in PPAR α -null mice?), and if so, whether the effect is species specific (*i.e.*, does the effect occur in hPPAR α -mice?). Studies using hPPAR α -transgenic mice have shown that human PPAR α mediates peroxisome proliferation and expression of genes involved in fatty acid metabolism, but not liver enlargement or hepatocyte proliferation (Cheung *et al.*, 2004; Yang *et al.*, 2008).

There are also differences among the four PFCs at issue in their ability to activate PPAR α , with PFOA being the most potent agonist (Vanden Heuvel *et al.*, 2006; Takacs and Abbott, 2007; Wolf *et al.*, 2008). In a study with COS-1 cells transiently transfected with either mouse or human PPAR α , PFBA was approximately 5- to 10-fold less potent than PFOA, PFOS was approximately 15-fold less potent, and PFBS was approximately 50-fold less potent towards mouse PPAR α vs. approximately 10-fold less potent towards human PPAR α (Wolf *et al.*, 2008).

In addition to differences in potency, the evidence that PPAR α mediates critical effects for PFCs other than PFOA is limited. For example, Abbott *et al.* (2009) evaluated the involvement of PPAR α in PFOS-associated developmental effects in mice and concluded that PPAR α does not mediate the developmental effects of PFOS.⁴¹ For PFBA, there is limited evidence that hepatic focal necrosis is mediated by mouse but not human PPAR α , based on the absence of this effect in transgenic mice expressing human PPAR α . However, this finding may have been a result of PFBA liver concentrations in mice expressing human PPAR α being lower than those in wild-type mice (Foreman *et al.*, 2009). Bijland *et al.* (2011) found that whereas PFOS and PFHxS significantly increased expression of PPAR α -responsive genes, PFBS either had no gene expression effect (*e.g.*, for Acox1), or the effect was less pronounced than for PFOS or PFHxS (*e.g.*, Ehhadh). Although PFOS increased expression of PPAR α -responsive genes in the study by Bijland *et al.* (2011), as noted above, PFOS is less potent as a PPAR α activator than PFOA.

Below, I discuss evidence supporting a role for PPAR α in mediating the hepatic, immunological, and developmental effects of PFOA as well as evidence that these effects are less likely to occur in humans. I also discuss implications of PPAR α mediating PFOA's hepatic and developmental effects on the health-protectiveness the MDH drinking water guideline for PFOA, which is based on developmental effects and maternal liver effects.

5.3.1 PPAR α and PFOA Liver Effects

Both non-cancer and cancer liver effects associated with PFOA exposure (discussed in Section 6.1) are likely mediated, at least in part, by PPAR α activation. For example, Rosen *et al.* (2008b) found that, in mice exposed to PFOA in drinking water, 85% of gene expression changes in the liver were PPAR α -dependent. Although some studies have shown that the hepatic effects of PFOA observed in PPAR α -null

³⁹ The ligand-binding domain is the portion of the PPAR α receptor that binds to ligands, such as PFOA, which activate the PPAR α receptor to bind to DNA and elicit changes in gene expression.

⁴⁰ Note that I used the terms PPAR α -knockout and PPAR α -null mice interchangeably in this report, in general deferring to the terminology used in the specific study under discussion.

⁴¹ MDH's and US EPA's RfD for PFOS is based on developmental effects in rats (Luebker *et al.*, 2005a).

mice are less robust than effects in wild-type mice (*e.g.*, Zhao *et al.*, 2010; Albrecht *et al.*, 2013; Nakamura *et al.*, 2009), other studies have observed hepatic effects of comparable magnitude in wild-type and PPAR α -null mice (*e.g.*, Abbott *et al.*, 2007; Yang *et al.*, 2002a; Minata *et al.*, 2010; Filgo *et al.*, 2015; Das *et al.*, 2017). In terms of human relevance, interpretation of results from studies with PPAR α -null mice should consider inherent differences between wild-type and PPAR α -null mice that could influence response to chemical exposure. For example, PPAR α -null mice have been shown to have defective mitochondrial fatty acid metabolism and to accumulate intracellular lipid droplets in their liver when exposed to hypolipidemic agents⁴² (Lee *et al.*, 1995; Aoyama *et al.*, 1998), which could make them susceptible to disruption of fatty acid homeostasis.

In light of the potential differences between wild-type and PPAR α -null mice, studies with hPPAR α mice – which maintain normal fatty acid metabolism – would be more informative regarding potential effects in humans (Yang *et al.*, 2008). Results from several *in vitro* studies provide evidence that humans are likely less susceptible than rodents to PFOA's hepatic effects. For example, studies by Maloney and Waxman (1999) and Wolf *et al.* (2008) found that mouse PPAR α was more sensitive than human PPAR α to activation by PFOA by approximately 2-3-fold. Bjork and Wallace (2009) observed increased expression of the PPAR α target genes Acox1 and acyl coenzyme A thioesterase in PFOA-treated primary rat hepatocytes, but not in human-derived cells treated with PFOA.

Taken together, results from *in vitro* and *in vivo* studies provide evidence that PPAR α is involved in mediating at least some of the liver effects of PFOA, and that humans would be less sensitive than rats and mice to these effects.

5.3.2 PPAR α and PFOA Immunological Effects

As discussed by Yang *et al.* (2002b), a role for PPAR α in mediating the immunological effects of PFOA is suggested by the observation that peroxisome proliferation precedes PFOA-associated splenic and thymic atrophy in mice. In a subsequent study, Yang *et al.* (2002a) evaluated the immunotoxic effects of PFOA in wild-type and PPAR α -null mice. In contrast to PFOA-treated wild-type mice, for which spleen weight and the number of spleen cells was reduced significantly, there were no effects on the spleen in PFOA-treated PPAR α -null mice. Similarly, whereas the response of lymphocytes to specific activators was significantly reduced in cells isolated from PFOA-treated wild-type mice, there was no effect on lymphocytes isolated from PFOA-treated PPAR α -null mice.⁴³ Although thymus weight and the number of thymus cells were significantly reduced by PFOA in both wild-type and PPAR α -null mice, the extent of reduction in the PPAR α -null mice was approximately two-fold less than that in the wild-type mice (*i.e.*, reductions of ~40% *vs.* ~80-85%, in PPAR α -null *vs.* wild-type mice). The findings from this study by Yang *et al.* (2002a) thus indicate that PPAR α mediates the effects of PFOA on splenic atrophy, reductions in splenocytes, and suppression of splenocyte response to T- and B-cell activators, and partially mediates the effects of PFOA on thymic atrophy and reductions in thymocytes.

Using small interference RNA (siRNA) for PPAR α , which reduced PPAR α expression by 32%, Corsini *et al.* (2011) demonstrated that PPAR α mediates PFOA-associated suppression of cytokine release *in vitro* from human promyelocytic cells. In this study, PFOA suppressed cytokine release by approximately 40-50% in cells with fully functioning PPAR α *vs.* by approximately 80% in cells for which PPAR α expression was reduced with the siRNA.

Consistent with the study by Yang *et al.* (2002a), DeWitt *et al.* (2016) found that relative spleen weight and thymus weight were reduced significantly in PFOA-treated wild-type mice but not in PPAR α -

⁴² Hypolipidemic agents reduce levels of lipids, such as cholesterol.

⁴³ Spleen cells were stimulated with the T-cell activator concanavalin A (ConA), and the B-cell activator lipopolysaccharide.

knockout mice. However, DeWitt *et al.* (2016) found that PFOA-associated suppression of the T-cell-dependent antibody response (TDAR) to sheep red blood cells (SRBCs) was not dependent on PPAR α expression.

Collectively, findings from the studies by Yang *et al.* (2002a), Corsini *et al.* (2011), and DeWitt *et al.* (2016) indicate that PPAR α mediates or partially mediates the effects of PFOA on splenic and thymic atrophy and suppression of some, but not all, lymphocyte responses.

5.3.3 PPAR α and PFOA Developmental Effects

Using PPAR α -knockout mice, Abbott *et al.* (2007) demonstrated that PPAR α is involved in various developmental effects of PFOA, including both pre-natal and post-natal mortality, delayed eye-opening, and reduced post-natal pup weight gain. In this study, wild-type and PPAR α -knockout pregnant female mice were treated daily with PFOA during the gestational period. As shown in Table 5.7, developmental effects occurred either at higher PFOA concentrations in PPAR α -knockout female mice, or did not occur at all.

Table 5.7 Comparison of Developmental Effects in Wild-type vs. PPAR α -knockout Mice

Effect ^a	Wild-type Mice	PPAR α -knockout Mice
Pre-natal mortality	Statistically significant increase at ≥ 0.6 mg/kg-day.	Statistically significant increase at ≥ 5 mg/kg-day.
Reduced post-natal survival	Statistically significant increase at 0.6 and 1 mg/kg-day.	No significant effect (up to 3 mg/kg-day).
Delayed eye opening ^b	Trend for a delay at 0.6 mg/kg-day. Statistically significant increase at 1 mg/kg-day.	No significant effect up to 1 mg/kg-day. Initial eye opening delayed by 1 day, but mean for all pups was not significantly different from control at 3 mg/kg-day. ^b
Reduced post-natal body weight gain ^c	Statistically significantly reduced at 1 mg/kg-day.	No significant effect up to 3 mg/kg-day.

Notes:

PPAR α -knockout Mice = Transgenic mice in which the mouse gene for peroxisome proliferator-activated receptor α is deleted. As reported by Abbott *et al.* (2007).

(a) Prenatal mortality was evaluated up to 20 mg/kg-day PFOA, in both wild-type and PPAR α -knockout mice; other effects were evaluated up to 1 mg/kg-day in wild-type mice, and up to 3 mg/kg-day in PPAR α -knockout mice

(b) Whereas eyes were fully opened for some of the pups on post-natal day 13 at PFOA doses of ≤ 1 mg/kg-day, eyes were not fully opened for any of the pups at 3 mg/kg-day. On post-natal day 14, the percentage of pups with fully opened eyes in the 3 mg/kg-day treatment group was comparable to that in the control group.

(c) Although there was no significant effect of PFOA on body weight gain at any single dose, Abbott *et al.* (2007) reported there were dose-related trends for both wild-type and PPAR α -knockout mice.

Findings from the study by Abbott *et al.* (2007) that PPAR α mediates PFOA's effects on perinatal mortality are supported by findings from a study by Albrecht *et al.* (2013), who observed that post-natal survival was reduced in PFOA-treated wild-type mice but not in either PFOA-treated PPAR α -null or hPPAR α mice, provides evidence that some of PFOA's developmental effects may be species-specific. As with the study by Abbott *et al.* (2007), pregnant female mice were treated daily with 3 mg/kg-day PFOA during the gestational period. PFOA had no effect on pup weight gain, delayed eye opening, or mammary gland development in wild-type, PPAR α -null, or hPPAR α mice. Thus, it is not possible to evaluate whether these effects are also mediated by PPAR α . As discussed by Albrecht *et al.* (2013), the reduced post-natal survival in the wild-type mice may be due either to differences in gene regulation between the mouse vs. human PPAR α or to reduced potency of human vs. mouse PPAR α .

Together, the studies by Abbott *et al.* (2007) and Albrecht *et al.* (2013) provide good evidence that the effects of PFOA on perinatal mortality are mediated by PPAR α , and thus would not be expected to occur in humans. The study by Abbott *et al.* (2007) indicates that PPAR α also mediates delayed eye opening and reduced body weight gain.

5.3.4 Implications of a PPAR α Mode of Action for Minnesota's PFOA Drinking Water Guideline

The PFOA drinking water guideline developed by MDH is based on developmental and maternal effects in mice as observed in a study by Lau *et al.* (2006), including delayed ossification in the fetus, accelerated preputial separation in male pups, a trend for decreased pup body weight, and increased maternal liver weight (MDH, 2017b). To the extent that PPAR α is involved in these effects, and the fact that humans are less susceptible to PPAR α -mediated effects than rodents, using the effects observed in the study by Lau *et al.* (2006) as a basis for the Minnesota drinking water guideline for PFOA overestimates risk, because the effects observed by Lau *et al.* (2006) would either not occur in humans or would occur at higher doses.

6 Animal Studies

Although potential health effects in humans can be directly evaluated in epidemiology studies, it is generally not possible or feasible to measure actual exposures or completely control for factors, independent of the exposure in question, that could also influence health outcomes. Results from studies in laboratory animals conducted under controlled exposure conditions are useful for identifying potential health effects that may occur in humans and helping determine whether effects observed in humans are biologically plausible. Results from animal studies can also help identify exposure levels at which health effects are not likely to occur in humans and are used to derive regulatory criteria for chemicals, such as drinking water limits (as I discuss in Section 8 of my report). When interpreting animal studies, it is important to consider that such studies are typically conducted at exposure levels that are much higher than what humans might experience and that there may be important differences in toxicological responses across species. I have incorporated these considerations when interpreting the animal studies discussed in this section.

3M, academic investigators, and government agencies have been evaluating the animal toxicology of PFCs, particularly PFOA and PFOS, for several decades (see for example, 3M *et al.*, 2003; US EPA, 2003b; DeWitt, 2015). Investigations have assessed PFC toxicity in several different species, including monkeys, rats, and mice, over a wide range of treatment durations (*i.e.*, single dose or acute exposures through 2 years of exposure). 3M in particular conducted numerous studies for routine toxicity testing and follow-up study. In this section, I discuss the animal studies of PFOA, PFOS, PFBA, and PFBS specifically, because MDH has set health-based criteria for these compounds.

Authors of animal studies often report NOAELs or LOAELs. US EPA (2011c) defines a NOAEL as "the highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect... some effects may be produced at this level, but they are not considered adverse or precursors of adverse effects." US EPA (2011c) defines a LOAEL as "the lowest dose or exposure level at which a statistically or biologically significant effect is observed." In this analysis, however, I report findings as NOELs and LOELs, because I conclude that many of the PFC effects reported at low doses are not adverse. Exposures are reported as a dose in mg/kg-day, *i.e.*, the amount of PFC (in mg) per the body weight of the animal (in kg) per day (for the study duration). When available, exposures are also presented as the PFC concentration in the animals' serum. As I discussed in Section 5.1.4, serum measurements are important because they are a measure of internal dose and more easily allow for extrapolating the relationship between exposure and effects across different species (including humans).

With respect to the animal studies, the most sensitive and generally reliable endpoints for PFCs are changes in liver weight, serum lipids, and serum thyroid hormone levels. In a number of cases, such findings are not necessarily indicative of adverse effects and may be non-adverse, adaptive effects. For example, increased liver weight following exposure to a chemical may be an adaptive response involving increased enzyme production. Typically, once the chemical is out of the animal's system, the liver returns to normal size (Hall *et al.*, 2012). I note that while some agencies have recently focused on reproductive/developmental and immune endpoints as being of particular relevance to PFOA and PFOS, I do not consider these findings to be conclusive, as discussed in this section.

6.1 PFOA

6.1.1 Key Endpoints

My analysis of PFOA toxicity studies shows that liver effects are the most sensitive reliably established endpoint for rats, mice, and monkeys. Effects on serum lipids and thyroid hormones have also been observed in some studies at doses that are comparable to those associated with liver effects.

While effects on the immune system have been observed in some studies in certain mouse strains at low doses, these studies have important limitations and uncertainties such as inconsistent findings across studies and lack of replication of certain results. Thus, these studies do not provide reliable evidence to support PFOA causing effects on the immune system in humans.

Reproductive and developmental toxicity are among the most widely studied PFOA endpoints in animals. Although US EPA and MDH both based their drinking water guidelines for PFOA on developmental endpoints, it is my opinion that much of the scientific evidence supporting the developmental endpoints is weak, inconsistent, and does not constitute adverse effects (discussed in more detail in this section and in Section 8.2). Moreover, in most studies, developmental and reproductive effects have been observed at higher exposure levels than effects on the liver, serum lipids, or the immune system, and many appear to be mediated by PPAR α .

In general, the liver, serum lipid, thyroid hormone, immunotoxicity, and developmental effects are observed at lower PFOA exposure doses than those associated with other endpoints, are reversible, and are likely not adverse.

Both US EPA and MDH have derived guidance for daily intake of PFOA and for concentrations of PFOA in drinking water. In addition, MDH has guidance for PFOA in soil. The development of this guidance is discussed in Section 8.1. In this section, I have focused on changes in liver weight, serum lipids, thyroid hormone levels, immunotoxicity, and reproductive and developmental endpoints for the following reasons. Liver weight, serum lipids, thyroid hormone levels, and immunotoxicity are the most sensitive, reliable endpoints, and constitute the basis of some of the Minnesota drinking water criteria described in Section 8.1. These endpoints, plus reproduction and development, are discussed at length in the US EPA Health Effects Support Document for the PFOA Lifetime HA for drinking water (US EPA, 2016a). In addition, reproductive and developmental endpoints are the basis of the most recent Minnesota drinking water guidance for PFOA (MDH, 2017b) and the US EPA Lifetime HA for PFOA in drinking water (US EPA, 2016c).

6.1.1.1 Liver Effects

Findings in Monkeys, Rats, and Mice

Both short- and longer-term animal studies have indicated that liver effects are generally the most sensitive endpoint for PFOA toxicity. Interestingly, however, in studies of rats and monkeys that have allowed for a recovery period (*i.e.*, a time without PFOA exposure), changes in liver size and enzyme levels revert to control levels after PFOA exposure has ceased. For example, Perkins *et al.* (2004) exposed male Crl:CD rats to PFOA *via* the diet at levels of 0.06, 0.6, 1.8, or 6 mg/kg-day (1, 10, 30, and 1,000 ppm in food, respectively). Dosing continued for 13 weeks; some animals were sacrificed at the end of dosing, while a subgroup of animals from each dose group was allowed to recover for 8 weeks. Although the investigators observed some decreased body weights during the study, along with liver

effects, including increased hepatic palmitoyl coenzyme A oxidase activity (a measure of peroxisome proliferation), increased liver weights, and hepatocellular hypertrophy (*i.e.*, increase in liver cell size), all effects were reversible, with no significant differences between the control and exposed animals after the 8-week recovery period. This study, along with multiple others, has demonstrated that PFOA is a potent peroxisome proliferator⁴⁴ in rodents (Sohlenius *et al.*, 1992, 1993; Kashiwima *et al.*, 1994; Intrasuksri *et al.*, 1998; Kudo *et al.*, 2000). The toxic effects associated with peroxisome proliferation occur in rats and mice but have limited human relevance, as discussed in more depth in Section 5.3.

In a 28-day rat study, which did not include a recovery period, Liu *et al.* (1996) dosed CD rats orally for 14 days with 0, 0.2, 2, 20, or 40 mg/kg-day PFOA (in the form of ammonium perfluorooctanoate, or APFO, which dissociates to PFOA). Body weight was statistically significantly decreased in the rats that received 20 mg/kg-day PFOA. The 40 mg/kg-day dose group did not have a statistically significantly different body weight from the pair-fed control group. Increases in absolute and relative liver weights were noted at 2 mg/kg-day PFOA and above, and increases in protein yield of hepatic microsomes⁴⁵ were noted at all dose levels. At 2 mg/kg-day PFOA and above, there were also increases in estradiol, hepatic aromatase activity, and hepatic peroxisomal β -oxidation activity. In a similar study by Iwai and Yamashita (2006), Sprague-Dawley rats were dosed orally for 14 days with 0, 0.5, 5, or 50 mg/kg-day PFOA (in the form of APFO). While all the dose groups showed a tendency for increased absolute and relative liver weights, only the 50 mg/kg-day group had statistically significantly higher relative liver weights as compared to non-treated controls. Increased hepatic peroxisomal β -oxidation activity was also seen at 50 mg/kg-day. Another 14-day study in rats by Loveless *et al.* (2006) reported statistically significant increases in relative liver weights and hepatic peroxisomal β -oxidation activity at 1 mg/kg-day PFOA (in the form of APFO).

Butenhoff *et al.* (2002) conducted a study in which cynomolgus monkeys (4-6 per dose group) received oral doses of PFOA at 0, 3, 10, or 30 mg/kg-day (reduced to 20 mg/kg-day after 12 days) for 26 weeks. Monkeys in the 3 and 10 mg/kg-day dose groups had enlarged livers (135% and 138% of control values, respectively) but did not exhibit any other effects (*i.e.*, no liver tissue injury or changes in hormones, lipids, or other clinical chemistry). Two monkeys from each group that were allowed to recover had normal liver weights after the 13-week recovery period. In the high dose group, the monkeys showed signs of toxicity, including weight loss and lower food consumption. Two monkeys in the high dose group were sacrificed due to moribund condition, and the dosing in this group was discontinued before the end of the 26-week period. The remaining monkeys in the high dose group appeared to recover within 3 weeks after the end of dosing. In 2008, Minnesota based its HRL for PFOA on the liver enlargement effects in this study (MDH, 2008a). This is discussed further in Section 8.1.

Butenhoff *et al.* (2012b) conducted a 2-year study in Sprague-Dawley rats in which groups of male and female rats (50 per sex) received diets of 0, 30, or 300 ppm PFOA, equivalent to 0, 1.3, or 14.2 mg/kg-day for males and 0, 1.6, or 16.1 mg/kg-day for females. No changes in liver weights were observed, but the males in the high dose group and all the females exhibited liver cell enlargement, and the males in the high dose group also had microcysts in the liver. The LOEL for this study was identified by the authors as 300 ppm for both males and females based on histological changes in the liver (males), reduced body weight gain (both sexes), and increased Leydig cell adenomas (males).

⁴⁴ A peroxisome proliferator is a chemical that causes peroxisomes to multiply within a cell. A peroxisome is a cellular organelle involved in cellular metabolism (carbohydrate, lipid, and protein) and cell differentiation. Uncontrolled peroxisome proliferation can lead to certain types of tumors of the liver, testes, and pancreas in rats; however, such findings are not relevant to humans.

⁴⁵ Microsomes are fragmented pieces of endoplasmic reticulum, the cellular structure that is a site of synthesis of enzymes and other proteins (Merriam-Webster, Inc., 2016). Increased enzyme synthesis can occur as an adaptive response to a chemical agent, as described previously in this section.

Butenhoff *et al.* (2004b) conducted a two-generation reproductive study of PFOA in Sprague-Dawley rats. Both males and females in the parental generation (F0) received oral doses of APFO at 0, 1, 3, 10, or 30 mg/kg-day for 10 weeks prior to mating. The F0 males were sacrificed after mating. The F0 females were dosed until after weaning. Their offspring, the first generation (F1), were dosed similarly starting at weaning. Absolute and relative liver weights increased at all doses in the F0 males, and the LOEL for liver effects in the F0 rats in this study was 1 mg/kg-day, resulting in liver weights that were 120% of the controls (males only). The females in the F0 generation did not have enlarged livers. The F1 generation males and females had similar liver effects as those in the F0 generation. The second generation (F2, the offspring of F1) showed no treatment-related effects at any dose.

Short-term studies of PFOA in mice have also demonstrated liver effects. Son *et al.* (2008) dosed ICR mice with 0, 0.5, 2.6, 17.6, or 47.2 mg/kg-day PFOA (0, 2,000, 10,000, 50,000, or 250,000 ppb, respectively, in drinking water) for 21 days. The severity of liver effects was dose-dependent, with increased relative liver weights at 0.49 mg/kg-day PFOA and above, increased alanine aminotransferase (ALT) activity at 2.6 mg/kg-day PFOA and above, and increased aspartate aminotransferase (AST) activity at 17.6 mg/kg-day PFOA and above. Decreased body weight gain and enlarged liver cells also occurred at 17.6 mg/kg-day, and both diffuse hepatic damage and liver necrosis were noted in animals from the 47.2 mg/kg-day group. Loveless *et al.* (2006) noted increased absolute and relative liver weights and increased hepatic peroxisomal β -oxidation activity in a 14-day study of PFOA in Crl:CD-1(ICR)BR mice. In contrast to studies with rats and monkeys, the liver effects in mice in the Loveless *et al.* (2006) study were not reversible in the relatively short recovery period. DeWitt *et al.* (2008) treated mice by oral gavage with 30 mg/kg-day PFOA for 10 days, followed by a recovery period of 5 days. Liver weights remained elevated in the recovery group, while other effects, such as decreased body weights and spleen weights, were reversible. Additional studies by DeWitt *et al.* (2008) in C57BL/6J and C57BL/6N mice using lower doses of PFOA also showed reversibility of the spleen effects seen at doses of 3.75 mg/kg-day and above, but not of the liver effects seen at 0.94 mg/kg-day and above. The lack of reversibility of the liver effects in these studies may be due to the relatively short duration of the recovery period (5 days) as compared to studies in rats and monkeys that had recovery periods of 2-3 months.

Overall Lack of Adversity of Liver Effects at Low Doses

In considering the liver effects observed in monkeys and rodents, an important question is whether effects such as liver cell enlargement, increased liver weight, and lipid accumulation are adverse. Specifically, if increased liver weight is reversible and not accompanied by significant necrotic or inflammatory changes, then an increase in liver weight by itself may not be adverse.

In a guidance document regarding hepatocellular hypertrophy (liver cell enlargement), the US EPA Health Effects Division Toxicology Science Advisory Council (US EPA, 2002b) noted that a statistically significant increase in liver size alone is not a reliable indicator of hepatic toxicity. Similarly, Williams and Iatropoulos (2002) described increases in liver size (and liver cell enlargement) as an adaptive effect, noting that hepatocyte viability will not be compromised if liver changes are within the context of maintaining homeostasis. This position is consistent with that of the ATSDR. When evaluating liver effects as an endpoint for establishing health criteria for any chemical, ATSDR notes that effects such as increased liver weight and elevated liver enzymes are likely adaptive changes, which should be categorized as "less serious" if they do not occur in conjunction with "other effects showing a threat to the organism from serious damage to the liver" (Pohl and Chou, 2005).

A 2012 report by the European Society of Toxicologic Pathology also evaluated adverse vs. adaptive changes in the liver. This report concluded that "[h]epatomegaly [*i.e.*, enlarged liver] as a consequence of hepatocellular hypertrophy [*i.e.*, enlarged liver cells] without histologic or clinical pathology [*i.e.*, cell damage or disease] alterations indicative of liver toxicity is considered an adaptive and a nonadverse

reaction" (Hall *et al.*, 2012). The analysis further noted that "liver weight increases up to 150% of control values may be considered nonadverse in the context of safety evaluation for a chemical," and the authors ultimately proposed a framework for assessing the biological significance of liver changes whereby liver weight changes would only be considered adverse when accompanied by histological evidence of structural degeneration (*e.g.*, hepatocyte necrosis, fibrosis, steatotic vascular degeneration) and changes of sufficient magnitude⁴⁶ in markers of liver dysfunction (*e.g.*, elevated serum concentrations of enzymes indicative of liver damage) (Hall *et al.*, 2012). The above characterizations of what constitutes an adverse effect in the liver are consistent with general principals regarding determination of adversity (Lewis *et al.*, 2002; Palazzi *et al.*, 2016).

The conclusions of Hall *et al.* (2012) and others are applicable to the findings in the 13-week rat study by Perkins *et al.* (2004) and the 26-week monkey study published by Butenhoff *et al.* (2002), as well as additional studies described in the sections below for other PFCs, in which increases in liver weight were not associated with a functional deficit and were completely reversible after a sufficient recovery period.

6.1.1.2 Serum Lipid Effects

Serum lipids are comprised of cholesterol, triglycerides, and phospholipids, which collectively serve a variety of important functions in the body. Cholesterol is a component of cell membranes and also a precursor of bile salts (which are important for digestion and absorption of dietary fats) and various hormones. Phospholipids are primarily used as a component of cell membranes. The majority of cholesterol, as well as some triglycerides, are synthesized in the liver, for distribution to other tissues. Triglycerides are mainly used as an energy source (Guyton and Hall, 2000). Whereas modest, transient reductions in serum lipids may not be adverse, persistent reductions of sufficient magnitude could be adverse, given that lipids are essential for various processes in the body. As discussed below, it is not clear that the serum lipid reductions observed in the PFOA animal studies were sufficiently persistent or of sufficient magnitude to be considered adverse.

Short-term (14- to 29-day) studies in Sprague-Dawley rats showed reduced serum cholesterol (total, high-density lipoprotein [HDL] and non-HDL) and triglycerides at oral PFOA doses as low as 0.3 mg/kg-day (Loveless *et al.*, 2006, 2008). In a high-dose study, Sprague-Dawley rats that were administered PFOA at 20 mg/kg-day by oral gavage had decreased serum cholesterol as early as 1 day after dosing (Martin *et al.*, 2007). In a second high-dose study, Sprague-Dawley rats that were fed 300 ppm PFOA in their diets (about 20 mg/kg-day) had increased triglycerides at 1 day after the start of dosing and decreased total cholesterol and triglycerides after 7 or 28 days (Elcombe *et al.*, 2010).

Rosen *et al.* (2007) reported activated genes for fatty acid catabolism, cholesterol and bile acid biosynthesis, and metabolism of lipoproteins, steroids, and glucose in the lungs and livers of CD-1 mouse fetuses when dams were dosed orally with 1-10 mg/kg-day PFOA during gestation. Most of the affected genes were associated with lipid homeostasis. Rebholz *et al.* (2016) also reported increased serum cholesterol and decreased activation of genes associated with steroid metabolism in C57BL/6 and BALB/c mice that were given a high-fat diet with 0.5 mg/kg-day PFOA. The degree of effects was dependent on the mouse strain.

⁴⁶ According to Hall *et al.* (2012) these changes include 2- to 3-fold increases in the liver enzyme ALT and biologically significant changes in other markers, such as cholesterol, triglycerides, and the liver enzymes AST and alkaline phosphatase (ALP).

In an effort to determine the role of PPAR α in lipid response to PFOA, Rosen *et al.* (2008b) studied gene activation in either 129S1/SvImJ wild-type or PPAR α -null⁴⁷ adult mice that were administered PFOA by oral gavage. Fourteen percent of genes that were activated in the wild-type mice were also activated in the PPAR α -null mice, and many of those genes were associated with lipid metabolism.

Nakamura *et al.* (2009) tested serum and liver lipid response to 2-week oral dosing of 0.1 or 0.3 mg/kg-day PFOA in 129/Sv wild-type mice, PPAR α -null mice, and hPPAR α mice. These authors reported that at 0.3 mg/kg-day, the wild-type mice had increased cholesterol and triglycerides in the liver but not in serum compared to controls, and the liver and serum lipid levels in the PPAR α -null and hPPAR α mice were unchanged. Minata *et al.* (2010) also studied plasma lipids in response to 4 weeks of PFOA exposure at higher oral doses (5.4, 10.8, and 21.6 mg/kg-day) in mice. In wild-type 129S4/SvImJ mice, total serum cholesterol decreased at ≥ 10.8 mg/kg-day and triglycerides increased at 5.4 and 10.8 mg/kg-day but not 21.6 mg/kg-day. In PPAR α -null mice, total cholesterol decreased at 5.4 and 10.8 mg/kg-day but increased at 21.6 mg/kg-day, and triglycerides increased at all doses. The authors suggested that liver injury accounted for the anomalous results at the highest dose (Minata *et al.*, 2010).

In a study in cynomolgus monkeys, males were dosed with PFOA (in the form of APFO) *via* oral capsule for 6 months at doses of 3, 10, or 20-30 mg/kg-day (Butenhoff *et al.*, 2002). There were small, transient increases in triglycerides in the 20-30 mg/kg-day dose group on days 31, 63, and 90, but no significant changes in cholesterol in any dose group throughout the duration of the study.

6.1.1.3 Thyroid Hormone-related Effects

Thyroid hormones, which include thyroxine (commonly called T4) and triiodothyronine (commonly called T3), are synthesized by the thyroid gland. The thyroid is a small, butterfly-shaped structure located in front of the trachea, just below the Adam's apple (Capen, 2000). The thyroid synthesizes all of the T4 present in the body but only approximately 20% of the T3. The remaining T3 is produced from conversion of T4 to T3 outside the thyroid gland in tissues in which thyroid hormones are utilized (Chopra and Sabatino, 2000). Thyroid hormones are essential for a variety of functions in the body, including metabolism, development and function of the brain, and function of other organs such as the skin, heart, and intestines (Johns Hopkins Medicine, 2015). The majority of circulating thyroid hormones in the blood are bound to proteins and thus are not available to enter cells in tissues in which thyroid hormones are utilized. The small portion of unbound thyroid hormones in the blood, referred to as "free" thyroid hormones (*e.g.*, free T3 and T4) do enter into cells and thus are biologically active (ATA, 2014; Johns Hopkins Medicine, 2015). Thyroid hormone levels are regulated by thyroid-stimulating hormone (TSH), which is produced in the pituitary gland (Carrasco, 2000; Taurog, 2000; ATA, 2014). TSH levels increase in response to reductions in circulating free thyroid hormones and decrease in response to elevated levels of circulating thyroid hormones (ATA, 2014). Both excess production of thyroid hormones (hyperthyroidism) and insufficient production of thyroid hormones (hypothyroidism) can be associated with clinical symptoms such as rapid heartbeat, tremor, increased heat sensitivity, difficulty sleeping, and brittle hair (in the case of hyperthyroidism), and fatigue, increased cold sensitivity, dry skin, elevated blood cholesterol, slowed heart rate, depression, and impaired memory (in the case of hypothyroidism) (Mayo Clinic, 2015, 2017). As such, TSH plays a critical role in maintaining thyroid hormone levels within the appropriate range. Changes in TSH and in free T4 are typically used to determine whether the thyroid is functioning properly. Free T3 levels may also be useful for diagnosing hyperthyroidism but not hypothyroidism (ATA, 2014). Substantial and sustained reductions in thyroid hormone levels, accompanied by increased TSH levels, can lead to enlargement of the thyroid gland,

⁴⁷ A wild-type animal is an animal model that is not genetically altered with respect to the gene of interest. In contrast, a "null" or "knock-out" animal has been genetically changed to have a certain gene or set of genes deleted. A PPAR α -null or -knock-out mouse is a mouse without a functional PPAR α gene, and thus will not respond to PPAR α agonists.

which is considered potentially adverse. Small, transient changes in thyroid hormone levels are not necessarily adverse.

The effects of PFOA on thyroid hormones in animals are not well characterized. Only one subchronic monkey study (Butenhoff *et al.*, 2002) and one short-term rat study (Martin *et al.*, 2007) of thyroid hormone effects were identified, and effects were only observed at high PFOA doses (20 mg/kg-day) in both studies.

In the monkey study by Butenhoff *et al.* (2002), male monkeys were dosed with PFOA (in the form of APFO) *via* oral capsule for 6 months at doses of 3, 10, or 20-30 mg/kg-day. In the highest dose group (20-30 mg/kg-day), serum concentrations of T3 and free T3 were lower but still within normal variation, while total T4, free T4, and TSH were unchanged. Serum thyroid hormone levels recovered after treatment stopped. Because T4 levels are expected to decrease before T3 levels (ATA, 2014), the decrease in T3 levels without a corresponding decrease in T4 levels is difficult to interpret. There were no changes to the thyroid gland. The authors attributed changes in thyroid hormone levels to normal variation or stress, not treatment with PFOA.

Martin *et al.* (2007) gave male Sprague-Dawley rats PFOA at 20 mg/kg-day by oral gavage for 1-5 days and reported that total T4 and free T4 were reduced by about 75% at all time points. Serum T3 was reduced by 25% after 1 day and continued to decrease over the later time points.

A committee convened by the US National Research Council (NRC) considered whether changes in serum thyroid hormone and TSH concentrations are adverse effects (NRC, 2005). The NRC Committee, which included three endocrinologists and three pediatricians, as well as veterinarians, toxicologists, and epidemiologists, concluded that decreases in thyroid hormones and increases in TSH are not adverse in and of themselves. Rather, the Committee concluded that hypothyroidism, in which the thyroid is enlarged subsequent to a sufficient decrease in T3 and T4, and an increase in TSH were the first biologically adverse effects associated with perchlorate exposure. Furthermore, there is no evidence in animal studies of thyroid effects at PFOA doses that have been associated with other, more-sensitive effects, such as developmental effects or changes in serum lipids.

6.1.1.4 Immunotoxicity Effects

A number of studies have been conducted in *in vitro* systems and in whole animals to test the potential for PFOA to affect immune function. These studies have been conducted using an array of different types of tests of immune function. Immune effects may be categorized as primary or secondary. Primary outcomes are effects on functional aspects of immunity, such as immunosuppression, hypersensitivity, or autoimmunity. Secondary outcomes include more indirect evidence that is less indicative of overall immunotoxicity, such as lymphocyte counts, serum antibody levels, and weights of organs associated with immune function (NTP, 2016). Many of the results presented here are measures of secondary outcomes and do not necessarily indicate that immune function has been compromised.

It is my opinion that the scientific evidence discussed here does not provide convincing evidence that PFOA would be immunotoxic in humans. I note that there have been some findings in mouse studies that are suggestive of immunotoxicity. However, there are important limitations and uncertainties in these studies (*e.g.*, limited evidence for a dose-response effect in the absence of systemic toxicity), as discussed in this section. These uncertainties and limitations preclude drawing reliable conclusions from these studies' findings.

Evaluating Immune System Effects

Immunotoxicity adversely affects an organism's immune system. Immunotoxicity effects can manifest as immunosuppression (a reduced immune response), hypersensitivity (enhanced immune response), or autoimmunity (in which an individual's immune system reacts against their own cells and tissues) and can occur without clinical illness (Kaplan *et al.*, 2013; Burleson and Dean, 1995). The cells responsible for immunity are primarily found in the blood, spleen, and lymph nodes. The tests used to evaluate toxic effects on these cells primarily evaluate the function of a subset of immune cells called lymphocytes, which include natural killer (NK) cells, T cells, and B cells.

When considering findings from tests of immune function, it is important to remember that the normal immune response is dynamic and complex. At any one time, an individual's immune response may be suppressed or activated due a number of environmental factors, including stress, diet, medications, or infection (Karol, 1998; Kaplan *et al.*, 2013). This normal intra-individual variability is observed within all organisms and can complicate the interpretation of variable responses in association with environmental exposures. Thus, alterations in immune function tests do not necessarily indicate that an individual's immune system would be compromised from exposure to the chemical of interest. Such a determination must be made in the context of a number of factors, including the host factors noted above, as well as the strengths and limitations of the particular test and the magnitude and duration of chemical exposure (Karol, 1998).

Below, I provide descriptions of the specific tests that have been used to evaluate the potential immunotoxicity of PFOA, before discussing the results of studies specific to the chemical. The array of different tests used to study PFOA provide information on different aspects of immune function.

The NK activity assay is immunologically simple and evaluates the function of NK cells, which are important for immediate immune responses that can occur without prior exposures to a pathogen (*i.e.*, an innate immune response). A reduction in NK activity may reflect immunosuppression and impaired innate immunity (Djeu, 1995) and is considered a primary outcome. In humans, a reduction in NK activity of sufficient magnitude could impair innate immunity, leading to increased susceptibility to infections by certain intracellular parasites or bacteria and an increased risk of cancer (Janeway *et al.*, 2001a; Kaplan *et al.*, 2013).

Tests of antibody production to an antigen,⁴⁸ such as SRBCs, are immunologically complex, because this response requires a host of immune cells, including specialized T cells, antigen-presenting cells, and B cells (Holsapple, 1995; Kaplan *et al.*, 2013). Antigen-presenting cells activate T cells and share antigen with B cells, with activated T cells being necessary for B cells to make antibodies. TDAR is termed an adaptive (or humoral) immune response and is the basis of effective vaccination. In this assay, known as the SRBC assay and sometimes referred to as the plaque-forming cell assay, rodents are immunized with SRBC and the resulting B-cell-mediated production of antibodies⁴⁹ against SRBCs is evaluated by the *in vitro* lysis or destruction of SRBCs and the formation of "plaques" (Holsapple, 1995; Karol, 1998; Kaplan *et al.*, 2013). This is considered a primary outcome, reflecting immune response function. In humans, altered adaptive immune responses of sufficient magnitude and over sufficient duration can be reflected in increased susceptibility to infection or reduced response to vaccination.

If an effect on B cell function or survival is suspected, B cell activation can be evaluated *in vitro* independently of other cells using a process termed T-cell-independent antibody response (TIAR) (Snow,

⁴⁸ An antigen is a substance that can induce an immune response, specifically, the production of antibodies.

⁴⁹ Antibodies are also called immunoglobulins (Igs), with IgG and IgM being two types of antibodies produced by B cells. Antibodies are specific for small molecules and are an important component of the immune response against pathogens and other foreign substances.

1995). In this assay, mice are injected with trinitrophenyl (TNP), which activates B cells independently of T cells or antigen-presenting cells. The TNP-reactive B cells are then highly enriched and restimulated *in vitro* to assess proliferation and function. Unlike the SRBC assay, the TIAR assay does not provide evidence of compromised adaptive immunity in humans or animals but is a useful *in vitro* screening assay to identify potential B cell toxicity and is considered a primary outcome because it measures B cell function. In humans, TIAR occurs in response to bacterial infection. Thus, similar to NK deficiency, B cell toxicity could increase susceptibility to bacterial infections (Janeway *et al.*, 2001b).

Effects of PFOA on the Immune System

Several mouse studies show that mice appear to be more sensitive than rats to immune effects from PFOA. However, in most studies, the doses that elicited responses in the mice were also associated with systemic toxicity and were higher than any human exposures that would be expected from environmental exposure to PFOA. Such findings would be of limited relevance to humans potentially exposed to PFOA. There is little information on immune effects from PFOA in monkeys.

PFOA's effects on the immune system have not been observed in rats. Iwai and Yamashita (2006) dosed Sprague-Dawley rats orally with PFOA up to 50 mg/kg-day for 14 days and found no changes in the numbers of circulating T cells, B cells, NK cells, helper T cells, or killer T cells. Loveless *et al.* (2008) reported no immune system effects in CD rats dosed orally with PFOA at up to 30 mg/kg-day for 29 days, even though systemic toxicity (reduced body weight) was evident in the 10 and 30 mg/kg-day dose groups.

Yang *et al.* (2001, 2002b) reported a decrease in the humoral and T-cell-dependent immune response of C57BL/6 mice that were fed PFOA at approximately 40 mg/kg-day for 10 days. The authors administered only a single dose level of PFOA, and therefore, a dose-response cannot be evaluated. In addition, this dose was associated with about 10% loss of body weight in mice, indicating possible systemic toxicity.

Vetvicka and Vetvickova (2013) reported reduced antibody production and cellular immune response (phagocytosis and NK cell activity) in BALB/C mice given PFOA at 20 mg/kg-day by oral gavage for 7 days. The authors also observed reduced thymus cellularity in the treated mice. Because only a single dose of PFOA was tested, a dose-response cannot be evaluated. In addition, the treated mice had reduced body weights by the fourth day of treatment, indicating systemic toxicity at this dose.

DeWitt *et al.* (2008) reported reduced antibody immunoglobulin M (IgM), but not immunoglobulin G (IgG), production in C57BL/6N mice given PFOA in drinking water for 15 days compared to controls. The LOEL for suppressed IgM antibody production was identified by the authors as 3.75 mg/kg-day, and the NOEL was identified as 1.88 mg/kg-day. While there was a dose-related effect on antibody production, this was a secondary outcome (*i.e.*, indirect evidence of immunotoxicity) and no functional deficits were evident, as measured by the SRBC assay. Furthermore, as noted by the authors, the experimental serum PFOA concentration associated with significant antibody reduction was 74,000 ng/mL, which the authors identified as being 15,000 times the serum concentrations of the general population and 150 times higher than concentrations in individuals living near a PFOA production site.

In a second study by the same group (DeWitt *et al.*, 2009), in which PFOA was administered to the same C57BL/6N strain of mice for 10 days *via* drinking water, the authors identified a LOEL for IgM antibody production of 15 mg/kg-day and a NOEL of 7.5 mg/kg-day. IgG levels were not reported. Antibody production is a secondary outcome and does not necessarily indicate a functional deficit. The dose associated with reduced antibody production was also associated with a 10% reduction in body weight, indicating possible systemic toxicity. This study is inconsistent with the LOEL value reported in the

DeWitt *et al.* (2008) study, and the authors did not discuss this discrepancy. Because changes in IgM represent a secondary outcome, the clinical significance of reduced IgM is not established.

In a third study with C57BL/6-Tac mice, DeWitt *et al.* (2016) administered PFOA *via* drinking water at 0, 7.5, or 30 mg/kg-day for 15 days. The authors again reported a NOEL for TDAR of 7.5 mg/kg-day and a LOEL of 30 mg/kg-day. Again, this is a secondary outcome and its clinical significance is not established. At the LOEL, body weights were reduced by 15-20%, indicating systemic toxicity. Spleen weights were reduced at 30 mg/kg-day, and thymus weights were reduced at 7.5 but not at 30 mg/kg-day. In a separate experiment using C57BL/6N mice, TIAR was reduced in mice given ≥ 1.88 mg/kg-day *via* drinking water; the NOEL for this response was 0.94 mg/kg-day. Reduced TIAR is a primary outcome (*i.e.* may indicate a functional deficit). However, the reduction was roughly equivalent at all doses above the NOEL. The TIAR at 1.88, 3.75, and 7.5 mg/kg-day was reduced by 10.3, 9.4, and 10.7%, respectively, indicating a lack of a dose-response.

Hu *et al.* (2010) investigated the developmental effects of PFOA on the immune system in female C57BL/6 mice. This group observed no effects on SRBC-specific antibody response in female C57BL/6 offspring following dam exposure *via* drinking water of up to 1 mg/kg PFOA from gestational days (GDs) 6-17. Litter weights were reduced by 10% in the 1 mg/kg-day dose group.

In CD-1 mice administered PFOA by oral gavage at 0, 0.3, 1, 10, or 30 mg/kg-day, antibody production in response to SRBC (a primary effect) decreased only at 10 and 30 mg/kg-day, doses that were also associated with systemic toxicity (reduced body weight and increased serum corticosterone levels, a sign of stress) (Loveless *et al.*, 2008). Spleen weights (a secondary effect) were reduced in the 1 mg/kg-day dose group. The authors considered the LOEL for this study to be 10 mg/kg-day and the NOEL to be 1 mg/kg-day based on suppression of anti-SRBC IgM antibodies. They did not address the immunological significance of a reduction in relative spleen weight observed at 1 mg/kg-day, which may also be considered an adverse immune effect. However, both of these effects are difficult to interpret because they occurred in conjunction with systemic toxicity.

Little information on the immune effects of PFOA in monkeys is available. In the Butenhoff (2002) study, doses of up to 20-30 mg/kg-day administered to male cynomolgus monkeys for 26 weeks had no effect on spleen weight or spleen tissue damage. In a study by Griffith and Long (1980), monkeys that received PFOA at 30 mg/kg-day by stomach tube had some spleen and lymph node effects (atrophy of lymphoid follicles) and bone marrow effects (reduced cellularity). Neither of these studies tested for functional effects of PFOA on the immune system.

Recently, NTP (2016) conducted a systematic review of the immunotoxicity of PFOA and concluded that PFOA is presumed to be an immune hazard to humans based on a high level of evidence that PFOA suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. The NTP (2016) review has many limitations; specifically, most of the animal studies reviewed in the evaluation that reported PFOA dose-dependent immune effects also reported systemic toxicity at the same doses (see, for example, DeWitt *et al.*, 2009, 2016). Thus, while the NTP (2016) review is comprehensive in its coverage of the immunotoxicology of PFOA, much of the animal evidence it relied on does not provide reliable support for its conclusions. These limitations are also discussed in Gradient's comments submitted to NTP in response to NTP's draft systematic review of PFOA and PFOS immunotoxicity (Gradient, 2016).

Overall, the studies that show an association between PFOA and reduced antibody production in mice provide very limited evidence for a dose-response effect in the absence of systemic toxicity. I have not found any scientific evidence indicating PFOA immunotoxicity in rats. In addition, as discussed in

Section 5.3 of this report, some of the PFOA-associated immune responses in mice appear to be mediated by PPAR α , thus reducing the relevance of the mouse findings for humans.

6.1.1.5 Developmental and Reproductive Effects

Several studies examining the developmental and reproductive toxicity of PFOA have been conducted in rats and mice. Overall, these studies do not provide reliable evidence that PFOA can cause overt malformations at doses that are not associated with maternal toxicity. In other words, malformations may occur as a consequence of effects on the dams (mothers) and would not occur at doses at which the dams are unaffected.

In a two-generation study, F0 Sprague-Dawley rats were dosed by oral gavage through pregnancy, birth, and lactation with 0, 1, 3, 10, or 30 mg/kg-day PFOA (in the form of APFO) (Butenhoff *et al.*, 2004b). While offspring were not directly dosed with PFOA until after they weaned, they were exposed to PFOA *via* lactation transfer from the mother until weaning. Overall, growth deficits and changes in organ weights (*i.e.*, liver, kidney, and spleen) were observed in F0 and F1 rats upon direct dosing with PFOA. Female rats experienced reduced weight gain and increased kidney weight only at the highest dose. Despite the toxicity observed, PFOA did not affect reproduction (*i.e.*, mating and fertility). Also, at birth, the F1 offspring as a whole did not have any adverse health outcomes, except in the highest dose group. Among these effects were decreased birth weight and reduction in pup viability. Any other adverse effects in the offspring in the lower dose groups only became evident post-weaning, when direct dosing of PFOA commenced. While some measures of reproductive development were affected (delayed vaginal opening in females at 30 mg/kg-day and delayed preputial separation in males at 30 mg/kg-day), there were no effects on mating or fertility, and no dose-related effects were seen in the F2 offspring for any of the parameters examined (Butenhoff *et al.*, 2004b). The authors identified a NOEL of 10 mg/kg-day and a LOEL of 30 mg/kg-day for reproductive effects. The LOEL for systemic effects (changes in organ weights in the F0 and F1 generations) was 1 mg/kg-day.

Lau *et al.* (2006) examined the effects from PFOA on CD-1 mice during pregnancy. Animals were dosed by oral gavage during gestation at 0, 1, 3, 5, 10, 20, or 40 mg/kg-day, with one group of animals sacrificed on GD 18 and the other group allowed to deliver normally. Some skeletal variations (reduced ossifications and enlarged fontanel) occurred in all the dose groups in fetuses of dams sacrificed on GD 18, but these effects did not occur in a normal dose-response pattern, and the authors did not consider them to be toxicologically significant. In mice allowed to deliver normally, dose-dependent growth deficits were seen at 3 mg/kg-day PFOA and above; these resolved by 6.5 weeks of age for females and 13 weeks of age for males. Increases in full litter resorptions and neonatal mortality occurred at 5 mg/kg-day PFOA and above. For the pups that survived, limb defects and delays in eye opening were seen at 5 mg/kg-day PFOA and above. Male offspring in all groups showed accelerated sexual maturation (Lau *et al.*, 2006) in contrast to the Butenhoff *et al.* (2004b) study in rats, which showed delayed sexual maturation. However, the dose-response for sexual maturation in males was irregular, with the largest effect at the lowest dose and the smallest effect at the highest dose. The LOEL for maternal effects in this study is 1 mg/kg-day for increased liver weight. While this study is the basis for both US EPA's Drinking Water Health Advisory (HA) (US EPA, 2016a) and Minnesota's HBV for PFOA (MDH, 2017b), it should be noted that, as discussed in Section 8.1, these agencies' interpretation of the study is not consistent with that of the study authors. The significance of the developmental endpoints in Lau *et al.* (2006) as the basis of the regulatory guidelines for PFOA will be discussed in Section 8.1.

White *et al.* (2007) dosed CD-1 mice by oral gavage with 5 mg/kg PFOA on GDs 1-17, 8-17, or 12-17. In the dams dosed with PFOA on GDs 1-17 or 8-17, the authors observed a reduction in mammary gland development. This condition could interfere with a dam's ability to nurse her pups, although this effect was not tested by White *et al.* (2007). Offspring of dams dosed during these periods had reduced body

weights on PNDs 1, 5, 10, and 20. On PNDs 10 and 20, female offspring of dams in all the treatment groups showed stunted mammary epithelial branching and growth. The LOEL for this study was 5 mg/kg-day (the only dose tested) for both dams and offspring. Wolf *et al.* (2007) conducted a cross-fostering study using 3 or 5 mg/kg-day PFOA. Whether pups were exposed *in utero* only, during lactation only, or both *in utero* and during lactation, the pups (and dams) in both dose groups experienced increased relative liver weights. Birth weight and pup survival were reduced in the 5 mg/kg-day group. The LOEL for reproductive and developmental effects was 5 mg/kg-day.

Several subsequent studies also addressed mammary gland development in mice, with reported LOELs ranging from 0.01-3 mg/kg-day, depending on the mouse strain (White *et al.*, 2009, 2011a; Macon *et al.*, 2011; Albrecht *et al.*, 2013; Tucker *et al.*, 2015). The delays in mammary gland development did not correlate with any functional deficit, however, as measured by body weight gain of pups nursing from dams that exhibited mammary gland effects (Macon *et al.*, 2011; White *et al.*, 2011a). US EPA noted that it was not possible to assess the quality of many of these studies, including those with the lowest reported LOELs (Macon *et al.*, 2011; Tucker *et al.*, 2015), because the authors did not describe the methodology used to score the tissue for histological criteria (US EPA, 2016a). US EPA did not consider mammary gland development as an endpoint in its HA derivation, because it noted that there is uncertainty regarding the functional impact (*i.e.*, adversity) of this endpoint (US EPA, 2016a).

6.1.2 Cancer

The International Agency for Research on Cancer (IARC) (2016a) recently classified PFOA as Group 2B, *i.e.*, possibly carcinogenic to humans. This classification was based on "limited evidence" for the carcinogenicity of PFOA in both humans and animals. It should be emphasized that IARC did not identify PFOA as a known human carcinogen. The finding for animals was based primarily on evidence of testicular Leydig, liver, and pancreatic cell adenomas in male rats. Overall, the carcinogenicity studies in animals, described in this section, do not provide reliable evidence that PFOA is carcinogenic to humans.

In a study that was conducted in 1981-1983 and published by Butenhoff *et al.* in 2012, investigators fed Sprague-Dawley rats a diet containing PFOA for 2 years at 30 ppm and 300 ppm (1.3 and 14.2 mg/kg-day for males; 1.6 and 16.1 mg/kg-day for females) in order to investigate the potential carcinogenicity of PFOA (Butenhoff *et al.*, 2012b). Both low-dose and high-dose females were originally recorded as having increases in the number of mammary fibroadenomas, but a reanalysis of pathology slides by a panel of experts (Hardisty *et al.*, 2010; Butenhoff *et al.*, 2012b) resulted in reclassification of the lesions and indicated that the appearance, incidence, and distribution of the neoplasms were not different from control and were not treatment-related. There was an increase in benign Leydig cell tumors (a type of tumor in the testis) in the male rats that received 300 ppm PFOA. Because PFOA is not considered genotoxic, the authors hypothesized that the increase in Leydig cell tumors was a result of hormonal changes brought about by enzyme induction that occurs at high doses of PFOA (Butenhoff *et al.*, 2012b).

Another 2-year feeding study in CD rats (Biegel *et al.*, 2001) confirmed only some of the tumor findings in the Butenhoff *et al.* (2012b) study. This study included only one dose of PFOA – the high dose used in the Butenhoff *et al.* (2012b) study. There was an increase in liver adenomas, but not carcinomas, in the PFOA group. At 24 months, PFOA-treated animals also had increases in Leydig cell hyperplasia and adenomas, as well as increased acinar cell hyperplasia and adenomas. One treated animal had a pancreatic carcinoma. Liver adenomas, Leydig cell tumors, and pancreatic acinar tumors often occur together in rodents and have come to be known as a "tumor triad." These tumors are thought to occur together because they are believed to share a common MoA that may involve PPAR α agonism and thus may have limited human relevance (Biegel *et al.*, 2001; Kennedy and Symons, 2015).

There are important qualitative differences between pancreatic acinar tumors in rodents and human pancreatic tumors. For example, as discussed in Caverly Rae *et al.* (2014), rodent pancreatic acinar tumors are histologically distinct from the most common type of pancreatic tumors observed in humans and, while rodent pancreatic tumors generally have no effect on mortality, humans with pancreatic tumors have very low survival rates.

Liver tumors were also investigated in mice exposed to PFOA in a developmental study by Filgo *et al.* (2015). In this study, three different strains of mice, including one PPAR α -knockout strain plus wild-types CD-1 and 129/Sv, received PFOA *via* oral gavage at doses ranging from 0-5 mg/kg-day during gestation and lactation. The study authors assessed the mice for liver tumors after maturity. There were no consistent and dose-related increases in any type of liver tumor in any of the strains. The authors did not report the incidence of any other tumor type.

In another developmental study, Ngo *et al.* (2014) examined the effect of PFOA (0-3 mg/kg-day on GDs 1-17 by oral gavage) on the numbers of intestinal tumors in the offspring in both wild-type C57BL/6J-Apc^{+/+} mice and a mouse strain (C57BL/6J-Min/+) that is sensitive to spontaneous (*i.e.*, without known causes) development of tumors in the intestines. The authors did not observe any increase in intestinal tumors in either strain and did not report the incidence of any other tumor type.

Overall, the cancer study results do not support a role for PFOA in cancer development in humans. Liver adenomas associated with PFOA in rats are likely mediated by PPAR α and are therefore not relevant to humans. In addition, Leydig cell, and pancreatic acinar adenomas associated with PFOA in rats may be mediated by PPAR α , in which case they would not be relevant to humans.

In addition, agency guidelines by US EPA (2005), NTP (2015), and IARC (2016b) all require findings of malignant tumors or a combination of benign and malignant tumors for sufficient evidence of carcinogenicity. Adenomas are benign tumors and carcinomas are malignant. The findings of benign adenomas only in the Butenhoff *et al.* (2012b) study and only one carcinoma in the Biegel *et al.* (2001) study do not provide strong or sufficient evidence of PFOA carcinogenicity according to US EPA, NTP, and IARC guidance. Other types of cancer in exposed animals have not been observed following PFOA exposure.

6.1.3 Overall Conclusions for PFOA

I conclude that the most sensitive and generally reliable endpoints for PFOA in the animal studies are changes in liver weight, serum lipids, and serum thyroid hormone levels. In a number of cases, such findings are not adverse and may be adaptive. Some reproductive and developmental studies reported effects at doses lower than those affecting other endpoints. It is my opinion, however, that these findings are not reliable, due to either a lack of a dose-response (Lau *et al.*, 2006), or to questions regarding the quality of the studies and the functional impact of the effects (*e.g.*, Macon *et al.*, 2011; Tucker *et al.*, 2015). As discussed in Section 5.3, some of the liver, immune system, and developmental effects observed in rodents as a result of PFOA exposure are mediated, at least in part, by PPAR α , and thus either would not occur in humans or would occur at higher PFOA doses than in rodents.

6.2 PFOS

6.2.1 Key Endpoints

A review of PFOS toxicity studies shows that, whereas effects on the liver and serum lipids are comparably sensitive endpoints for monkeys (*i.e.*, effects on the liver and serum lipids occur at lower PFOS exposure levels than effects on other endpoints), liver effects are the most sensitive endpoint for rats, and effects on the immune system are the most sensitive endpoint for mice. Effects on thyroid hormones have also been observed in monkeys and rats in some studies, at doses comparable to those associated with liver effects.

While effects on the immune system have been observed in some studies in mice at low doses, relative to the monkey studies, these studies have important limitations and uncertainties, such as inconsistent findings across studies and lack of replication of certain results. These studies do not provide reliable evidence of PFOS causing immune system effects in humans.

Developmental and reproductive toxicity are together the most widely studied effects of PFOS in animals, with more than 15 studies evaluating these related endpoints in either rats, mice, or rabbits. Although US EPA and MDH both based their drinking water guidelines for PFOS on developmental endpoints (discussed in Section 8.2), in most studies, developmental and reproductive effects have been observed at higher exposure levels than effects on the liver, serum lipids, or the immune system, and in general are observed only at doses that are associated with maternal toxicity.

Because changes in liver weight, serum lipids, and thyroid hormone levels are sensitive endpoints, and because such changes constitute the basis for some of the Minnesota drinking water criteria described in Section 8.2, I have focused on these endpoints in this section. I have also focused on reproductive and developmental endpoints, because they are the basis of the US EPA Lifetime HA (US EPA, 2016d) and the most recent MDH drinking water guidance (MDH, 2017c).

In general, the liver, serum lipid, thyroid hormone, and developmental effects are observed at lower PFOS exposure doses than those associated with other endpoints, are reversible, and are likely not adverse.⁵⁰

Both US EPA and MDH have derived guidance for daily intake of PFOS and for concentrations in drinking water. In addition, MDH has guidance on PFOS in soil. The development of this guidance is discussed in Section 8.2. In this section, I focus on changes in liver weight, serum lipids, thyroid hormone levels, immunotoxicity, and reproductive and developmental endpoints for the following reasons. Liver weight, serum lipids, thyroid hormone levels, and immunotoxicity are the most sensitive, reliable endpoints and constitute the basis of some of the Minnesota drinking water criteria described in Section 8.2. These endpoints, plus reproduction and development, are discussed at length in the US EPA Health Effects Support Document for the PFOS HA for drinking water (US EPA, 2016b). In addition, reproductive and developmental endpoints are the basis of the most recent Minnesota drinking water guidance (MDH, 2017c) and the US EPA HA (US EPA, 2016d).

⁵⁰ I note that potentially adverse effects on the liver (*e.g.*, cell death) have been observed in rats (but not monkeys) at higher PFOS doses of at least 5 mg/kg-day (for a 28-day exposure period), corresponding with serum concentrations of at least 72 µg/mL.

6.2.1.1 Liver Effects

Effects of PFOS on the liver have been studied in monkeys, rats, and mice. Common findings across species include enlarged cells, lipid accumulation (referred to as vacuolation), and increased liver weight, observed at doses of 0.24 mg/kg-day (in rats) and higher (in rats, monkeys, and mice). These effects appear to be reversible after a recovery period (*e.g.*, 7 months or less in the monkey, which is <10% of their lifespan [University of Wisconsin-Madison, 2014]), and it is my opinion and consistent with criteria for determining whether findings are adverse, as discussed below, that these effects are likely not adverse.

Findings in Monkeys, Rats, and Mice

Covance Laboratories, Inc. (Thomford, 2002a) conducted a 6-month (182-day) oral toxicity study in cynomolgus monkeys. This study was subsequently published by scientists at Covance Laboratories, Inc. and 3M as Seacat *et al.* (2002). Monkeys received PFOS doses of 0.03, 0.15, and 0.75 mg/kg-day by gastric intubation of a capsule once per day. For the 0.15 and 0.75 mg/kg-day dose groups, recovery animals were monitored for 1 year after treatment cessation. Liver effects, including enlarged cells, lipid accumulation, and increased liver weight, were observed in monkeys in the 0.75 mg/kg-day dose group. In the recovery animals, hepatic effects were completely reversed within 211 days (approximately 7 months) post-exposure.

Covance Laboratories, Inc. (Thomford, 2002b) also conducted a 2-year chronic dietary study (subsequently published by scientists at Covance Laboratories, Inc. and 3M as Butenhoff *et al.*, 2012a), in which Sprague-Dawley rats were fed diets with PFOS levels of 0, 0.5, 2, 5, and 20 ppm. These dietary levels correspond to average doses of 0.02, 0.10, 0.24, and 0.98 mg/kg-day in male rats, and 0.03, 0.12, 0.30, and 1.25 mg/kg-day in female rats. An additional group of male and female rats received the 20 ppm PFOS dietary level for 52 weeks, followed by a 52-week recovery period. Liver effects, observed in males and females in the 5 ppm and 20 ppm dose groups, were the main findings of this study. These effects included slight macroscopic changes (*e.g.*, enlarged, mottled liver), enlarged cells, pigmentation changes, and lipid accumulation. ALT, which is a liver enzyme released into the blood as a result of liver injury (A.D.A.M. Medical Encyclopedia, 2017), was slightly elevated and both relative and absolute liver weight (assessed at week 53) were increased in males at 20 ppm PFOS. Although relative liver weight was increased in females at 20 ppm PFOS, the study authors questioned the toxicological significance of this finding, given that body weight was also significantly decreased in females at 20 ppm. Additional liver effects observed in treated males at 2, 5, and 20 ppm (0.10, 0.24, and 0.98 mg/kg-day) include altered foci and cellular degeneration, but the study authors did not consider these effects to be adverse. Liver effects, including enlarged cells, pigmentation changes, and lipid accumulation, were reduced in severity for the recovery group, with incidences comparable to those observed in the control animals. I identified 2 ppm (approximately 0.1 mg/kg-day) as a NOEL for this study, based on the liver effects at 5 and 20 ppm (*i.e.*, at 0.24/0.30 mg/kg-day and 0.98/1.25 mg/kg-day in males/females, respectively).⁵¹ At the dietary LOEL of 5 ppm, average doses, in males and females respectively, were 0.24 ± 0.10 and 0.30 ± 0.10 mg/kg-day.

Seacat *et al.* (2003) published interim results from the 2-year Covance Laboratories, Inc. study in Sprague-Dawley rats (that was subsequently published by Butenhoff *et al.*, 2012a). Following subchronic PFOS exposure *via* the diet over 14 weeks (98 days), enlarged cells and lipid accumulation were observed at 5 and 20 ppm (0.34 ± 0.09 and 1.33 ± 0.38 mg/kg-day) in male rats and at 20 ppm (1.56 ± 0.35 mg/kg-day) in female rats, relative liver weights were increased in both male and female rats at 20 ppm, and absolute liver weight and ALT were increased in male rats at 20 ppm. Because the liver effects in the male rats at 5 ppm PFOS were considered to be marginal and liver weight was not increased, I identified

⁵¹ Thomford (2002b) considered 2 ppm to be a NOAEL.

5 ppm (0.34 ± 0.09 and 0.40 ± 0.08 mg/kg-day in males and females, respectively) as a NOEL for this study.⁵² Average doses at the dietary LOEL of 20 ppm were 1.3 ± 0.4 and 1.6 ± 0.4 mg/kg-day in males and females, respectively. Goldenthal *et al.* (1978a) also conducted a subchronic, 90-day study in rats in which increased liver weight was observed at doses of approximately 2 mg/kg-day (the lowest dose tested) and 7 mg/kg-day *via* the diet; focal necrosis was also observed at the higher dose.⁵³

I identified three studies that evaluated PFOS liver effects in rats following a subchronic exposure over 28 days. Curran *et al.* (2008) observed increased relative liver weight in male Sprague-Dawley rats at dietary PFOS exposures of 1.33 mg/kg-day and in females Sprague-Dawley rats at 0.15 mg/kg-day (the lowest dose tested). Increased relative liver weight, liver cell enlargement, lipid accumulation, and cell death were observed at 5 mg/kg-day PFOS (the lowest dose tested) given by oral gavage to Sprague-Dawley rats in a study by Cui *et al.* (2009). Elcombe *et al.* (2012a) observed increased relative liver weight and enlarged liver cells in Sprague-Dawley rats exposed to 20 ppm PFOS in the diet (1.3 mg/kg-day). Although proliferation of liver cells was increased following 7 days of PFOS exposure, it was not significantly increased following 28 days of exposure. In addition to the effects observed at 20 ppm in the diet, absolute liver weight and liver cell proliferation were increased in rats exposed to 100 ppm PFOS in the diet for 28 days (5.6 mg/kg-day).

I identified two studies that evaluated liver effects following short exposures to PFOS of 3-7 days. Elcombe *et al.* (2012b) observed increased relative liver weight, enlarged liver cells, and increased proliferation of liver cells in Sprague-Dawley rats following 7 days of exposure to 20 ppm PFOS in the diet (1.9 mg/kg-day). In addition to the effects observed at 20 ppm PFOS in the diet, absolute liver weight was increased in rats exposed to 100 ppm PFOS in the diet (10.1 mg/kg-day). With the exception of the enlarged liver cells, the liver effects were reversed within 28 days following cessation of exposure; liver cells remained enlarged through at least 12 weeks after cessation of exposure. Liver cell enlargement was also observed in a study by Martin *et al.* (2007), following 1, 3, and 5 days of exposure to 10 mg/kg-day PFOS by oral gavage.

Liver effects have also been observed in mice exposed to PFOS. Bijland *et al.* (2011) observed increased liver weight and increased liver triglyceride levels in E3L.CETP mice that were fed PFOS at 3 mg/kg-day for 28 days. Studies by Cohen *et al.* (2006) and Rebholz *et al.* (2016) in mice that were fed high-fat diets that included approximately 0.5-3 mg/kg-day PFOS for 5-10 weeks showed increased liver weight, liver triglycerides, fatty acid oxidation in the liver, and increased blood cholesterol. In the study by Rebholz *et al.* (2016), the degree of response was dependent on the strain of mouse tested. Wang *et al.* (2014a) observed increased liver weight and fat content and decreased liver glycogen content in mice that received either a high-fat or a regular diet and PFOS at 5 or 20 mg/kg-day by oral gavage for 14 days compared to controls.

Overall Lack of Adversity of Liver Effects Observed at Low Doses

The discussion presented in Section 6.1.1.1 regarding the nonadverse nature of effects such as liver cell enlargement, increased liver weight, and lipid accumulation following exposure to PFOA is applicable to PFOS as well. Specifically, if increased liver weight is reversible and not accompanied by significant necrotic or inflammatory changes, then an increase in liver weight by itself may not be adverse.

The conclusions of US EPA (2002b), Williams and Iatropoulos (2002), ATSDR (Pohl and Chou, 2005), and Hall *et al.* (2012), as presented in Sections 6.1.1.1, are applicable to the findings for PFOS effects in

⁵² Seacat *et al.* (2003) considered 5 ppm to be a NOAEL.

⁵³ Average doses for males and females were 2.1 and 2.3 mg/kg-day, respectively, based on average food consumption at a dietary PFOS level of 30 ppm. At a dietary PFOS level of 100 ppm, average doses were 6.9 and 7.2 mg/kg-day for males and females, respectively (see Table 4 in Goldenthal *et al.*, 1978a).

the 6-month monkey study published by Seacat *et al.* (2002) and the 2-year rat study published by Butenhoff *et al.* (2012a), in which increases in liver weight were not associated with a functional deficit and were completely reversible after a sufficient recovery period.

Lipid accumulation, if not severe or persistent, is also reversible and not adverse (*i.e.*, it is not associated with necrosis or inflammation) (Gopinath and Mowat, 2014).

6.2.1.2 Serum Lipid Effects

Reductions in serum cholesterol have been observed in monkeys, rats, and mice at PFOS doses ranging from approximately 1-4 mg/kg-day. Reductions in serum triglycerides have been observed in rats and mice at PFOS doses of approximately 3 mg/kg-day or greater. As discussed by Seacat *et al.* (2003), the effect of PFOS on serum lipids may reflect effects occurring in the liver, such as reduced synthesis of cholesterol and metabolism of fatty acids that form triglycerides. In addition, as discussed in this section, it has not been established whether the serum lipid reductions observed in the PFOS animal studies were sufficiently persistent or of sufficient magnitude to be considered adverse. Effects of PFOS on cholesterol and triglycerides are discussed in more detail in the paragraphs that follow.

In the 6-month monkey study by Seacat *et al.* (2002), both total cholesterol and HDL were decreased in monkeys dosed with 0.75 mg/kg-day PFOS. Total cholesterol decreased by almost 70% in males (from a mean of 152 mg/dL in control monkeys to a mean of 48 mg/dL in treated monkeys) and by approximately 50% in females (from a mean of 160 mg/dL in control monkeys to a mean of 82 mg/dL in treated monkeys). HDL decreased by 80% in male monkeys (from a mean of 63 mg/dL in control monkeys to a mean of 13 mg/dL in treated monkeys) and by approximately 60% in female monkeys (from a mean of 56 mg/dL in control monkeys to a mean of 21 mg/dL in treated monkeys). Both total cholesterol and HDL levels returned to pretreatment levels within 36 days after PFOS treatment ended. Because PFOS concentrations in both the liver and serum remained elevated 36 days after treatment, Seacat *et al.* (2002) concluded that PFOS may not be "a major contributor" to reduced cholesterol levels. In males, total cholesterol and HDL were decreased at 0.03 mg/kg-day PFOS but not at 0.15 mg/kg-day. The authors concluded this decrease was not treatment-related, but due to an inherently lower level of cholesterol in the 0.03 mg/kg-day group, because it did not appear to be dose-related (Seacat *et al.*, 2002). While HDL was also decreased at 0.15 mg/kg-day PFOS in female monkeys, the authors considered this effect to be of questionable toxicological significance, because the decrease was modest (*i.e.*, values for two of six monkeys were slightly below the reference range, while mean levels were still within the normal range) and there was no concurrent decrease in total cholesterol, which would be expected to decrease in conjunction with the decreases in HDL (Seacat *et al.*, 2002). Because the effects on cholesterol in the Seacat *et al.* (2002) study at 0.03 mg/kg-day PFOS in male monkeys and at 0.15 mg/kg-day PFOS in female monkeys was of uncertain toxicological significance, I identified 0.75 mg/kg-day as a LOEL for decreased serum cholesterol.⁵⁴ Minnesota chose the cholesterol endpoint, together with alterations in thyroid hormones, in the Seacat *et al.* (2002) study as the basis of the 2009 Minnesota HRL for PFOS (MDH, 2008b). The significance of this choice will be discussed in Section 8.2.

The study by Seacat *et al.* (2002) had some limitations that led to uncertainties in the interpretation of the data. For example, there were no baseline measurements for HDL, nor were any HDL measurements taken until day 153 of the study. In addition, the methods used for thyroid hormone measurement were not optimized for cynomolgus monkeys, and the hormone data were considered unreliable after a re-evaluation of a subset of samples by a clinical reference laboratory (discussed in Section 6.2.1.3).

⁵⁴ Seacat *et al.* (2002) considered 0.75 mg/kg-day to be a LOAEL.

Recently, Chang *et al.* (2017) conducted a similar cynomolgus monkey study in order to address some of the uncertainties and limitations associated with the Seacat *et al.* (2002) study. Chang *et al.* (2017) used doses that were calculated to match the serum PFOS concentrations in the previous study but with a dose administration regimen that was designed to be less stressful to the monkeys. In the Seacat *et al.* (2002) study, monkeys were dosed by gastric intubation of a capsule once per day for 182 days. Chang *et al.* (2017) also administered PFOS by gastric intubation, but each monkey received only one to three doses over the course of 1 year, calculated to achieve serum concentrations that were equivalent to those reported in the Seacat *et al.* (2002) study. The control group (treatment group 1) received a control dose with no PFOS on days 1, 43, 106, 288, and 358. Treatment group 2 received one dose of 9 mg/kg on day 106, calculated to achieve a serum PFOS concentration of 70 µg/mL, comparable to the 0.15 mg/kg-day group in the Seacat *et al.* (2002) study at 6 months. Treatment group 3 received 14 mg/kg (both sexes) on day 43; 14.8, and 17.2 mg/kg for males and females, respectively, on day 288; and 11 mg/kg (both sexes) on day 358, calculated to achieve serum concentrations that were comparable to the 0.75 mg/kg-day dose group in the Seacat *et al.* (2002) study over the course of the year.

Chang *et al.* (2017) found only small treatment-related changes to serum HDL concentrations that the authors did not consider toxicologically or clinically relevant (the authors did not report whether the treatment-related change in HDL was statistically significant). The small effects Chang *et al.* (2017) observed for HDL were reversed after PFOS dosing stopped. The authors calculated the serum PFOS benchmark concentration for a 5% response (BMCL₅), based on the small reduction in HDL, to be 74 and 76 µg/mL for male and female monkeys, respectively. These values are four orders of magnitude higher than the geometric mean PFOS serum concentrations in the US population in 2013-2014 of 0.00499 µg/mL (CDC, 2017a).

In the 2-year rat toxicity study published by Butenhoff *et al.* (2012a), decreases in serum total cholesterol occurred in male Sprague-Dawley rats fed a 20 ppm PFOS diet at weeks 14, 27, and 53, and in female rats fed diets with 2, 5, and 20 ppm at week 27. The maximum decrease in cholesterol, of almost 60%, was observed in male rats in the 20 ppm group at week 53. Although not statistically significant, Butenhoff *et al.* (2012a) noted that total cholesterol also appeared to be lower in the females on the 20 ppm diet at 53 weeks and in both males and females fed the 20 ppm diet at the end of the study, and concluded that the effect on serum cholesterol "likely represents a treatment related effect." Based on these findings, I identified a PFOS dietary level of 20 ppm as the LOEL for serum lipid effects in the Butenhoff *et al.* (2012a) study.⁵⁵ This LOEL corresponds with average daily intakes of 0.98 and 1.3 mg/kg-day PFOS in males and females, respectively.

In rats exposed for shorter durations, the LOEL for decreases in serum cholesterol are generally observed at higher PFOS doses. In the 14-week rat dietary study by Seacat *et al.* (2003), the LOEL for decreased serum cholesterol in males was 1.3 mg/kg-day.⁵⁶ Cholesterol was not decreased in females at the highest exposure level of 1.6 mg/kg-day. In the 28-day rat dietary study by Curran *et al.* (2008) the LOEL was 3.2 and 3.7 mg/kg-day PFOS in males and females, respectively. In the 28-day rat dietary study by Elcombe *et al.* (2012a), serum cholesterol was reduced at an average dose of 1.3 mg/kg-day PFOS, and both cholesterol and serum triglycerides were reduced at an average dose of 5.6 mg/kg-day. In the 7-day rat dietary study by Elcombe *et al.* (2012b), serum cholesterol was reduced at an average dose of 1.9 mg/kg-day PFOS, and both cholesterol and serum triglycerides were reduced at an average dose of 9.7 mg/kg-day. Serum cholesterol was decreased following 5 days of exposure to 10 mg/kg-day PFOS in the rat oral gavage study by Martin *et al.* (2007) (measured following 3 days of PFOS exposure).

Decreased serum lipids have also been observed in mice in response to PFOS exposure. Wang *et al.* (2014a) reported dose-dependent reductions in serum triglycerides, total cholesterol, HDL, and LDL in

⁵⁵ Thomford (2002b) considered 2 ppm to be a NOAEL.

⁵⁶ Seacat *et al.* (2003) considered doses of 0.34 mg/kg-day (in males) and 0.40 mg/kg-day (in females) to be NOAEL doses.

mice fed either a regular or high-fat diet and given PFOS at 5 or 20 mg/kg-day, compared to controls. Cohen *et al.* (2006) observed decreased plasma cholesterol and triglycerides in mice treated for 10 weeks with approximately 3 mg/kg-day PFOS *via* the diet. Bijland *et al.* (2011) observed decreased plasma cholesterol and triglycerides in mice that were genetically engineered to metabolize lipids in a manner comparable to that of humans and treated for 28 days with 3 mg/kg-day PFOS *via* the diet.

6.2.1.3 Thyroid Hormone-related Effects

A discussion of the functional and clinical importance of thyroid hormones and of the toxicological significance of small changes in hormone levels is presented in Section 6.1.1.4.

In subchronic studies, thyroid hormone effects have been observed in monkeys and rats exposed to PFOS doses of approximately 1 mg/kg-day, at which effects on the liver and serum lipids were also observed. Thyroid hormone effects have also been observed in rat dams and their offspring following PFOS exposure during pregnancy at doses of 0.4 mg/kg-day and greater. Effects on thyroid hormones were not accompanied by effects on the size or appearance of the thyroid gland.

Seacat *et al.* (2002) reported changes in thyroid hormone levels in a subchronic study in which PFOS was administered to monkeys *via* gastric intubation and capsule delivery. Thyroid-related hormone concentrations were analyzed by both Ani Lytics and the Mayo Clinic (Thomford, 2002a).

I consider the Ani Lytics TSH values unreliable for several reasons.⁵⁷ Average TSH values reported by Ani Lytics included values of zero (Thomford, 2002a). Given the critical role of TSH in maintaining thyroid hormone levels within the appropriate range, TSH values of zero are not biologically plausible. Moreover, the method used by this group for TSH measurement was not optimized for cynomolgus monkeys, and a crosscheck by the Mayo Clinic of a subset of samples produced different results (Chang *et al.*, 2017). My conclusions regarding TSH effects are thus based on the values reported by the Mayo Clinic, although I use the results from both Ani Lytics and the Mayo Clinic with respect to findings on T3 and T4.

Thyroid effects observed in males dosed with 0.75 mg/kg-day PFOS included increased TSH and decreased total and free T3. There were no changes in either total or free T4 or in thyroid weight and no microscopic histological changes. Total and free T3 were also decreased in females dosed with 0.75 mg/kg-day PFOS, with no significant changes in TSH and either total or free T4. Total, but not free, T3 was decreased in both males and females dosed with 0.15 mg/kg-day PFOS. Seacat *et al.* (2002) considered the increases in TSH at 0.75 mg/kg-day to be "slight," which is consistent with the absence of thyroid gland enlargement or histological changes. Because T4 levels are expected to decrease before T3 levels (ATA, 2014), the decrease in T3 levels without a corresponding decrease in T4 levels is difficult to interpret, although Seacat *et al.* (2002) noted that decreased T3 levels may be associated with non-thyroidal illness. The biological significance of decreased T3 is particularly questionable for animals dosed with 0.15 mg/kg-day PFOS, given that decreases in T3 levels were modest (*e.g.*, levels in treated monkeys were 80 and 75% of values in male and female control monkeys, respectively) and that T3 levels as reported by the Mayo Clinic were not significantly reduced at 0.15 mg/kg-day. It is also important to note that total T3 is not a useful measurement to use for thyroid function, because it includes >99% of protein-bound T3, which is inactive (Chang *et al.*, 2017).

⁵⁷ As reported by Ani Lytics (Thomford, 2002a), TSH was significantly reduced in males dosed with 0.15 and 0.75 mg/kg-day PFOS and in females dosed with 0.75 mg/kg-day PFOS.

Minnesota chose the alterations in thyroid hormones, together with the cholesterol endpoint, in the Seacat *et al.* (2002) study as the basis of the 2008 Minnesota HRL for PFOS (MDH, 2008b). The significance of this choice is discussed in Section 8.2.

The recent monkey study by Chang *et al.* (2017) investigated thyroid hormone levels in order to address the methodology issues in the Seacat *et al.* (2002) study. Chang *et al.* (2017) used a more reliable method to measure thyroid hormones that was optimized for cynomolgus monkeys, which is described in Section 6.2.1.2. In this study, there was a slight reduction in serum total T4, but not free T4, TSH, or T3 levels in monkeys at the highest serum PFOS concentration (165 µg/mL). The total T4 levels were still within normal range, and the authors did not consider the change to be clinically relevant, because 99.9% of total T4 is protein-bound and therefore not metabolically active.

In the 28-day rat study by Curran *et al.* (2008), total T4 decreased in rats dosed with dietary PFOS levels of 20 mg/kg or higher (corresponding to doses of 1.33 and 1.43 mg/kg body weight per day for males and females, respectively). The thyroid gland was not enlarged. Neither TSH or free T4 were measured in the Curran *et al.* (2008) study. Both total and free T4 were reduced after 3 days and total T3 was reduced after 5 days in rats exposed to 10 mg/kg-day PFOS (Martin *et al.*, 2007). In both dams and neonatal rats exposed to PFOS during pregnancy, decreases in total and free T4 were also observed at all treatment doses, starting at 1 mg/kg-day, with no changes in either total T3 or TSH (Lau *et al.*, 2003; Thibodeaux *et al.*, 2003). A study by Chang *et al.* (2007) determined that reductions in free T4 are likely to be an artifact of the analytical method used to quantify free T4, rather than an effect of PFOS. Studies that evaluated free T4 levels using an alternative method did not observe decreased T4. For example, Luebker *et al.* (2005b) observed decreased total T4, but no change in free T4 or TSH, in rat dams exposed during pregnancy to PFOS doses of 0.4 mg/kg-day and greater. As discussed by Chang *et al.* (2008b), PFOS likely reduces total T4 by displacing free T4 on serum protein binding sites, such that free T4 levels can be sustained, or even increased, while total T4 is reduced due to increased turnover of free T4. Importantly, PFOS does not cause hypothyroidism, a condition in which TSH increases and in which the thyroid gland may become enlarged.

In a follow-up to the Curran *et al.* (2008) study, Dong *et al.* (2016) looked at gene expression in rats exposed to 50 mg/kg PFOS in the diet, corresponding to 3.32 mg/kg body weight per day. Changes occurred in the expression of genes involved in thyroid hormone metabolism and clearance, but not in genes involved in transport or thyroid hormone target genes. The authors concluded that PFOS did not cause changes that could lead to hypothyroidism (in which the thyroid gland is enlarged subsequent to a sufficient decrease in T3 and T4 and an increase in TSH).

A committee convened by the NRC to review the health effects of perchlorate, which specifically targets the thyroid, considered whether changes in serum thyroid hormone and TSH concentrations are adverse effects (NRC, 2005). The NRC Committee, which included three endocrinologists and three pediatricians, as well as veterinarians, toxicologists, and epidemiologists, concluded that decreases in thyroid hormones and increases in TSH are not adverse in and of themselves. Rather, the Committee concluded that hypothyroidism was the first biologically adverse effect associated with perchlorate exposure.

There is no indication that the changes in thyroid hormone levels and TSH observed in monkeys or rats exposed to PFOS were of sufficient magnitude to result in hypothyroidism (identified by the NRC Committee as the first biologically adverse effect associated with exposure to an agent that affects thyroid hormone levels) or that hypothyroidism occurred in studies that looked for it. Moreover, with respect to the thyroid hormone effects observed in rats, it is important to keep in mind that rodents are more susceptible to thyroid hormone perturbations than humans (*e.g.*, Lewandowski *et al.*, 2004; CalOEHHHA, 2012; Fisher *et al.*, 2012).

6.2.1.4 Immunotoxicity Effects

A number of studies have been conducted in *in vitro* systems and in whole animals to test the potential for PFOS to affect immune function. These studies have been conducted using an array of different types of tests of immune function. It is my opinion that the studies described do not provide reliable evidence that PFOS would be immunotoxic in humans. Similarly, the Danish Environmental Protection Agency (Danish EPA) (2015b) has also concluded that the animal data are inadequate for demonstrating that PFOS is potentially immunotoxic in humans. I note that there have been some findings in mice that are suggestive of immunotoxicity. However, there are important limitations and uncertainties in these studies (*e.g.*, more potent responses were seen from exposures with a feeding tube than with a physiologically normal dietary exposure). These uncertainties and limitations preclude drawing reliable conclusions from study findings.

A description of immunologic processes and tests of immune function is presented in Section 6.1.1.4. Immune effects may be categorized as primary or secondary. Primary outcomes are effects on functional aspects of immunity, such as immunosuppression, hypersensitivity, or autoimmunity. Secondary outcomes include more indirect evidence that is less indicative of overall immunotoxicity, such as lymphocyte counts, serum antibody levels, and weights of organs associated with immune function (NTP, 2016). Many of the results presented here are measures of secondary outcomes and do not necessarily indicate that immune function has been compromised.

Effects of PFOS on the Immune System

The immunotoxicity of PFOS is not as well-studied as other endpoints. I identified five studies in which immunologic endpoints were evaluated following oral administration of PFOS – four in mice and one in rats. An important finding from studies that have evaluated the immunotoxicity of PFOS is that the doses at which effects are observed appear to depend on how animals are exposed to PFOS, with much lower effect levels observed when PFOS is administered *via* gavage (*i.e.*, *via* a tube inserted into the esophagus) than when PFOS is administered in the diet. Whereas effects (consisting of a reduction in SRBC-specific antibodies) were observed at 0.0017 mg/kg-day PFOS following gavage administration in B6C3F1 mice (Peden-Adams *et al.*, 2008), effects following dietary administration (consisting of increased programmed cell death in the thymus) were observed at doses of 3.2 mg/kg-day and higher (*i.e.*, more than 1,000-fold higher than following gavage administration). Because drinking water ingestion is more analogous to dietary administration than to gavage administration, I consider the effect levels following dietary administration more appropriate than those following gavage administration for evaluating exposure to PFOS in drinking water, surface water, sediments, and fish in Minnesota.

In a study by Keil *et al.* (2008), B6C3F1 mouse dams received PFOS by oral gavage at doses of 0, 0.1, 1, or 5 mg/kg-day on GDs 1-17. There were no significant effects on immunological endpoints in the offspring at 4 weeks of age. At 8 weeks of age, effects included decreased NK cell activity in males dosed with 1 and 5 mg/kg-day PFOS and in females dosed with 5 mg/kg-day PFOS. However, the dose-response for reduced NK cell activity was equivocal. In the male mice, NK cell activity (a primary outcome, indicating a functional deficit) was slightly lower at 1.0 mg/kg-day compared to 5 mg/kg-day; in the female mice, NK cell activity at 0.1 mg/kg-day was comparable to that at 1.0 mg/kg-day. Compared to the positive control, in which NK cell activity was almost completely eliminated after administration of anti-asialo-GM1, decreases in NK cell activity in the PFOS-treated mice were modest (*e.g.*, less than 50%). Antibody production after immunization with SRBCs decreased (a primary outcome), and the numbers of CD3+ and CD4+ thymocytes also decreased (secondary outcomes) in males dosed with 5 mg/kg-day PFOS. Keil *et al.* (2008) note that observing decreased NK cell activity at 8 weeks of age but not 4 weeks of age was "an unusual observation." The authors also note that the

observation of sex-specific effects cannot be explained by differences in PFOS concentrations, but could be due to sex-specific alterations in endocrine function.

In a study by Peden-Adams *et al.* (2008), adult B6C3F1 mice received PFOS by oral gavage for 28 days at doses of 0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, and 0.17 mg/kg-day over the course of 28 days. The observed effects were sex-specific. I identified a LOEL for this study of 0.0017 mg/kg-day, based on decreased SRBC-specific antibody production in males. The mean serum PFOS concentration for animals at this dose level was 0.092 µg/mL (Peden-Adams *et al.*, 2008). Additional effects observed in the male mice included altered T cell subpopulations in the spleen at ≥ 0.0033 mg/kg-day and increased NK cell activity at ≥ 0.017 mg/kg-day. In the female mice, SRBC-specific antibody production was decreased at ≥ 0.017 mg/kg-day and T cell subpopulations in the thymus were altered at ≥ 0.033 mg/kg-day. In an additional group of female mice exposed to 0.31 mg/kg-day PFOS over 21 days, TNP-specific antibody production was decreased. Because antibody production was suppressed for both T-cell-dependent (SRBC) and T-cell-independent (TNP) antigens, the study authors concluded that the B cell is a potential target of PFOS. The increase in NK cell activity in association with PFOS in this study is in contrast to a reduction in NK cell activity observed by Keil *et al.* (2008). However, sex-specific differences in the effects of PFOS treatment were observed in both studies. In the Peden-Adams *et al.* (2008) study, an apparent threshold for effects at a PFOS dose of 0.0033 mg/kg-day in male mice is supported by the reduction in SRBC-specific antibody response (a primary outcome) and altered lymphocyte numbers in the spleen (a secondary outcome). However, a dose-response was not observed; none of these changes increased with increasing PFOS doses. Furthermore, the altered lymphocyte numbers in the spleen that were observed in PFOS-treated male mice were comparable to the values in female control mice, suggesting that the changes are well within the normal range. Considering the absence of a dose-response, the discordance between the male and female mice, the relatively small number of animals per dose (5), and the absence of similar effects in other immunotoxicity studies, results from this study would need to be further validated before the findings can be generally accepted.

Dong *et al.* (2009) administered PFOS to adult male C57BL/6 mice by oral gavage at daily doses of 0, 0.0083, 0.083, 0.42, 0.83, and 2.08 mg/kg-day for 60 days. The LOEL for this study was 0.083 mg/kg-day, based on increased liver weight and reduced SRBC antibody response. Systemic toxicity, manifested by dose-dependent reductions in body weight gain and food consumption beginning around day 36, occurred at ≥ 0.42 mg/kg-day PFOS. Decreases in specific cell populations in the spleen and thymus also occurred at ≥ 0.42 mg/kg-day PFOS. At ≥ 0.88 mg/kg-day, serum corticosterone⁵⁸ levels increased, and both NK cell activity and lymphocyte proliferation decreased. The authors suggested that the immunologic effects may have been secondary to liver effects, because the liver mass in the mice increased at the same doses that immunologic effects occurred. However, they did not discuss a possible mechanism for this hypothesis. The study by Dong *et al.* (2009) does not provide conclusive evidence that PFOS induces immunotoxicity in the absence of hepatic or other systemic effects.

In contrast to the studies by Peden-Adams *et al.* (2008) and Dong *et al.* (2009), in which PFOS was administered *via* gavage, similar effects were not observed in B6C3F1 mice exposed to PFOS *via* the diet. Qazi *et al.* (2010) observed no effect on either SRBC- or TNP-specific antibody production (primary outcomes) and no alterations in either thymic or splenic immune cell populations (secondary outcomes) in mice exposed to 0.20 mg/kg-day PFOS administered in the diet for 28 days (corresponding with a PFOS serum concentration of 11 µg/mL). As discussed by Qazi *et al.* (2010), the difference in effect levels for gavage *vs.* dietary administration cannot be explained either by serum PFOS concentrations (which were slightly higher following dietary administration) or stress (as indicated by cortisone levels, which can impair adaptive immunity but were not elevated following gavage administration). Rather, Qazi *et al.* (2010) hypothesized that the difference in response between dietary *vs.* gavage administration may be

⁵⁸ Corticosterone is a steroidal hormone produced by the adrenal glands that is important in protein and carbohydrate metabolism (Merriam-Webster, Inc., 2016).

explained by differences in pharmacokinetics, with gavage administration yielding higher peak concentrations of serum PFOS.

Lefebvre *et al.* (2008) evaluated the immunological effects of PFOS in rats. In this study, there were no changes in lymphocyte subpopulations or levels of immunoglobulins (Igs) up to the highest dose tested (means of 6.3 and 7.6 mg/kg-day in males and females, respectively, corresponding with mean serum PFOS concentrations of 30 and 43 µg/g, respectively) following 28 days of dietary exposure to PFOS. The only effect observed in the study by Lefebvre *et al.* (2008) was an increase in programmed cell death in the thymus, which occurred at ≥ 3.2 mg/kg-day in males and at 7.6 mg/kg-day (the highest dose tested) in females, corresponding with mean PFOS serum concentrations of ≥ 21 and 43 µg/g, respectively.

In addition to the observation that immunological effects occur at much lower doses following exposure to PFOS *via* gavage (*vs.* dietary exposure), it should also be noted that the toxicological significance of a reduction in plaque-forming cells in response to SRBCs is uncertain. Specifically, it is unclear if decreases in antibody production (assessed *in vitro* – outside the animal) will manifest as an adverse clinical outcome (Luster *et al.*, 1993). Although they are considered primary outcomes, ATSDR (2007) classifies an altered humoral or cell-mediated response to SRBC as "less serious," meaning the effect may not be adverse in the absence of other physiological deficits. Furthermore, the observations by Dong *et al.* (2009) provide evidence that underlying hepatic effects and systemic toxicity cannot be excluded as the causes of immunologic alteration.

Considering the lack of conclusive evidence of immunotoxicity in humans (discussed in Section 7.2.4.5), the human relevance of the immune effects in response to PFOS exposure observed in mice is questionable. In addition, the mouse studies have a number of limitations and uncertainties that make drawing overall conclusions from their results difficult. Depending on the particular study, these include inconsistencies across studies, differences in findings between gavage exposure and dietary exposure, questions as to whether effects are due to direct immunotoxicity or secondary to liver toxicity, and a lack of a dose-response. It is my conclusion that the mouse studies do not provide reliable evidence that PFOS would be immunotoxic in humans. I note that the Danish EPA (2015b) similarly concluded that, considering the lack of evidence for immunotoxic effects in humans, the animal data were inadequate for demonstrating that PFOS is potentially immunotoxic in humans.

Recently, NTP (2016) conducted a systematic review of the immunotoxicity of PFOS and concluded that PFOS is presumed to be an immune hazard to humans based on a high level of evidence from animal studies that PFOS suppressed the antibody response and a moderate level of evidence of the same from studies in humans. The NTP (2016) review has many limitations; specifically, most of the animal studies reviewed in the NTP evaluation that reported PFOS dose-dependent immune effects also reported systemic toxicity at the same doses (see, for example, Dong *et al.*, 2009, 2011; Zheng *et al.*, 2009). The only animal study that reported a PFOS dose-dependent reduction in immune response (Peden-Adams *et al.*, 2008) has not been replicated in publications by this or other research groups. Thus, while the NTP (2016) review is comprehensive in its coverage of the immunotoxicology of PFOS, much of the animal evidence it relied on does not provide reliable support for its conclusions. These limitations are also discussed in Gradient's comments submitted to NTP in response to NTP's draft systematic review of PFOA and PFOS immunotoxicity (Gradient, 2016).

Overall, because the studies that show an association between PFOS and reduced antibody production in mice provide very limited evidence for a dose-response effect in the absence of systemic toxicity, the studies do not provide reliable evidence that PFOS is immunotoxic.

6.2.1.5 Developmental and Reproductive Effects

Multiple studies examining the developmental and reproductive toxicity of PFOS have been conducted with rats, mice, and rabbits. As with the PFOA studies, these studies do not provide evidence that PFOS can cause overt malformations at doses that are not associated with maternal toxicity. Neurobehavioral findings and mortality have also been observed in the offspring of rodents following prenatal exposure. However, these findings generally occur at doses that are higher than those associated with the effects on liver weight, serum lipids, and thyroid hormone levels, as discussed earlier. Thus, criteria that protect against those endpoints would also be protective against developmental and reproductive effects. The bases for these conclusions are described in the following paragraphs.

In general, the studies reviewed reported few teratological findings (*i.e.*, gross abnormalities or abnormalities with regard to soft tissue or skeletal development); when they were reported, these effects generally occurred at relatively high doses in conjunction with maternal toxicity (*e.g.*, Wetzel *et al.*, 1983; Case *et al.*, 2001; Stump, 2008; Yahia *et al.*, 2008). If a teratological effect occurs in conjunction with maternal toxicity, it is difficult to determine whether the developmental effect is due to *in utero* exposure or if it is secondary to maternal toxicity. For example, in a developmental toxicity study by Case *et al.* (2001) in rabbits, fetal body weight was reduced and ossification was delayed at 2.5 and 3.75 mg/kg-day PFOS, while maternal weight gain (attributable to reduced food consumption) was reduced at 1 and 2.5 mg/kg-day PFOS (data regarding maternal effects were not provided for the 3.75 mg/kg-day dose group). There were no gross, soft tissue, or skeletal malformations. Case *et al.* (2001) concluded that PFOS was not a selective developmental toxicant in rabbits. At higher doses, somewhat more severe teratological effects have been observed. In a study by Thibodeaux *et al.* (2003), PFOS was administered orally to Sprague-Dawley rats and CD-1 mice on GDs 2-20 and GDs 1-17, respectively. Fetuses exposed to the highest dose levels for both species (10 mg/kg-day for rats, 20 mg/kg-day for mice) exhibited delayed ossifications with several malformations, including cleft palate and cardiac abnormalities (rats only). Maternal signs of toxicity included decreased maternal weight gain (≥ 2 mg/kg-day for rats, 20 mg/kg-day for mice), increased liver weight (10 mg/kg-day only in mice), and thyroid effects (≥ 1 mg/kg-day for rats, 20 mg/kg-day for mice) as evidenced by reduced T4 and T3 with no change to TSH. The relationship between PFOS and cleft palate was examined in ICR mice that were dosed from GDs 1-17 (Era *et al.*, 2009); cleft palate was correlated with modest increases of PFOS in fetal serum, with increased incidence as maternal doses increased from 13 to 20 mg/kg-day.

Several studies have examined the potential for PFOS exposure during gestation to cause neurobehavioral effects. Neurobehavioral studies are of interest because PFOS can cross both the placental and blood-brain barriers. Studies were identified for both mice and rats. For mice, a study by Johansson *et al.* (2008) showed changes in spontaneous behavior (*e.g.*, hyperactivity and a hypoactive response to nicotine) in 2- and 4-month-old NMRI mice dosed at 10 days of age with a single dose of PFOS by oral gavage at 0.75 or 11.3 mg/kg. In offspring of CD-1 mice treated with 6 mg/kg-day PFOS by oral gavage, Fuentes *et al.* (2007a,b) observed a transient decrease in body weight and delayed neuromotor maturation in pups (Fuentes *et al.*, 2007a) and behavioral effects and decreased corticosterone levels in response to stress in adult female offspring (Fuentes *et al.*, 2007b). In rats, Wang *et al.* (2014b) reported reduced spatial learning and memory abilities in offspring of Wistar rat dams given PFOS at 5 or 15 mg/L in drinking water (equivalent to about 0.5 or 1.5 mg/kg-day). There was no discussion of any maternal effects in these three studies.

Butenhoff *et al.* (2009a) observed increased motor activity in male Sprague-Dawley rats at PND 17 but not at PNDs 13, 21, or 61, following a maternal oral dose of 0.1 mg/kg-day. Maternal body weight was also reduced (relative to control animals) at 1 mg/kg-day. While this may appear to be a relatively low dose, it is not clear whether the increased motor-activity observed by Butenhoff *et al.* (2009a) is actually related to PFOS exposure. Specifically, there are more than 200 statistical comparisons in this study, with

no adjustment or corrections for multiple comparisons. At a significance level of 0.05, one could expect to find about 10 results that are statistically significant by chance alone. Other endpoints evaluated, which were not affected by PFOS treatment, include birth weight, growth, age and weight at sexual maturation, brain weight, learning and memory, acoustic startle response, and other behavioral endpoints. Moreover, the increased motor activity effect was transient, occurring on PND 17 but not PNDs 13, 21, or 61. Regardless of whether this effect was related to PFOS exposure, I identified a NOEL for this study of 0.3 mg/kg-day, based on maternal body weight reductions.

Luebker *et al.* (2005a,b) conducted two developmental studies in rats in which PFOS was administered by oral gavage prior to and during pregnancy and lactation. In the first study (Luebker *et al.*, 2005a), male and female Crl:CD (SD)IGS BR VAF rats were given 0, 0.1, 0.4, 1.6, or 3.2 mg/kg-day PFOS for 6 weeks prior to mating, during mating, and through gestation and lactation, across two generations. In the F2 rats, only 0, 0.1, or 0.4 mg/kg doses were given by oral gavage, due to substantial neonatal toxicity at higher doses. The study authors observed reduced weight gain in the F0 males and in the F2 pups at 0.4 mg/kg-day. The reduced weight gain in the F2 pups was a transient effect noted at days 7 and 14 but not at day 21 post-partum. The authors did not consider this to be a toxicologically significant effect, because it was transient, could have been related to litter size (*i.e.*, larger litters tend to have smaller pups), or could have been a random effect of culling. The F1 generation pups did not exhibit this effect; the NOEL for the F1 generation was 0.4 mg/kg-day (the highest dose tested in the F1 generation). F0 dams dosed with PFOS at 3.2 mg/kg-day had reduced length of gestation, reduced number of implantation sites, and increased numbers of dams with stillborn pups or with all pups dying on lactation days 1-4. Luebker *et al.* (2005a) considered the NOEL for offspring effects to be ≥ 0.4 mg/kg-day. This study is the basis for both US EPA's Drinking Water HA (US EPA, 2016b) and Minnesota's HBV for PFOS (MDH, 2017c). The significance of the developmental endpoints in Luebker *et al.* (2005a) as the basis of regulatory guidelines will be discussed in Section 8.2.

The second study by Luebker *et al.* (2005b) was a one-generation reproduction and development study in rats of the same strain as those used by Luebker *et al.* (2005a). In this study, females only were dosed for 6 weeks prior to mating through day four of lactation with PFOS at 0, 0.4, 0.8, 1.0, 1.2, 1.6, or 2.0 mg/kg-day by oral gavage. In the 0.4 mg/kg-day dose group the F1 pups exhibited lower birth weight and reduced weight gain. This is different from the results of the first study (Luebker *et al.*, 2005a), in which the F1 pups did not exhibit effects at this dose. The authors did not address the differences in results between the two studies. Length of gestation was reduced at 0.8 mg/kg-day PFOS and above, and pup viability was reduced at 1.6 mg/kg-day PFOS and above. The authors identified a LOEL for this study of 0.4 mg/kg-day based on pup weight.

Dose-dependent increases in mortality were observed in Sprague-Dawley rats and CD-1 mice exposed to PFOS during gestation, reaching statistical significance at 2 mg/kg-day for rats and 10 mg/kg-day for mice (Lau *et al.*, 2003). Pup mortality was also observed in the two-generation reproductive toxicity study in rats at doses of 1.6 mg/kg-day PFOS and greater (Luebker *et al.*, 2005a). While pup mortality was observed at lower dose levels in the two-generation reproduction toxicity in comparison with the study conducted by Lau *et al.* (2003), offspring in the two-generation study were exposed to PFOS for a longer duration (*i.e.*, during gestation and lactation) than in the prenatal developmental study (*i.e.*, gestation only).

Increased pup mortality after PFOS exposure during gestation is thought to be related to lung maturation, a key event in development that occurs late in gestation. Specifically, PFOS is thought to interfere with the surfactant needed for the dilation of the alveoli. This hypothesis is supported by evidence that PFOS has been shown to interact with dipalmitoylphosphatidylcholine, a major component of pulmonary surfactant (Grasty *et al.*, 2005). In exploration of this hypothesis, Grasty *et al.* (2003) reported that PFOS induced 100% pup mortality in fetuses exposed for 2 days late in gestation. In a subsequent study by

Grasty *et al.* (2005), the alveolar wall was observed to be thicker and the ratio of tissue to airway space was increased in the rat neonates exposed to PFOS at up to 50 mg/kg-day. The authors suggested that these results showed that lungs were immature and potentially indicted an interference with surfactant. Adverse effects on lung development (*i.e.*, lung atelectasis or collapsed lung) also occurred in mice that were orally dosed with PFOS at 1-20 mg/kg-day from GDs 0-18 (Yahia *et al.*, 2008); these effects corresponded to an increased incidence of mortality. These data are supportive of PFOS's involvement with the inhibition of prenatal lung maturation.

6.2.2 Morbidity and Mortality

In the subchronic toxicity study by Seacat *et al.* (2002), compound-related mortality or morbidity was observed in two (of six) male monkeys in the 0.75 mg/kg-day PFOS dose group (the highest dose tested). The probable cause of death in one of the monkeys was acute, recurring pulmonary inflammation. In the other monkey, which was found in a moribund condition and sacrificed on day 179, the available information indicated that morbidity may have been due to hyperkalemia.⁵⁹ Decreased body weight and reduced estradiol (females only) was also observed in the high dose group. No clinical effects were observed in the recovery animals.

In a 90-day subchronic toxicity study with rhesus monkeys, Goldenthal *et al.* (1978b) initially administered PFOS by gavage at very high doses, ranging from 10-300 mg/kg-day, resulting in 100% mortality within 20 days. Goldenthal *et al.* (1978b) subsequently administered PFOS at lower doses (but still high relative to a clear NOEL) of 0, 0.5, 1.5, and 4.5 mg/kg-day. Monkeys in the 4.5 mg/kg-day dose group either died or were sacrificed *in extremis* (at the point of death) between weeks 5 and 7 of the study with signs of gastrointestinal tract toxicity (*e.g.*, anorexia, emesis, black stool, and dehydration) as well as decreased level of activity, decreased body weight, and decreased serum cholesterol and serum alkaline phosphatase (ALP).⁶⁰ Although the animals were clearly stressed in this study, because of the high PFOS dose at which mortality occurred, it has limited relevance for assessing potential PFOS health effects in humans.

The more-recent monkey study by Chang *et al.* (2017) employed dosing methods that were less stressful to the monkeys than those used in the studies by Seacat *et al.* (2002) and Goldenthal *et al.* (1978b) (*i.e.*, 1-5 doses over the course of one year rather than daily gastric intubation), but still achieved serum concentrations similar to those in Seacat *et al.* (2002). In the Chang *et al.* (2017) study, the monkeys did not exhibit any mortality or overt toxicity. Thus, it is likely that the morbidity and death of the monkeys in the Seacat *et al.* (2002) study were not treatment-related.

6.2.3 Cancer

In a 2-year rat study, Butenhoff *et al.* (2012a) reported a statistically significant increase in liver tumors in Sprague-Dawley rats dosed with 20 ppm PFOS in the diet (means of 0.98 mg/kg-day in males and 1.25 mg/kg-day in females). In males, there was an increase in benign liver tumors, and in females, there was an increase in both benign and combined benign/malignant liver tumors (with a malignant tumor observed in only 1 of 60 rats in the 20 ppm dose group). As discussed by Elcombe *et al.* (2012b), benign liver tumors in rats may be due to PFOS activation of a receptor, the PXR, which stimulates proliferation of liver cells in rats. Because human liver cells are much less responsive to the proliferative effects of PXR,

⁵⁹ Hyperkalemia refers to high levels of potassium in the blood, which may be caused by kidney dysfunction (A.D.A.M. Medical Encyclopedia, 2015a).

⁶⁰ Reduced ALP is associated with a variety of medical conditions, including nutritional deficiency (*e.g.*, of magnesium, vitamin C, zinc, and protein/calories in general), severe or pernicious anemia, and hypothyroidism (*e.g.*, Lum, 1995).

benign liver tumors in humans, if they occur at all, would likely occur at only at much higher PFOS doses than in rats.

Butenhoff *et al.* (2012a) also observed a statistically significant increase in thyroid follicular cell tumors (*i.e.*, arising from follicle cells in the thyroid) in female rats dosed with 5 ppm PFOS (mean of 0.3 mg/kg-day) for 2 years and in male rats dosed with 20 ppm PFOS for 1 year, followed by a 1-year recovery period. Thyroid follicular cell tumors were not increased in male or female rats dosed with 20 ppm PFOS for 2 years. The study authors considered that the increases in thyroid follicular tumors were most likely spurious findings, because they occurred only in the rats treated with PFOS for 1 year and not in the rats treated with PFOS for 2 years. Other tumor findings included a statistically significant increase in mammary gland fibroadenomas⁶¹ in females dosed with 0.5 ppm PFOS and statistically significant decreases in both mammary fibroadenomas and thyroid cell tumors in female rats dosed with 20 ppm PFOS. Given that mammary fibroadenomas (which are benign) are very common in rats (Mann *et al.*, 1996) and that these tumors were increased in females dosed with 0.5 ppm PFOS, but not at higher doses, the evidence indicates that the increase in mammary fibroadenomas may not be PFOS-related.

In a developmental study, Ngo *et al.* (2014) examined the effect of PFOS (0-3 mg/kg-day on GDs 1-17) on the numbers of intestinal tumors in the offspring in both wild-type C57BL/6J-Apc^{+/+} mice and a mouse strain that is sensitive to spontaneous intestinal tumorigenesis (C57BL/6J-Apc^{Min/+}). The authors did not observe any increase in intestinal tumors in either strain at any dose.

Wimsatt *et al.* (2016) also studied intestinal tumors in response to PFOS administration in mice. In this study, the authors investigated whether PFOS had an inhibitory effect on tumor development, based on an observation in a human population of a lower incidence of colorectal cancer among individuals with higher serum PFOS concentrations. Using a mouse strain that is susceptible to intestinal tumors, the authors observed that PFOS at doses of 20-250 mg/kg total, administered *via* drinking water over the course of 8 weeks (equivalent to 0.4-4.5 mg/kg-day) were associated with a dose-dependent reduction in numbers and size of tumors. The authors also reported a loss in body weight in the highest dose group, indicating possible systemic toxicity. No other indicators of toxicity were reported.

While Butenhoff *et al.* (2012a) observed increased benign liver tumors in rats at a PFOS dose of approximately 1 mg/kg-day, there is evidence that rats may be more susceptible to developing liver tumors from PFOS exposure than humans due to the likely involvement of the PXR as discussed above, which is not as active in humans as in rats (Elcombe *et al.*, 2012b). Other tumor findings were likely spurious or of questionable toxicological significance. Because of the potential for important species differences in the hepatic response to PFOS and because the tumors were benign, these findings in the rodents are unlikely to be relevant to humans.

6.2.4 Overall Conclusions for PFOS

I conclude that the most sensitive and generally reliable endpoints for PFOS in the animal studies are changes in liver weight, serum lipids, and serum thyroid hormone levels. In a number of cases, such findings are not adverse and may be adaptive. Developmental effects at lower doses are transient and not adverse.

⁶¹ Fibroadenomas are benign tumors with a large amount of fibrous tissue.

6.3 PFBA

There is relatively little information on the toxicity of other PFCs compared to PFOA and PFOS. Studies of PFBA report some of the same endpoints as PFOA and PFOS but with higher LOELs and NOELs in general. MDH has derived guidance for daily intake of PFBA and for concentrations in drinking water and soil.

6.3.1 Liver Effects

Short-term (5- to 14-day) studies in rats report that PFBA had no effect on liver weight or changes in liver tissue at doses up to 184 mg/kg-day (Ikeda *et al.*, 1985; Charles River Laboratories, 2007). In C57Bl/6 mice, a dose of 78 mg/kg-day PFBA for 10 days caused a 63% increase in absolute liver weight (Permadi *et al.*, 1992, 1993). In two intermediate-duration studies, PFBA was associated with increased liver weights in rats. In a 28-day 3M study conducted by NOTOX B.V. (2007a) and later reported by Butenhoff *et al.* (2012c), Sprague-Dawley rats received doses of 0, 6, 30, or 150 mg/kg-day PFBA. Liver weights increased at ≥ 30 mg/kg-day, and hepatocyte hypertrophy was observed at 150 mg/kg-day. All liver effects were reversible and resolved during a 21-day recovery period. The authors identified a NOEL for this study of 6 mg/kg-day. In a 90-day study, also sponsored by 3M (NOTOX B.V., 2007b; Butenhoff *et al.*, 2012c), Sprague-Dawley rats received 0, 1.2, 6, or 30 mg/kg-day PFBA. Increased liver weight and hepatocyte hypertrophy occurred at the 30 mg/kg-day dose; after a 21-day recovery period, no liver effects were observed. The authors identified a NOEL for this study of 6 mg/kg-day as well. The liver weight changes in this study, along with histological changes in the liver and thyroid gland and alterations in thyroid hormones, were selected by Minnesota as the basis of the PFBA HRL (MDH, 2011a), which is discussed in Section 8.3.

6.3.2 Serum Lipid Effects

In the 28-day rat study of PFBA described above (NOTOX B.V., 2007a; Butenhoff *et al.*, 2012c), male rats receiving 30 or 150 mg/kg-day had reduced serum total cholesterol. No statistically significant changes in serum cholesterol occurred in the 90-day version of this study (NOTOX B.V., 2007b; Butenhoff *et al.*, 2012c), although the mean total cholesterol in the 30 mg/kg-day dose group was 15% lower than in the controls.

6.3.3 Thyroid Hormone-related Effects

In the aforementioned 28- and 90-day studies of PFBA in rats (NOTOX B.V., 2007a,b; Butenhoff *et al.*, 2012c), there was some hyperplasia and hypertrophy of the thyroid gland at doses of 30 mg/kg-day and above. The authors suggested that this occurred in response to an increased turnover of T4 by the hypertrophic hepatocytes (noted in Section 6.3.1). After a 3-week recovery period, the thyroid gland tissue was normal.

Serum concentrations of TSH were unchanged at all doses in the 28-day rat study (NOTOX B.V., 2007a; Butenhoff *et al.*, 2012c). There were dose-dependent reductions in total T4 and free T4 at all doses; however, these differences were not apparent after the 21-day recovery period, except in the highest dose group. The LOEL for thyroid hormones in this study was 6 mg/kg-day. Serum concentrations of TSH were also unchanged at all doses in the 90-day rat study (NOTOX B.V., 2007b; Butenhoff *et al.*, 2012c). Serum total T4 was reduced in the highest dose group only, but returned to control values after the recovery period. Free T4 was not measured in this study. The NOEL for thyroid hormones in the 90-day study was 6 mg/kg-day, and the LOEL was 30 mg/kg-day.

6.3.4 Developmental and Reproductive Effects

I identified only one developmental study and three reproductive studies of PFBA. In a developmental study of PFBA (Das *et al.*, 2008), pregnant CD-1 mice received 0, 35, 175, or 350 mg/kg PFBA on GDs 1-17 by oral gavage. There was no effect on maternal weight gain, number of implantations, fetus viability, fetus weight, or incidence of fetal malformations. Maternal liver weights were increased at ≥ 175 mg/kg-day PFBA and full-litter loss increased at 350 mg/kg-day PFBA. There was no effect on pup survival or growth. There was a transient increase in pup liver weights on PND 1 that disappeared by day 10. Pups in all three dose groups exhibited a delay in eye opening, and pups in the two highest groups had slight delays in puberty onset. I identified a LOEL for this study of 35 mg/kg-day based on delayed eye opening. No effects on any reproductive tissues were observed in Sprague-Dawley rats after administration of PFBA up to 184 mg/kg-day for 5 days (Charles River Laboratories, 2007), 150 mg/kg-day for 28 days (NOTOX B.V., 2007a; Butenhoff *et al.*, 2012c), or 30 mg/kg-day for 90 days (NOTOX B.V., 2007b; Butenhoff *et al.*, 2012c).

6.4 PFBS

I located only four studies of PFBS toxicity. They include 90-day and two-generation studies in Sprague-Dawley rats by Lieder *et al.* (2009a,b), a 28-day Sprague-Dawley rat study by Primedica Redfield (2001), and a recent reproductive and developmental study in ICR mice by Feng *et al.* (2017). Some of the endpoints are the same as endpoints for PFOA and PFOS but with higher LOELs and NOELS in general. Both US EPA and MDH have derived guidance for daily intake of PFOA and for concentrations in drinking water. In addition, US EPA has guidance on PFOA in soil.

6.4.1 Liver Effects

Lieder *et al.* (2009a,b) conducted two studies of the effects of PFBS on rats. In a 90-day study (Lieder *et al.*, 2009a), rats received 0, 60, 200, or 600 mg/kg-day PFBS by oral gavage. There were no changes in absolute or relative liver weights or liver pathology in males or females at any dose. In a two-generation study (Lieder *et al.*, 2009b), F0 male and female rats received PFBS at 0, 30, 100, 300, or 1,000 mg/kg-day for 10 weeks by oral gavage prior to and through mating, and dosing continued for females through gestation and lactation. Upon weaning, the F1 pups were dosed according to the same schedule. The F0 and F1 male rats in the 300 and 1,000 mg/kg-day dose groups had increased liver weights and minimal-to-mild hepatocellular hypertrophy. No liver effects were noted in the F2 pups. The authors identified a NOEL for liver effects of 100 mg/kg-day for the F0 and F1 males.

In a 28-day study by Primedica Redfield (2001), rats received 0, 100, 300, or 900 mg/kg-day PFBS by oral gavage. Some animals in the control and high dose groups were allowed a 14-day recovery period. In males, liver weights (both absolute and relative) increased 25-30% in the 900 mg/kg-day PFBS group, with no changes in body weight compared to controls. The increased liver weights in the treated animals were not accompanied by any gross or histopathological changes and were not observed in the recovery animals, and may thus be considered an adaptive, rather than a toxicological, response. The NOEL for this response was 300 mg/kg-day.

6.4.2 Serum Lipid Effects

In the 90-day study by Lieder *et al.* (2009a), there were no changes in serum total cholesterol or triglycerides in either male or female rats at any dose of PFBS. The NOEL for serum lipid changes in this

study was 600 mg/kg-day. Likewise, there were no effects on serum total cholesterol or triglycerides in either male or female rats given up to 900 mg/kg-day PFBS for 28 days; the NOEL for serum lipid changes in this study was 900 mg/kg-day (Primedica Redfield, 2001).

6.4.3 Kidney Effects

In the 28-day rat study by Primedica Redfield (2001), female rats that received 900 mg/kg-day PFBS had increased kidney weights (9-11%) but no changes in body weight compared to controls. There were no gross or histopathological effects and recovery animals had normal kidney weights. The increased kidney weights may thus be considered an adaptive, rather than a toxicological, response. The NOEL for this response was 300 mg/kg-day.

In the 90-day study by Lieder *et al.* (2009a), there were no changes in kidney weights, but the authors reported minimal-to-mild kidney hyperplasia in rats that received PFBS at 600 mg/kg-day. This dose, however, had no effect on kidney function as measured by clinical chemistry parameters in the serum. I identified a NOEL for kidney effects of 200 mg/kg-day. US EPA chose the kidney changes in this study as a basis for its water and soil guidance for PFBS.

Minimal-to-mild kidney hyperplasia (in the absence of kidney weight changes) also occurred in the F0 and F1 rats in the 300 and 1,000 mg/kg-day dose groups of the two-generation study by Lieder *et al.* (2009b). The authors identified a NOEL for kidney effects of 100 mg/kg-day in this study.

6.4.4 Blood Effects

Lieder *et al.* (2009a) reported a decrease in hemoglobin and hematocrit levels in male rats that received PFBS at 200 or 600 mg/kg-day for 90 days and a decrease in absolute numbers of red blood cells in the male rats that received 600 mg/kg-day PFBS. There were no corresponding changes in bone marrow histopathology. The authors identified a NOEL for this effect of 60 mg/kg-day. Minnesota chose blood effects in the Lieder *et al.* (2009a) study as the basis of the HRL for PFBS (MDH, 2011b), discussed in Section 8.4. There were no hematological changes in rats in the 28-day study by Primedica Redfield (2001) at any dose, up to 900 mg/kg-day PFBS. The NOEL for blood effects in this study was 900 mg/kg-day.

6.4.5 Thyroid Hormone-related Effects

There were no changes in thyroid gland weight or histopathology at any dose in either the 90-day study by Lieder *et al.* (2009a) or the 28-day study by Primedica Redfield (2001). Neither of these studies reported thyroid hormones measurements.

Feng *et al.* (2017) noted thyroid hormone effects in pregnant mice that received 200 or 500 mg/kg-day PFBS by oral gavage. Total T4, free T4, and total T3 were lower in the serum of treated dams on GD 20 than in controls, and free TSH was higher. The authors did not comment on whether the thyroid hormone values in the treated mice were outside the range of normal values and low enough to constitute hypothyroidism. The NOEL value for this study was 50 mg/kg-day.

6.4.6 Developmental and Reproductive Effects

Mice may be more sensitive than rats to developmental effects from PFBS. Feng *et al.* (2017) administered 50, 200, or 500 mg/kg-day PFBS to pregnant mice by oral gavage on GDs 1-20 and studied

developmental effects in female offspring. Effects at ≥ 200 mg/kg-day included decreased body weight and delayed eye opening and vaginal opening in the pups, and delayed first estrus, prolonged diestrus, reduced relative ovarian and uterine weights, and reduced follicle and corpus luteum numbers in adult offspring. In addition, the authors reported changes in sex hormones, decreased total T4 and T3, and a slight increase in TSH and thyrotropin-releasing hormone (TRH) in the serum of adult offspring. The dams also had decreased total T4, total T3, free T4, and increased TSH in serum. The NOEL for this study was 50 mg/kg-day. It is notable that the body weights of the offspring in the 200 and 500 mg/kg-day PFBS dose groups were lower than the body weights of the controls and mice in the 50 mg/kg-day PFBS dose group and remained lower throughout development and adulthood. This may indicate that the endpoints reported by the authors are due to systemic, rather than developmental, toxicity. Toxicity to the dams was not monitored except for body weight, which did not change at the higher PFBS doses, and thyroid hormone levels, which were altered at doses ≥ 200 mg/kg-day (described in Section 6.4.5). Because the effects in the pups occurred at the same dose of PFBS as thyroid effects in the dams, the effects on the pups may have been caused indirectly by toxicity to the dams at the high dose of PFBS. In other words, the pups were not more sensitive than the dams.

In the two-generation rat study by Lieder *et al.* (2009b), there were no effects on reproductive parameters including fertility, sperm production, and estrus cycling, and no histological effects in reproductive tissues in F0 or F1 male and female rats that were dosed with PFBS up to 1,000 mg/kg-day for 10 weeks prior to and through mating. There were also no effects on pup survival, body weight, or development at any dose. The NOEL for developmental and reproductive effects in this study was 1,000 mg/kg-day.

6.5 Overall Conclusions for Animal Toxicity

In general, the most commonly observed endpoints in animals exposed to PFOA, PFOS, PFBA, and PFBS at sufficiently high doses are changes in liver weight, serum lipids, and serum thyroid hormone levels. In addition, some PFBS studies showed blood and kidney effects. For several endpoints, mice appear to be more sensitive to PFCs than rats. In many cases, the findings are not adverse and may be adaptive. In most cases, effects were reversed when the animals were allowed a recovery period. Developmental findings at lower doses were transient and not adverse. In addition, the animal findings occurred at doses that are much higher than those that humans would plausibly experience.

7 Human Studies

7.1 PFOA

The following subsections discuss PFOA exposure levels over time and across populations, and the association between PFOA and numerous health endpoints in humans, including reproductive and developmental toxicity, thyroid hormones, serum lipids and CVD, immunological effects, liver effects, kidney effects, and cancer. Many of these endpoints are commonly assessed, and have been the focus of recent agency reviews by the US EPA and NTP. Human studies are important to consider to fully understand the toxicity associated with PFOA, since these studies require no species extrapolation, and represent a range of environmentally-relevant exposure levels. In general, exposure is substantially higher in occupationally-exposed individuals relative to those living in communities with a nearby point source of PFOA (*e.g.*, a production plant), and the general population with no nearby sources of exposure (who are exposed to PFOA mainly through low levels in food, water, and consumer products). In contrast, most animal studies are conducted at dose levels far higher than would be expected in any human population. These studies can be used to inform the relevance of the animal toxicity studies, and also to determine the likelihood of adverse health effects in populations exposed to PFOA. It is critical to consider that while PFCs have been found in the serum of people around the world, as noted by the Centers for Disease Control and Prevention (CDC), "[f]inding a measureable amount of PFCs in serum does not mean that the levels of PFCs can cause an adverse health effect" (CDC, 2009a).

Overall, these studies demonstrate no consistent health effects or changes in clinical chemistry parameters in workers who have been exposed to high levels of PFOA for long periods of time, in residents with PFOA in their drinking water, or in populations with background exposure to PFOA. In many cases, where health effects were seen, the changes were of unclear clinical significance, or in some cases, were not consistent with animal or mechanistic data for the same endpoints. In some cases, findings in human studies could be explained by "reverse causation,"⁶² and did not represent an effect of PFOA.

7.1.1 Serum Concentrations in Workers

3M has been conducting worker health studies for several decades. As part of these assessments, 3M has monitored workers' PFOA serum concentrations. For PFOA, the primary facilities that have been monitored by 3M include those in the Mid-Ohio Valley; Cottage Grove, Minnesota; Decatur, Alabama; and Antwerp, Belgium. In addition, DuPont has conducted monitoring of its Washington Works facility in West Virginia. Additional serum data is also provided for a well-studied cohort in Italy (Costa *et al.*, 2009). Exposure varies substantially across populations, with the highest exposures in the Italian cohort and the lowest at the Washington Works facility. In general, the serum concentrations of PFOA measured in these working populations are associated with exposure to high concentrations of PFOA, often several orders of magnitude higher than expected in the general population and communities with point sources of PFOA exposure. Below is a summary of the serum concentrations of PFOA over time in these facilities (Table 7.1).

⁶² Reverse causation occurs when the likelihood of an outcome is causally to the exposure of interest. For example, menopause may cause an increased level of PFOA in the blood relative to women still menstruating, because there is no longer a menstrual blood pathway of excretion. Thus, it may appear from a study that increasing PFOA contributes to menopause onset, when in fact, menopause causes an increase in serum PFOA.

Table 7.1 Serum PFOA (ng/mL) in Representative Occupational Populations

Cohort/Population	N	Range	Arithmetic Mean	Geometric Mean	Reference
Male PFOA Production Workers, Italy					
2000	25	1,540-86,300	18,800	11,700	Costa <i>et al.</i> (2009)
2001	42	730-91,900	19,700	10,200	
2002	46	340-91,900	19,300	9,300	
2003	41	380-74,700	13,700	6,900	
2004	34	540-46,300	11,400	6,500	
2006	49	540-41,900	10,800	5,800	
2007	50	200-47,00	11,600	5,400	
2007 (Current Exposure)	39	200-47,040	12,930 (SD: 14,430)	4,020	
2007 (Former Exposure)	11	530-18,660	6,810 (SD: 6,060)	3,760	
3M PFOA Production Workers (Employed between 1985-1989)					
Cottage Grove, MN plant	115	ND-26,000 ^a	NR	3,300 (SD: 4,680)	Gilliland and Mandel (1996)
Male 3M PFOA Production Workers					
1993 (Cottage Grove, MN Plant)	111	ND-80,000	5,000 ^{b,c} (SD: 12,200)	1,100 ^{c,d}	Olsen <i>et al.</i> (1998)
1995 (Cottage Grove, MN Plant)	80	ND-114,100	6,800 ^{b,c} (SD: 16,000)	1,200 ^{c,d}	
1997 (Cottage Grove, MN Plant)	74	100-81,300	6,400 ^b (SD: 14,300)	1,300 ^d	Olsen <i>et al.</i> (2000)
2000 (Cottage Grove, MN Plant)	122	10-92,030	4,630 ^b (SD: 12,530)	950 ^d	Olsen and Zobel (2007)
2000 (Antwerp, Belgium Plant)	196	10-7,040	1,020 ^b (SD: 1,060)	650 ^d	
2000 (Decatur, AL Plant)	188	40-12,700	1,890 ^b (SD: 1,610)	1,510 ^d	
2000 (All Plants Combined)	506	10-92,030	2,210 ^b (SD: 6,400)	1,100 ^d	
Washington Works Facility Workers, West Virginia					
2004 (Current Exposure to PFOA)	259	17.4-9,500	NR	494 ^d	Sakr <i>et al.</i> (2007a)
2004 (Intermittent Current Exposure to PFOA)	160	8.1-2,070	NR	176 ^d	
2004 (Past Exposure to PFOA)	264	8.6-2,590	NR	195 ^d	
2004 (No PFOA Exposure)	342	4.6-963	NR	114 ^d	
2005-2006	1881	NR	325 (SD: 920)	113 ^d	Steenland and Winquist (2015)
C8 Health Project Cohort (2005-2006)					
Worker Cohort Only	3,713	55.9-256.2 ^e	324.6 ^b (SD: 920.6)	112.7 ^d	Winqvist and Steenland (2014a)

Notes:

N = Number of Participants/Samples; ND = Non-detectable; NR = Not Reported; PFOA = Perfluorooctanoic Acid; SD = Standard Deviation.

(a) Measured total fluorine as a surrogate for PFOA, because PFOA was the primary exposure at the plant.

(b) Unclear whether an arithmetic or geometric mean; because geometric means are more common, it was assumed to be a geometric mean (unless a median was also reported, in which case the mean value was assumed to be an arithmetic mean).

(c) Values reported in Olsen *et al.* (2000).

(d) Median values.

(e) 25th-75th percentile.

7.1.2 Occupational Health Studies

7.1.2.1 Reproductive and Developmental Effects

Very few studies are available regarding reproductive and developmental effects in workers exposed to PFOA. The only relevant studies I was able to find were of serum reproductive hormone levels, which may be associated with reproductive function (*i.e.*, the function of the organs, such as sperm production), but cannot be directly linked to reproductive success (*i.e.*, the ability to conceive a child).

In a prospective cohort study, Costa *et al.* (2009) investigated serum estradiol and testosterone levels in male workers at an Italian PFOA production plant with regular surveillance data collected between 2000 and 2007. The authors conducted cross-sectional analyses of PFOA and hormone levels in each year of the analysis period. Mean⁶³ serum PFOA concentrations were 12.93 µg/mL in exposed workers (n = 39) and 6.81 µg/mL in formerly exposed workers (n = 11). Serum PFOA was not associated with hormone levels in this cohort. In a cross-sectional analysis, Olsen (1998) assessed the association between PFOA and numerous serum hormones, including estradiol, follicle-stimulating hormone (FSH), dehydroepiandrosterone sulfate, 17 gamma-hydroxyprogesterone (a testosterone precursor), free testosterone, testosterone, luteinizing hormone (LH), prolactin, and sex-hormone-binding globulin (SHBG) in male workers at the Cottage Grove, Minnesota, plant from 1993-1995. There were no statistically significant associations between PFOA and any of these hormones. Finally, Sakr *et al.* (2007a) measured serum testosterone and estradiol in 1,025 workers at the DuPont Washington Works facility in West Virginia. The authors observed statistically significant cross-sectional associations between PFOA and these two hormones. The authors indicated that it was difficult to interpret the results, however, considering that the blood draws occurred at varying times of the day (which was not always reported), and these hormones are known to fluctuate within and across days.

Overall, based on three studies of serum hormones in male workers, the weight of evidence does not establish an association between occupational exposure to PFOA and reproductive hormones, or more broadly, reproductive and developmental effects.

7.1.2.2 Liver Enzymes and Disease

Liver effects are some of the most studied and sensitive endpoints of PFOA toxicity in animal studies. Consequently, liver toxicity has been the focus of numerous human studies of PFOA. Changes in liver enzymes in response to PFOA exposure have been tracked in several occupational cohorts. Some studies have also assessed the incidence of liver disease in PFOA-exposed workers.

Costa *et al.* (2009) analyzed serum liver enzymes in their cross-sectional assessment of 53 Italian male PFOA production workers. ALT, gamma-glutamyl transferase (GGT), and ALP were significantly increased per µg/PFOA in serum. Total bilirubin was significantly decreased per µg increase in PFOA, although conjugated bilirubin⁶⁴ was not significantly decreased. AST was not significantly altered by exposure to PFOA. The authors concluded that these results did not represent a "significant perturbation" of liver function (Costa *et al.*, 2009).

⁶³ Arithmetic mean. In all cases from here forward, it is assumed that the mean was arithmetic (since it is the most common metric), unless otherwise specified in the publication.

⁶⁴ Total bilirubin (also called direct bilirubin) is a measure of unconjugated bilirubin (which has not been conjugated in the liver), and conjugated bilirubin (the portion that has been processed by the liver; conjugated bilirubin only makes up about 10% of serum bilirubin in normal patients, because after conjugation, it is quickly excreted). Excess total or conjugated bilirubin could indicate that the liver is not properly processing and/or excreting the bilirubin (Murali and Carey, 2014).

In a cross-sectional study, Gilliland and Mandel (1996) evaluated health endpoints in workers at the Cottage Grove plant employed during the study period of 1985 through 1989. Exposure was estimated using total serum fluorine as a surrogate for PFOA.⁶⁵ Health information was collected *via* questionnaire, and blood was collected for measures of clinical chemistry, including measures of liver function (AST, ALT, and GGT). Levels of hepatic enzyme assays did not vary based on level of total serum fluorine and were in a clinically acceptable range. No workers reported hepatic disease, diagnoses or signs, or symptoms consistent with hepatic disorders.

Olsen *et al.* (2000) analyzed liver enzymes (including ALP, GGT, AST, and bilirubin) in a cross-sectional analysis using medical surveillance of 74-111 workers in the Cottage Grove Plant collected between 1993 and 1995. Increasing serum PFOA was associated with lower plasma cholecystokinin (CCK), a hormone associated with bile production in the liver (CCK, measured only in 1997); however, almost all of these measurements were within the assay's reference range. There were no statistically significant associations between increased serum PFOA and other liver enzymes. Overall, the authors concluded that there was no evidence of an association between PFOA and abnormal liver function, or cholestasis (reduced or complete blockage of flow of bile from the liver).

Olsen and Zobel (2007) conducted a cross-sectional analysis 506 workers who used or manufactured PFOA in 3M plants (Antwerp, Cottage Grove, and Decatur plants combined). Serum PFOA and clinical chemistry measures were collected in 2000. Serum PFOA ranged from 0.007-92.03 µg/mL (mean: 2.21 µg/mL). PFOA was positively associated with ALP, GGT, ALT, and total bilirubin only in the Decatur plant, but not for all plants combined or the other individual plants. There were no associations between PFOA and AST in any analysis. In an analysis of the risk of exceeding clinical reference points for ALT and GGT, adjusting for lactation, there were no statistically significant associations.

Steenland *et al.* (2015) conducted a retrospective cohort analysis of disease incidence in workers at the West Virginia Washington Works plant. The authors included all workers who had worked at least 1 day at the DuPont plant between 1948 and 2002 and who had sufficient work and residential history available (N = 3,713). Exposure in workers was estimated using serum PFOA concentrations over time and modeled residential exposures from drinking water data. The mean measured (for those surveyed in 2005 and 2006) and predicted serum PFOA concentrations were 325 ng/mL and 218 ng/mL, respectively. The authors estimated cumulative exposures (ppm-years, which was calculated by multiplying the concentration in ppm by the number of years exposed). Although there was a slight increase in risk of non-hepatitis liver disease lagged 10 years (*i.e.*, assuming 10 years of time between exposure and disease onset) as PFOA exposure increased, this relationship was not statistically significant.

Overall, the available occupational studies, which span decades of PFOA production, indicate that PFOA is not associated with clinically significant changes in liver enzymes or increased risk of liver disease.

7.1.2.3 Serum Lipids and Cardiovascular Disease

Serum Lipids and Cholesterol

I identified seven studies of serum lipids and cholesterol in workers exposed to PFOA (Costa *et al.*, 2009; Olsen *et al.*, 2000; Olsen and Zobel, 2007; Sakr *et al.*, 2007a,b; Winquist and Steenland, 2014a; Steenland *et al.*, 2015).

⁶⁵ The vast majority of exposure was to PFOA, so total serum fluorine should closely reflect total serum PFOA.

Gilliland and Mandel (1996) conducted a cross-sectional evaluation of workers at the Cottage Grove plant between 1985 and 1989. Exposure was estimated using total serum fluorine as a surrogate for PFOA.⁶⁶ Health information was collected *via* questionnaire, and blood was collected for measures of clinical chemistry, including cholesterol, LDL (*i.e.*, "bad" cholesterol), and HDL (*i.e.*, "good" cholesterol). None of these measures of cholesterol were significantly altered in more highly exposed workers compared with those with low exposure (exposure range: <1 ppm to >15,000-36,000 ng/mL). In analyses to determine the impact of different levels of drinking, an interaction between moderate alcohol use and serum fluorine was apparent, suggesting the beneficial effect (increases in HDL) observed between moderate drinking and PFOA was slightly blunted in more highly exposed workers. Similar relationships were not seen for light drinkers.

Olsen *et al.* (2000) analyzed serum lipids collected in health surveillance activities in 1993, 1995, and 1997 in Cottage Grove plant workers. Mean serum PFOA concentrations ranged from 6,000-6,800 ng/mL. The authors analyzed both cross-sectional associations as well as changes among the 63 participants that were sampled in both 1993 and 1995. There were no statistically significant associations between increased serum PFOA and total cholesterol, LDL, or HDL in cross-sectional analyses, nor total cholesterol in the longitudinal analysis. In contrast to the Gilliland and Mandel (1996), there was no interaction between drinking, PFOA and HDL in any year.

Olsen and Zobel (2007) assessed serum lipids collected during medical surveillance conducted in 2000 in a combined cross-sectional study of 506 workers at the Antwerp, Cottage Grove, and Decatur 3M plants. There were no statistically significant associations between PFOA and total cholesterol or LDL (all locations combined and for each plant individually). HDL was statistically significantly negatively associated with PFOA for all three facilities combined, but not when separated by facility. PFOA was also associated with high triglycerides in all facilities combined and with Antwerp individually. When assessed dichotomously (below or above the clinical reference point), the association with low HDL was not statistically significant for any decile of exposure. Similarly, there was no consistent association between PFOA exposure and clinical reference points for high triglycerides.

Sakr *et al.* (2007a) conducted a cross-sectional study of the association between PFOA exposure and serum lipids (and liver enzymes) in 1,025 workers at the Washington Works plant. Blood samples were collected and measured for PFOA, cholesterol, triglycerides, LDL, very low density lipoprotein (VLDL), and HDL. In the analysis that excluded workers on lipid-lowering medications, PFOA was statistically significantly associated with total cholesterol, LDL, and VLDL.

Sakr *et al.* (2007b) conducted a longitudinal study between PFOA exposure and serum lipids in the Washington Works plant. Blood lipid levels and serum PFOA concentrations were collected from employee medical records from the plant medical surveillance program. Workers needed to have at least two sets of measurements to be included in the study. The mean serum PFOA concentration was 1.13 ppm (range: 0-22.66 ppm). PFOA was not statistically significantly associated with any of the blood lipid measurements.

Costa *et al.* (2009), described in the previous two subsections, analyzed triglycerides and cholesterol in male workers at an Italian PFOA production plant between 2000 and 2007. There was a significant difference between total cholesterol levels in exposed vs. non-exposed workers (mean of 237 mg/dL vs. 206 mg/dL) but no differences in HDL or triglycerides. This association remained statistically significant when authors included all workers, present and former, who had concurrent serum PFOA measures.

⁶⁶ The vast majority of exposure was to PFOA, so total serum fluorine should closely reflect total serum PFOA.

Winqvist and Steenland (2014a) conducted a cohort study (with both retrospective and prospective analyses) of coronary artery disease, hypertension, and hypercholesterolemia (diagnosed high cholesterol) in more than 30,000 community residents and workers in the Mid-Ohio Valley (the C8 Health Project Cohort, hereafter referred to as the C8 Cohort).⁶⁷ The authors conducted both prospective (beginning 1 year after age at the time of C8 Cohort enrollment) and retrospective (beginning the year the individual turned 20 years old or 1952, whichever was later). Serum was either measured (prospective) or modeled (retrospective). In the combined cohort analysis of workers and community members, there were some statistically significant associations between PFOA and hypercholesterolemia. Stratified analysis by age group suggested the risk was predominantly increased in those aged 40 years and over. Note that the differences in the underlying characteristics of the worker vs. community cohorts likely made it difficult to properly control for potential confounding factors.

As discussed in the previous section, Steenland *et al.* (2015) conducted a prospective analysis of workers in the Washington Works plant. The authors found no association between estimated cumulative exposures (ppm-years) and high cholesterol (self-reported).

Overall, studies reported inconsistent findings regarding the association between PFOA and increased total cholesterol, HDL, and LDL, and triglycerides, even among studies with similar designs (i.e., when looking at cross-sectional and longitudinal assessments separately). An important limitation of several of these studies is the cross-sectional study design; serum lipid and cholesterol methods are highly variable over time and across individuals, and no baseline or follow-up measurements were available for comparison to the one-time measurements taken for most of these studies. Further, it is unclear whether the magnitude of changes observed in the studies that found associations would be sufficient to increase the risk of frank disease, such as coronary artery disease.

Cardiovascular Disease

Several studies examined death from cardiovascular conditions among PFOA-exposed workers at the West Virginia Washington Works plant (Leonard *et al.*, 2008; Sakr *et al.*, 2009; Steenland and Woskie, 2012), the Mid-Ohio Valley (Steenland *et al.*, 2015), and the 3M Cottage Grove plant in Minnesota (Gilliland and Mandel, 1993a; Lundin *et al.*, 2009). While PFOA exposures were "possible" in other plants (e.g., the 3M plant in Decatur, Alabama), the predominant exposure in these plants was PFOS. Please refer to Section 7.2.2 for a discussion of studies in Decatur plant employees.

Leonard *et al.* (2008) conducted a retrospective cohort mortality study of >6,000 male and female workers at the Washington Works plant employed between 1948 and 2002. Relative to the general population and the population of West Virginia, mortality from CVD (all types) and ischemic heart disease (IHD) was not elevated in the cohort. In an effort to minimize the healthy worker effect,⁶⁸ the authors also compared mortality in the workers relative to a regional worker population comprised of an eight-state DuPont worker population. In this analysis, there was a slight elevation in both CVD and IHD mortality, but the associations were not statistically significant (standard mortality ratio [SMR]⁶⁹ = 1.10, 95% confidence interval [CI]: 0.98-1.23, and SMR = 1.09, 95% CI: 0.96-1.24, respectively).

In a retrospective cohort study, Steenland and Woskie (2012) assessed mortality in 5,791 Washington Works employees exposed to PFOA between 1979 and 2004. In this study, the estimated averaged serum

⁶⁷ The authors did not present a worker-only analysis.

⁶⁸ Because severely ill and chronically disabled individuals are generally excluded from employment in certain fields, there are often fewer sick individuals in occupational cohorts, relative to the general population. This can result in lowered relative risk of disease/death when the general population is used as the reference population.

⁶⁹ An SMR is the ratio/percentage of mortality in a given population compared to a reference population, usually the general population of a state/province or the nation as a whole.

PFOA concentration was 350 ng/mL. The authors compared worker mortality to that of the general US population as well as updated version of the worker population used by Leonard *et al.* (2008). Death from IHD was not significantly increased in any exposure group, compared to the worker referent group, or for the entire cohort, compared to the US population. In fact, risk of IHD-related death, although not significant, was lower in the higher quartiles of exposure, indicating a lack of exposure-response patterns.

Sakr *et al.* (2009) also conducted a retrospective cohort study of workers ever employed at the Washington Works plant between 1948 and 2002. The main difference from the other recent studies of this cohort relates to the exposure assessment. The authors estimated PFOA exposure using an exposure reconstruction model developed from occupational information (detailed work histories) and serum PFOA data (from a previous study). Each person was then placed into the low (reference), medium, or high exposure group. The authors found no association between increasing PFOA exposure and higher risk of death from IHD.

Finally, as described above, Steenland *et al.* (2015) conducted a study of disease incidence (rather than mortality) in workers in the Washington Works plant. There were no consistent associations between estimated cumulative PFOA exposure and coronary heart disease, medicated hypertension, or stroke. While there was a slight increase in risk of stroke with no lag (*i.e.*, no time between exposure and disease), this was only for the lowest quartile of exposure, and not higher quartiles.

Winquist and Steenland (2014a), described above, found statistically significant associations between PFOA and hypertension (a risk factor for CVD) in the combined C8 Cohort worker and community cohort, but only in the two lower quintiles of exposure (risk was decreased, albeit not significantly, in the fourth and fifth quintiles). The authors reported a statistically significant association between PFOA and coronary artery disease in the entire group, as well as in males 20-39 years old, but only in the second quartile (there were no exposure-response patterns). Note that the differences in the underlying characteristics of the worker *vs.* community cohorts likely made it difficult to properly control for potential confounding factors.

In a retrospective cohort analysis of the 3M Cottage Grove, Minnesota, plant, Gilliland and Mandel (1993a) assessed cause of death in 2,788 male and 749 female workers employed between 1947 and 1983. CVD incidence was less than expected (based on the general US population) in both female employees (SMR = 0.81, 95% CI: 0.49-1.29) and male employees (SMR = 0.68, 95% CI: 0.57-0.83). The authors noted that the low SMRs may have resulted from the healthy worker effect.

Lundin *et al.* (2009) updated the original study by Gilliland and Mandel (1993a) to include additional employees, additional years of follow-up, and a job exposure matrix. In this retrospective cohort study, the authors reported a non-significant decrease in mortality from IHD and all heart disease. Risk of cerebrovascular disease⁷⁰ was slightly increased, but the association was not statistically significant (SMR = 1.6; 95% CI: 0.5-3.7). When stratified by low, moderate, and high exposure, however, the association was statistically significant for those with high PFOA exposure (defined as working on a job with definite exposure to PFOA for 6 months or longer; SMR = 4.6, 95% CI: 1.3-17.0). The results were again not statistically significant when the authors stratified by cumulative exposure to PFOA (*i.e.*, <1, 1-4.9, or ≥5 years of exposure). The authors noted that the inconsistencies in the cerebrovascular results across exposure groups led to some uncertainty with regard to their interpretation of the study results.

Overall, I conclude that the weight of evidence does not establish a causal relationship between occupational exposure to PFOA and increased risk of the incidence of CVD and mortality from CVD.

⁷⁰ Cerebrovascular disease is category of cardiovascular disease specific to brain circulation and resulting diseases and conditions, such as stroke. Risk of stroke is associated with diabetes, hypertension, and lifestyle factors such as diet and smoking.

The available studies show no increased incidence of death from CVD in workers at either the West Virginia Washington Works plant or the 3M Cottage Grove plant in Minnesota, a population whose serum PFOA concentrations were well above those of the general population (Mandel and Johnson, 1995). I only identified one study evaluating hypertension, which reported null results.

7.1.2.4 Immunological Effects

Steenland *et al.* (2013) conducted an analysis of autoimmune conditions in the Mid-Ohio Valley, including people who worked in PFOA production or lived in any of the PFOA-contaminated water districts (Steenland *et al.*, 2013). The authors assessed the risk of several immune-mediated conditions, including ulcerative colitis (a gastrointestinal condition), Crohn's disease (a gastrointestinal condition), rheumatoid arthritis, Type 1 diabetes, lupus, multiple sclerosis, and any other autoimmune conditions identified based on medical-record-validated self report. Exposure was assessed using serum PFOA measurements from 2005-2006 (mean: 325 ng/mL in workers). The only statistically significant association found was between PFOA and ulcerative colitis, in both unlagged models and those with 10-year lags for disease latency.⁷¹ In contrast, decreases in disease risk were apparent for Type 1 diabetes (particularly unlagged) and rheumatoid arthritis. The trends were generally not statistically significant, although individual quartiles did achieve significance (*e.g.*, fourth *versus* first quartile of exposure, for rheumatoid arthritis).

As discussed in previous sections, Steenland *et al.* (2015) conducted an analysis of various health effects in workers at the Washington Works plant. The authors included two autoimmune diseases, rheumatoid arthritis and ulcerative colitis. Exposure was assessed using a cumulative exposure metric calculated as the sum of yearly exposure estimates from birth through any given year. In addition to assessing exposure as a continuous measure of cumulative exposure, they conducted trend tests for categorical analyses (by quartile of cumulative exposure). Ulcerative colitis showed a statistically significant trend when assessed using cumulative exposure; however, the association was not statistically significant in the model using categories based on quartiles of exposure that had no lag for disease latency. The opposite patterns were seen for rheumatoid arthritis – while trends for categories of exposure showed statistically significant relationships (or nearly statistically significant, in the case of 10-year lag models), there were no statistically significant trends for measures of cumulative PFOA exposure. These analyses were limited by a small number of cases ($n = 23$ and 28 for rheumatoid arthritis and ulcerative colitis, respectively) for both of these diseases.

Overall, there is no consistent evidence of an association between PFOA and immune conditions in PFOA-exposed workers.

7.1.2.5 Kidney Effects

Several studies have assess the association between PFOA and biomarkers of kidney disease (uric acid) or kidney disease in several PFOA production plants in the US and Italy.

⁷¹ Unlagged models assume all exposure time is potentially relevant to disease development, *i.e.*, they assume the disease could have developed immediately or shortly after exposure. Lagged models exclude a defined period of time immediately preceding the outcome under the assumption that disease could not have developed that quickly (Richardson *et al.*, 2011). Lag times are informed by the pathophysiology of the disease.

Two studies analyzed serum uric acid levels, which are a biomarker of kidney function.⁷² Costa *et al.* (2009) found that increasing serum PFOA concentrations were associated with increasing uric acid ($p < 0.05$) in 56 current or former workers in an Italian production plant. The association remained in an analysis restricted to currently employed workers of the plant. Similar results were reported in the study of DuPont workers by Sakr *et al.* (2007a). Sakr *et al.* (2007a) noted, however, that they believed the associations between serum PFOA concentrations and uric acid concentrations were unlikely to be clinically significant at the levels of PFOA exposure measured, and the statistical significance could have resulted from chance.

Leonard *et al.* (2008), Steenland and Woskie (2012), Raleigh *et al.* (2014), and Steenland *et al.* (2015) investigated mortality from chronic kidney disease in PFOA-exposed workers.

Inconsistent associations were found at the Washington Works facility. In a retrospective cohort study, Leonard *et al.* (2008) reported no significant differences between death from nephritis and nephrosis⁷³ and exposed workers in comparisons with the US population, general West Virginia population, and non-PFOA exposed DuPont worker population. Steenland and Woskie (2012), however, reported an association between PFOA and mortality from chronic kidney disease in Washington Works employees exposed to PFOA between 1979 and 2004 (SMR = 3.11, 95% CI: 1.66-5.32). When separated by quartiles of exposure, the SMRs did not follow a clear exposure-response pattern.

Raleigh *et al.* (2014) conducted a retrospective cohort analysis of mortality and cancer incidence in workers employed for at least 1 year at the Cottage Grove plant. The authors compared health outcomes in production workers to another 3M cohort working in a tape and abrasives production facility in Minnesota. Exposure was estimated using daily time-weighted averages (TWAs) based on work history records, industrial hygiene monitoring data, and information from workers and industrial hygiene professionals. The authors found no associations between PFOA and chronic kidney disease, either by continuous exposure (increment of PFOA) or when stratified into quartiles of exposure.

Finally, Steenland *et al.* (2015) conducted a retrospective cohort study and identified 43 cases of chronic kidney disease in 3,713 workers in the Mid-Ohio Valley production plant 1948 and 2002; there was no association between PFOA and the incidence of chronic kidney disease in unlagged or 10-year lag models.

Overall, studies have suggested that small changes in uric acid following PFOA exposure were likely not clinically significant, and the majority of studies of chronic kidney disease showed no associations between PFOA and increased risk of mortality from this disease. Therefore, I conclude that the weight of evidence does not establish an association between PFOA and kidney function and disease in occupationally exposed populations.

7.1.2.6 Cancer

Several studies of workers at the Cottage Grove and Washington Works plants have examined mortality from cancer. I have focused on the recent analyses, because most are updates of previous occupational mortality studies (*e.g.*, Lundin *et al.*, 2009) analyzing the same populations but following them further in

⁷² Uric acid is formed from the metabolism of food and cells in the body. Uric acid is removed from the blood by the kidneys and is excreted in urine. Elevated serum uric acid concentrations may predict the development of chronic kidney disease (Johnson *et al.*, 2013; NLM, 2015).

⁷³ Nephritis is acute or chronic kidney inflammation; nephrosis is a non-inflammatory disease affecting the function of the kidney (Merriam-Webster, Inc., 2016).

time. Because cancer can take decades to develop, a longer follow-up time may better capture cancers that have a long latency period.

As noted previously, IARC (2016a) recently classified PFOA as Group 2B, *i.e.*, possibly carcinogenic to humans. This classification was based on "limited evidence" for the carcinogenicity of PFOA in both humans and animals. It should be emphasized that IARC did not identify PFOA as a known human carcinogen. The finding for humans was based primarily on evidence of testicular and kidney cancer. The epidemiological evidence, including the occupational studies described in the following section, does not provide reliable evidence that PFOA is carcinogenic to humans.

In a retrospective cohort study, Steenland and Woskie (2012), discussed in the previous section, assessed mortality in 5,791 Washington Works employees exposed to PFOA between 1979 and 2004. In this study, the estimated averaged serum PFOA concentration was 350 ng/mL. The authors compared worker mortality to that of the general US population as well as a worker referent population comprised of DuPont workers in eight states. There was no association between PFOA and all cancers combined. Death from liver cancer was slightly elevated in exposure quartile 3, but quartile 4 had a slight decrease in mortality compared to the worker referent population; neither association was statistically significant. There were no statistically significant associations between PFOA and death from lung, breast, pancreatic, prostate, testicular, or bladder cancer, nor with non-Hodgkin's lymphoma or leukemia.

Steenland and Woskie (2012) indicated that kidney cancer incidence was statistically significantly increased in all exposure groups relative to unexposed workers from other plants; however, CIs included 1.0 in all exposed groups except the fourth quartile ($\geq 2,700$ ppm-years; SMR = 2.66, 95% CI: 1.15-5.24). Further, there were no kidney cancers in the third quartile of exposure. The combined SMR for all exposure groups was 1.28 (95% CI: 0.66-2.24). Mortality from mesothelioma was also increased in the third and fourth quartiles of exposure, as well as in the analysis across all exposure groups (SMR = 2.85, 95% CI: 1.05-6.20, $p < 0.05$). The authors noted that the elevation in mortality from mesothelioma was likely the result of asbestos exposure.

Raleigh *et al.* (2014) conducted a retrospective cohort study of mortality and cancer incidence in workers employed for at least 1 year at the Cottage Grove plant. Across all the workers, there was no significant increase in all cancers or any type of specific cancer. The null associations and lack of apparent exposure-response relationship remained in analyses stratified by exposure quartile. A strength of this study is its use of an unexposed referent group of workers with similar job types, which reduced the healthy worker effect.

Finally, Steenland *et al.* (2015) conducted an analysis of disease incidence in a cohort of 3,713 workers at the Washington Works plant. Exposure was estimated using PFOA serum concentrations over time and modeled residential exposures from drinking water data, and the authors estimated cumulative exposures. The relative risk of prostate cancer was increased in more highly exposed workers relative to the lowest exposure group; however, there were no statistically significant associations or clear dose-response trends. In contrast, there was a statistically significant *decreased* risk of bladder cancer with increasing quartiles of exposure to PFOA. The risk of melanoma and colorectal cancer showed no statistically significant associations with PFOA.

Overall, there is little evidence of an increased risk of cancer in workers exposed to PFOA. The lack of a clear exposure-response relationship limits the ability to draw causal inferences from the Steenland and Woskie (2012) study.

7.1.3 Serum Concentrations in the General Population and Non-occupationally Exposed Cohorts

Exposure to PFOA in the general population is widespread, but may vary considerably based on factors such as age, sex, race, diet and other lifestyle factors, and geographical location. Higher concentrations are generally reported for males *vs.* females (Kato *et al.*, 2015). Associations between age and PFOA exposure have been reported, but patterns differ by sex. An analysis of data from four US National Health and Nutrition Examination Survey (NHANES) cycles (1999-2008) showed that serum PFOA concentrations in females increased as age increased, but for males, serum PFOA concentrations went down as age increased (Kato *et al.*, 2011). These differing trends may be related to sex-related differences in exposure based on physiology, including menstruation/menopause, pregnancy, and/or lactation.

Some of the differences in age groups may also be influenced by the patterns of PFOA usage over time; older individuals likely had higher exposures to PFOA in products than children born after the PFOA phase-out, which began in 2002 (3M, 2006a). Similarly, mean serum PFOA concentration has gradually declined over time in many populations due to the phase-out of PFOA, as shown across the NHANES data cycles. Table 7.2 summarizes PFOA biomonitoring data in representative studies of the general population and select cohorts across the world that were exposed to lower levels of PFOA (relative to the occupational cohorts) *via* drinking water (*i.e.*, the C8 cohort, discussed in Section 7.1.4) or other non-occupational routes, such as food and commercial products.

Table 7.2 Serum PFOA (ng/mL) in Representative Non-occupational Populations

Cohort/Population	N	Range	Arithmetic Mean	Geometric Mean	Reference
NHANES		50 th -95 th Percentile			
1999-2000	1,562	5.20-11.9	NR	5.21 (95% CI: 4.72-5.74)	CDC (2015)
Male	743	6.00-12.1		5.71 (95% CI: 5.17-6.31)	
Female	819	4.70-11.3		4.80 (95% CI: 4.32-5.34)	
2003-2004	2,094	4.10-9.80		3.95 (95% CI: 3.65-4.27)	
Pregnant Women	76	2.6-5.6		2.39 (SE: 0.24)	Woodruff <i>et al.</i> (2011)
Non-pregnant Women	400	3.2-8.4		3.19 (SE: 0.16)	
2005-2006	2,120	4.20-11.3		3.92 (95% CI: 3.48-4.42)	CDC (2015)
2007-2008	2,100	4.30-9.60		4.12 (95% CI: 4.01-4.24)	
2009-2010	2,233	3.20-7.50		3.07 (95% CI: 2.81-3.36)	
Male	743	6.00-12.1		3.53 (95% CI: 3.22-3.87)	CDC (2017a)
Female	1,158	2.70-6.90		2.69 (95% CI: 2.45-2.96)	
2011-2012	1,904	2.08-5.68		2.08 (95% CI: 1.95-2.22)	
Male	966	2.38-5.62		2.37 (95% CI: 2.22-2.53)	
Female	938	1.78-5.68		1.84 (95% CI: 1.68-2.01)	
2013-2014	2,165	2.07-5.57		1.94 (95% CI: 1.76-2.14)	
Male	1,031	2.37-5.67		2.29 (95% CI: 2.09-2.50)	
Female	1,134	1.67-5.07		1.66 (95% CI: 1.48-1.87)	
American Red Cross Donors		50 th -95 th Percentile			
2000-2001	645	4.7-12.0	NR	4.72 (95% CI: 4.52-4.93)	Olsen <i>et al.</i> (2017)
2006	600	3.6-7.9		3.44 (95% CI: 3.32-3.75)	
2010	600	2.5-5.6		2.44 (95% CI: 2.34-2.65)	
2015	616	1.1-3.2		1.08 (95% CI: 1.03-1.14)	
C8 Health Project Cohort (2005-2006) ^a					
Total Cohort (12 to ≥60 years old)	69,025	NR	82.9	32.9 (SD: 240.8)	Frisbee <i>et al.</i> (2009)
Male	33,240		98.2	39.4 (SD: 284.3)	
Female	35,785		68.8	27.9 (SD: 190.6)	
Pregnant Women	1,845	IQR: 10.3-49.8	NR	48.8 (SD: 77.8)	Stein <i>et al.</i> (2009)
Children (1 to <18 years old)	12,470	NR	NR	69.2 (SD: 111.9) ^a	Frisbee <i>et al.</i> (2010)
Aarhus Birth Cohort (2008-2013), Denmark					
Pregnant Women	1,533	IQR: 1.53-2.64	NR	2.01	Bjerregaard-Olesen <i>et al.</i> (2016)
Danish National Birth Cohort (1992-2002)					
Pregnant Women (First Trimester)	1,399	NR	NR	5.6 (SD: 2.5) ^a	Fei <i>et al.</i> (2007)
Pregnant Women (Second Trimester)	200			4.5 (SD: 1.9) ^a	
Infants (Cord blood)	50			3.7 (SD: 3.4) ^a	
Children (Average Age: 11, Born: 1998-2003)	973	Control IQR: 4.00-5.42	NR	3.88-4.06 ^b	Liew <i>et al.</i> (2015)

Cohort/Population	N	Range	Arithmetic Mean	Geometric Mean	Reference
Danish Diet, Cancer, and Health Cohort (1993-1997)					
Men and Women (50-65 years old)	753	NR	NR	7.1	Eriksen <i>et al.</i> (2013)
Decatur Cohort		95 th Percentile			
2010	153	61.1	NR	16.3 (95% CI: 13.2-19.6)	Worley <i>et al.</i> (2017)
2016	45	39.1	NR	11.7 (95% CI: 18.7-14.6)	
Flemish Environment and Health Study (2007-2015)					
2007-2011 (Infants: Cord Blood)	218	NR	NR	1.51 (95% CI: 1.43-1.59)	Schoeters <i>et al.</i> (2017)
2012-2015 (Infants: Cord Blood)	269	NR	NR	1.19 (95% CI: 1.12-1.26)	
Hokkaido Study on Environment and Children's Health (2002-2005)		25 th -75 th Percentile			
Pregnant Women	306	0.9-2.0	1.52 (SD: 0.89)	NR	Kishi <i>et al.</i> (2015)
HOME Study, Cincinnati, Ohio (2003-2006)		25 th -75 th Percentile			
Pregnant Women	204	3.7-7.7	NR	5.3	Braun <i>et al.</i> (2016)
Taiwan Birth Panel Study (2004-2005)					
Pregnant Women	429	NR	NR	1.84 (SD: 2.23)	Chen <i>et al.</i> (2012)
Washington County, Minnesota Communities (2008) ^b					
Men and Women (20-86 years old)	196	1.6-177	NR	15.4 (95% CI: 13.6-17.4)	Landsteiner <i>et al.</i> (2014)
Men	88	NR		16.6 (95% CI: 13.9-19.8)	
Women	108			14.4 (95% CI: 12.1-17.2)	
Young Taiwanese Cohort Study (2006-2008)		50 th -90 th Percentile			
Total (12-30 years old)	551	3.64-9.71	NR	2.67 (SD: 2.96)	Lin <i>et al.</i> (2013a)
Men	214	NR		2.71 (SD: 2.94)	
Women	337			2.64 (SD: 2.98)	
12-19 years old	212			2.80 (SD: 2.90)	
20-30 years old	339			2.59 (SD: 3.00)	

Notes:

CI = Confidence Interval; HOME = Health Outcomes and Measures of the Environment; IQR = Interquartile Range; NHANES = National Health and Nutrition Examination Survey; NR = Not Reported; PFOA = Perfluorooctanoic Acid.

(a) The C8 Health Project cohort includes residents of four water districts in Ohio (City of Belpre, Little Hocking Water Association, Tupper's Plains, and Village of Pomeroy), two water districts in West Virginia (Lubeck Public Service District, and Mason County), and some private wells in both states.

(b) Included current and former 3M workers (the authors reported that serum concentrations were not significantly different between those who worked at 3M and those who did not [17.0 vs. 15 ng/mL, respectively]).

Several large cohort studies have investigated the association between serum PFC concentrations, including PFOA, and various health effects in populations with low exposures associated with background environmental sources of PFOA (consumer products, food, water, and ambient air), or low-level contamination events (*e.g.*, exposure *via* drinking water from releases by a PFOA-associated industry). The most well-studied cohorts include the C8 Cohort, the Danish National Birth Cohort, the Norwegian Mother and Child Cohort, and NHANES in the US. The C8 Cohort is a cohort of 69,000 residents in Ohio and West Virginia living near a chemical plant who were exposed to PFOA in

contaminated drinking water. Participants were recruited in 2005-2006 and investigators intended to study PFOA primarily, though serum PFOS measurements were taken and associations with PFOS also analyzed. The Danish National Birth Cohort is a cohort of 100,000 pregnant women recruited between 2000 and 2002 and followed continuously since that time. Maternal serum and cord blood samples were collected, and interviews were conducted to collect information on factors such as diet and lifestyle. The Norwegian Mother and Child Cohort includes >90,000 pregnant Norwegian women recruited between 1999 and 2008, with collection of biologic samples and birth data through 2009. NHANES is an ongoing program designed to assess the health and nutritional status of a group of representative adults and children in the US. NHANES collects a variety of survey and health data, including serum concentrations of a number of substances, such as heavy metals and PFCs (CDC, 2014).

In general, I relied on these large, well-characterized cohorts for data-rich endpoints (*e.g.*, birth outcomes), unless other cohorts showed distinctly different results from the large cohorts (in which case, I included these divergent results as well). In areas in which data were lacking, I included discussion of smaller or less-well-studied cohorts, in addition to any studies in the larger cohorts. A summary of both large and small cohorts is presented below in Table 7.3. For several endpoints investigated in community and general population studies, only cross-sectional analyses are available, which limits the ability to determine whether a true cause and effect relationship exists. Overall, I conclude that the weight of evidence does not establish a causal association between non-occupational PFOA exposure and the endpoints examined.

Table 7.3 Non-occupational PFOA Cohorts

Cohort	Study Population	Location	PFOA Exposure Source	Outcomes Investigated
C8 Health Project Cohort	Ongoing study of 69,000 adults and children recruited from a community surrounding a PFOA plant	Mid-Ohio Valley, US	Contaminated drinking water	Developmental and reproductive, liver enzymes, thyroid hormones and disease, serum lipids, immunotoxicity, kidney effects, cancer
Danish National Birth Cohort	100,000 pregnant women enrolled in 2000-2002	Denmark	Background: Drinking water, food, products	Developmental and reproductive
Hokkaido Birth Cohort Study on the Environment and Child's Health	~500 mother-child pairs studied from 2002-2005	Japan	Background: Drinking water, food, products	Immunotoxicity
Norwegian Mother and Child Cohort	>90,000 pregnant women and their offspring enrolled in 1999-2008	Norway	Background: Drinking water, food, products	Developmental and reproductive, liver enzymes, thyroid hormones, serum lipids, immunotoxicity, kidney effects
NHANES	Ongoing representative sample of general US population	US	Background: Drinking water, food, products	Thyroid hormones and disease, serum lipids
INUENDO Cohort	>3,000 pregnant mothers and their children enrolled in 2002-2004	Sweden, Poland, Ukraine, and Greenland	Background: Drinking water, food, products	Developmental and reproductive, immunotoxicity
ALSPAC Cohort	>14,000 pregnant women and offspring recruited in 1991-1992	UK	Background: Drinking water, food, products	Developmental and reproductive
Odense Child Cohort	>2,000 pregnant women and children enrolled in 2010-2012	Denmark	Background: Drinking water, food, products	Developmental and reproductive, immunotoxicity
CHEF Project Cohort	Ongoing study of >1,000 children born beginning in 1986	Faroe Islands, halfway between Norway and Iceland	Background: Drinking water, food, products; diet high in fish and whale meat	Immunotoxicity
Taiwan Birth Panel Study	486 mother-infant pairs enrolled in 2004-2005	Suburban and Urban Taiwan	Background: Drinking water, food, products	Developmental and reproductive
Danish DCH Cohort	4,769 men and women >50 years old recruited 1993-1997	Denmark	Background: Drinking water, food, products	Serum lipids
European Youth Heart Survey	>1,700 boys and girls 9-15 years old beginning in 1997 ^a	Denmark, Estonia, Norway, and Portugal	Background: Drinking water, food, products	Serum lipids

Notes:

ALSPAC = Avon Longitudinal Study of Parents and Children; CHEF = Children's Health and the Environment in the Faroes; DCH = Diet, Cancer and Health; INUENDO = Biopersistent Organochlorines in Diet and Human Fertility; NHANES = National Health and Nutrition Examination Survey; PFOA = Perfluorooctanoic Acid; UK = United Kingdom; US = United States.

7.1.4 Studies in the General Population

7.1.4.1 Reproductive and Developmental Effects

Numerous studies are available that evaluate associations between PFOA exposure in the general population and various reproductive and developmental outcomes, including male and female fertility, miscarriage, timing of menopause, birth and growth outcomes, congenital anomalies (*i.e.*, birth defects), neurodevelopmental effects (*e.g.*, attention disorders), and timing of puberty. Most agency reviews (C8 Science Panel, 2011a,b; US EPA, 2016a) have focused on the studies of pregnancy-induced hypertension and preeclampsia, measures of fetal growth, and pubertal development. As such, I have focused on these endpoints for my analysis. Based on an extensive review of the large body of literature, I conclude that the weight of evidence does not establish an association between non-occupational PFOA exposure and developmental and reproductive effects.

Preeclampsia and Hypertension

Preeclampsia is characterized by protein in the urine and an increase in blood pressure after week 20 of pregnancy, which can harm both the mother and the developing infant. Gestational hypertension, also called pregnancy-induced hypertension, is the development of hypertension after week 20 of pregnancy without protein in the urine or other changes associated with preeclampsia (APA, 2015).

I identified one study of PFOA and preeclampsia in the general population. In a case-cohort analysis, Starling *et al.* (2014a) investigated the association between maternal serum PFOA concentration and preeclampsia in 976 first-time mothers enrolled in the Norwegian Mother and Child Cohort Study (a prospective cohort). Serum PFOA concentration (0.32-11.28 ng/mL) was not associated with an increased risk of confirmed preeclampsia (validated using medical records).

I identified three studies of PFOA and preeclampsia and gestational hypertension in exposed community members (Stein *et al.*, 2009; Savitz *et al.*, 2012a; Darrow *et al.*, 2013). Stein *et al.* (2009) investigated the association between PFOA and preeclampsia in 1,845 women in a retrospective cohort study of women enrolled in the C8 Cohort who gave birth between 2000 and 2006. In this study, serum PFOA concentrations (at interview, prior to birth) ranged from 0.25-894.4 ng/mL, with a mean of 48.8 ng/mL. The authors reported no association between any level of exposure to PFOA and preeclampsia. This study is limited by its cross-sectional design.

Savitz *et al.* (2012a) conducted the largest birth cohort study of the C8 Cohort, including 11,000 births occurring between 1990 and 2006. The primary analyses were based on modeled PFOA serum concentrations based on residential history and information on plant releases, air concentrations, and other environmental data, and applying assumptions related to air and water intake rates. The authors found no association between modeled serum PFOA concentration and preeclampsia per interquartile range (IQR) increase in PFOA (shift from 25th to 75th percentile of exposure); however, the association was statistically significant per 100 ng/mL increase in serum PFOA (odds ratio [OR] = 1.08, 95% CI: 1.01-1.15). Analyses by percentile of exposure (<40th through ≥80th) showed no increasing risk in more highly exposed groups compared to the less-exposed groups. The authors tested the correlation between actual and estimated serum concentrations using known serum measurements in 2005-2006. The correlation was low/moderate (0.67), indicating that exposure misclassification was likely.

Finally, Darrow *et al.* (2013) conducted a prospective cohort study of pregnancy outcomes in 1,630 births occurring between 2005 and 2010 in women enrolled in the C8 Cohort. Mean serum PFOA in this group of mothers ranged from 0.6-459.5 ng/mL, with a mean of 31 ng/mL. The authors reported a statistically

significant increase in pregnancy-induced hypertension across all births (OR = 1.27, 95% CI: 1.05-1.55 per log unit increase in PFOA). The association was not statistically significant when calculated per interquartile increase in serum PFOA concentration (22 ng/mL) or in a stratified analysis of first-time mother births only.

Overall, most of the available studies show no association between PFOA and preeclampsia and pregnancy-induced hypertension. Positive studies showed relatively weak associations (ORs < 1.5), and inconsistency across different analyses (*e.g.*, continuous *vs.* quartiles of exposure).

Birth and Growth Outcomes

General Population. Several studies have evaluated birth outcomes in children born to women enrolled in the Danish National Birth Cohort, which recruited pregnant women and their offspring between 1996 and 2002. In a prospective cohort study, Fei *et al.* (2007) evaluated the association of plasma concentrations of PFOA in 1,400 pregnant women from this cohort and their infants' birth weight and length of gestation. The mean concentrations of PFOA in maternal plasma were 5.6 ng/mL and 4.5 ng/mL for first and second trimester, respectively, and umbilical cord blood PFOA concentrations averaged 3.7 ng/mL. There was a statistically significant association between PFOA and the unadjusted mean birth weight in the second and third quartiles of exposure (5.21-6.96 and ≥ 6.97 ng/mL), but there was no exposure-response relationship. The difference in weight between the high and lower exposure groups was only 175 g, and average birth weight was well about the cutoff for low birth weight (<2,500 g). Indeed, in the analysis restricted to the clinical definition of low birth weight (<2,500 g), there were no statistically significant associations. There were no statistically significant associations between PFOA and small for gestational age (SGA), defined as birth weight <10th percentile at a specific gestational age in weeks.

In another analysis of the same cohort, Fei *et al.* (2008a) evaluated fetal growth by using several endpoints: placental weight, birth length, ponderal index (birth weight divided by cubed birth length),⁷⁴ and head and abdominal circumferences. PFOA was measured in maternal serum. In this study, PFOA concentrations were not associated with any one of the five fetal growth indicators. There was a significant decrement in birth length in the second exposure quartile (-0.21 cm), but not in other quartiles, and there was no exposure-response relationship. There was also a statistically significant association between continuous increases in serum PFOA concentration and decreased abdominal circumference, but this relationship was not statistically significant in analyses by quartile. No other outcomes were altered.

Recent prospective analyses of this cohort of children, followed further into childhood, have found conflicting results. Andersen *et al.* (2010) reported that maternal serum PFOA concentration was statistically significantly associated with lower birth weight in boys and girls (-12.8 g weight per 1 ng/mL increase in PFOA). In sex-specific analyses, weight and body mass index (BMI) in boys were significantly reduced at both 5 months and 1 year of age (1.1-5.8 g difference), but there were no associations with height at either age. There were no statistically significant associations in girls in the adjusted analyses. Andersen *et al.* (2013) reported that there was no association between maternal serum PFOA concentration and any anthropometric measures (including BMI, waist circumference, and risk of being overweight) in their children at 7 years of age. Note that neither of these studies had information on, and were thus unable to adjust for, post-natal PFC exposures or other potential confounders occurring after birth.

⁷⁴ Ponderal index, a measure of leanness, is a measure used to assess fetal growth, usually in SGA infants; it is similar to measurements of body mass index (BMI) in adults (Olsen *et al.*, 2017).

Because there were inconsistent findings reported in the larger cohorts, I expanded my search to smaller or less well-studied cohorts. Two studies of smaller cohorts reported no associations between PFOA and birth outcomes such as birth weight, SGA, and prematurity (Hamm *et al.*, 2010; Chen *et al.*, 2012). I also identified four smaller studies that reported some positive associations between PFOA and fetal growth (Apelberg *et al.*, 2007; Maisonet *et al.*, 2012; Li *et al.*, 2017a). Apelberg *et al.* (2007) conducted a cross-sectional study of singleton births in Baltimore, Maryland, and found that umbilical cord concentrations of PFOA were statistically significantly inversely associated with ponderal index and head circumference; however, there were no statistically significant associations between PFOA and birth weight, birth length, and gestational age. This study was limited by its cross-sectional design and lack of control for socioeconomic status, which is a potentially important confounder, because it is associated with both PFOA exposure and the outcome of birth weight. Further, all differences were very small (*e.g.*, the 0.10 cm difference in birth length), and may not have been clinically significant.

A cohort study of 447 mother-child pairs from the Avon Longitudinal Study of Parents and Children (ALSPAC) in the UK reported that increasing maternal serum PFOA was associated with lower birth weight (p for trend < 0.005), shorter birth length (p for trend < 0.01), and increased body weight at 20 months in female children (p for trend < 0.0001) (Maisonet *et al.*, 2012). However, because the actual growth parameter values were not provided for individual exposed groups (only for the population as a whole), it is impossible to determine whether the decrements seen (an average of 140 g birth weight) resulted in birth and growth parameters outside of normal population ranges. The authors also failed to adjust for socioeconomic status.

Chen *et al.* (2012) conducted a longitudinal cohort study of 429 mother-infant pairs that were part of the Taiwan Birth Panel Study. They evaluated associations between cord blood PFOA concentration and several developmental effects, including gestational age (weeks of pregnancy at birth), birth weight, birth length, head circumference, ponderal index, preterm birth (birth at gestational age < 37 weeks), low birth weight, and SGA at birth. In their analysis, using clinical definitions for prematurity, low birth weight, and SGA, cord blood PFOA concentration was not associated with any of these endpoints.

Li *et al.* (2017a) assessed fetal birth and growth outcomes in a prospective cohort study of 321 mother-child pairs in the Guangzhou Birth Cohort Study in China. After adjusting for potential confounders, total PFOA concentration was associated with lower birth weight (112 g less) in all babies, including in stratified analyses for boys and girls. There were no associations, however, between PFOA exposure and gestational age (adjusted for birth weight and other potential confounders). The authors did not analyze the association between PFOA and the clinical definition of low birth weight ($< 2,500$ g) or preterm status (< 37 weeks). Demographic data indicated that the average birth weight was 3,117 g, with 7.5% of the cohort considered to have a clinically low birth weight. As with Maisonet *et al.* (2012), it is impossible to tell, based on the analysis by Li *et al.* (2017a), whether PFOA was associated with clinically relevant changes in birth and growth parameters in this study.

Exposed Communities. Savitz *et al.* (2012b) assessed birth outcomes in 8,253 children born between 1990 and 2004 to mothers enrolled in the C8 Cohort, who were exposed to PFOA in their drinking water. Exposure was estimated based on environmental data and residential history, as described previously for Savitz *et al.* (2012a). There were no consistent associations between PFOA exposure and increased risk of preterm birth (< 37 weeks gestation) or extremely preterm birth (< 32 weeks gestation), term low birth weight, term SGA, birth weight, or change in term birth weight (*i.e.*, loss of weight immediately after birth).

Similarly, Darrow *et al.* (2013) conducted a study of pregnancy outcomes in 1,630 births occurring between 2005 and 2010 in women enrolled in the C8 Cohort (mean serum PFOA = 31 ng/mL). The

authors found no association between increasing maternal PFOA serum concentration and preterm birth or low birth weight.

Overall Conclusions. Associations between PFOA and birth and growth are inconsistent both within and across studies. In most cases, when positive associations were observed, the magnitude of change was low and not necessarily associated with clinical definitions of low birth weight or SGA. As noted by US EPA (2016a), "[w]hen analyzed as a continuous measure, changes in birth weight might not be clinically significant, as small changes in the distribution among term infants do not result in a shift into the distribution seen in preterm infants (Savitz 2007; Wilcox 2010)."

Further, it is important to consider factors that may be affecting the results of these studies. A recent PBPK analysis of the studies on birth weight reported that a substantial portion of observed associations between PFOA and birth weight may be attributable to confounding by GFR (Verner *et al.*, 2015). Low GFR is an indicator of reduced kidney function, and lowered kidney function reduces excretion of PFOA (*i.e.*, lower GFR is associated with higher blood concentrations of PFOA). GFR also rises during pregnancy, and pregnant women whose GFR remains low tend to have babies with lower birth weights. As such, women who have babies with lower birth weight (on account of low GFR) will also have higher serum PFOA concentrations.

Overall, I conclude that the weight of evidence does not establish an association between PFOA and birth outcomes and growth at levels associated with the general population and communities with PFOA exposure sources.

Timing of Puberty

I identified three studies that investigated age at puberty in girls and boys exposed to PFOS *in utero* and/or childhood (Christensen *et al.*, 2011; Lopez-Espinosa *et al.*, 2011; Kristensen *et al.*, 2013). Christensen *et al.* (2011) conducted a nested case-control study of 218 cases of earlier menarche (*i.e.*, commencement of menstruation before age 11.5 years) and 230 girls with menarche after 11.5 years of age enrolled in the ALSPAC in the UK. Analyses indicated that increasing gestational PFOA exposure was not associated with alterations in the timing of menarche.

Lopez-Espinosa *et al.* (2011) conducted a cross-sectional analysis of the association between concurrent serum PFOA concentrations and age at puberty in boys and girls aged 8-18 years enrolled in the C8 Cohort. Age at puberty was determined using age of menarche and serum estradiol in girls and serum testosterone levels in boys. No statistically significant associations were found between serum PFOA concentration and later puberty in boys; however, there was a statistically significant association between serum PFOA concentration and later puberty in girls. The median delay of puberty ranged from 79 to 138 days, and, as noted by authors, a delay in puberty of 3-6 months is of unclear clinical relevance. This study is also limited by its cross-sectional design; in many cases, the participants had already reached puberty when concentrations of PFOA in blood were measured.

Finally, Kristensen *et al.* (2013) assessed the association between prenatal exposure to PFOA and several parameters of female reproductive function around age 20 (age at menarche [collected retrospectively], menstrual cycle length, hormone levels, and number of follicles/ovary) in a prospective cohort study of the Aarhus Birth Cohort in Denmark. Prenatal PFOA exposure was assessed using maternal serum collected at 30 weeks of gestation. The authors reported that prenatal exposure to PFOA was associated with a 5.3-month delay in age at menarche (95% CI: 1.3-9.3 months) ($p = 0.01$). However, PFOA was not associated with statistically significant changes in any of the other reproductive parameters measured. As noted by the authors, the 5-month delay in menarche is of unknown clinical significance (*i.e.*, it is unclear if it would have any long-term health implications).

Overall, I conclude that the weight of evidence does not establish an association between either gestational or childhood exposure to PFOA and age at puberty. The single analysis of puberty in boys showed no associations. Three studies of puberty in girls provided conflicting evidence of an association between PFOA and early or late puberty. However, any association between PFOA and later age at menarche would likely be confounded by the pharmacokinetics of PFOA, in which case the association is unlikely to be causal. Specifically, because menstrual blood is one route of removal of PFOA from the body, girls with an earlier first menarche would have lower serum PFOA concentrations than those with later first menarche (Wong *et al.*, 2014; Wu *et al.*, 2015).

Conclusions for Reproductive and Developmental Effects

Overall, I conclude that the findings to date do not demonstrate a reliable association between PFOA exposure and declines in birth weight or other developmental parameters. The larger, well-characterized cohorts most often found no statistically significant associations between PFOA and fetal and childhood growth, and many of the effects observed in smaller cohort studies were so small that they may not have been clinically significant. Further, studies of exposed communities, who have higher serum PFOA relative to the general population, have been largely null for reproductive and developmental effects.

7.1.4.2 Liver Enzymes and Disease

I identified only two studies evaluating the association between PFOA and liver enzymes in the general population (Lin *et al.*, 2010; Gleason *et al.*, 2015). Lin *et al.* (2010) conducted a cross-sectional analysis of 2,216 adults in NHANES in 1999-2000 and 2003-2004. The authors reported that for each unit increase in log PFOA, there was a significant increase in ALT (1.86 units, $p = 0.005$) and GGT (0.08 units, $p = 0.019$). When analyzed by quartiles, the trend for ALT remained statistically significant, although ALT was higher in the first quartile relative to the second. One limitation of this study was the lack of control for medications that may have altered ALT or GGT and the possibility of reverse causation.

Similarly, Gleason *et al.* (2015) conducted a cross-sectional analysis evaluating liver enzymes in 4,33 participants of the 2006-2008 and 2009-2010 NHANES. Serum PFOA concentrations averaged 3.5 ng/mL (range: 3.4-3.7 ng/mL). Increases in PFOA were associated with increases in GGT, ALT, and total bilirubin.

I also identified several studies that measured liver enzymes in PFOA-exposed communities (Emmett *et al.*, 2006; Gallo *et al.*, 2012; Darrow *et al.*, 2016). In a cross-sectional study, Emmett *et al.* (2006) evaluated liver enzymes in residents in the Little Hocking water district in southeastern Ohio, where there has been substantial environmental exposure to PFOA.⁷⁵ Participants were required to have been residents of the district for at least 2 years. Questionnaires and blood samples were collected simultaneously. The authors found no statistically significant associations between PFOA and bilirubin, ALT, AST, or GGT. When the authors analyzed a subset of people falling outside of the clinically normal range of these endpoints, PFOA was statistically significantly associated with abnormal AST ($p = 0.03$). Finally, there was no statistically significant association between PFOA exposure and liver disease ($n = 13$ cases). Overall, the authors concluded there were no clear associations between PFOA and liver toxicity in this cohort.

⁷⁵ Note that these participants are part of the C8 Cohort, but this analysis was completed using data that the Little Hocking water district collected independently (Frisbee *et al.*, 2009).

Gallo *et al.* (2012) conducted a cross-sectional analysis of PFOA and markers of liver function in adults enrolled in the C8 Cohort between 2005 and 2006. Increasing serum PFOA was associated with a small increase in ALT but was not associated with GGT or bilirubin. The authors noted that the small changes in ALT may not lead to diagnosable conditions in the future, and it was unclear if these changes would be reversible.

Darrow *et al.* (2016) assessed the association between PFOA and physician-validated self-reported liver disease (hepatitis, fatty liver, enlarged liver, and cirrhosis) in >28,000 participants in the C8 Cohort. Blood samples were collected between 2005 and 2006 to measure liver enzymes and PFOA exposure. Information on self-reported liver disease was collected in surveys between 2008 and 2011 and validated by medical record review. The authors also included workers in previous cohorts drawn from the local PFOA production plant (Leonard *et al.*, 2008). Cross-sectional analysis of liver enzymes and PFOA in 2005/2006 and cumulative serum PFOA concentration showed a statistically significant association between increasing PFOA concentration and increased ALT, but no consistent associations with bilirubin and GGT (note, however, that there was a significant *decrease* in bilirubin the fifth quintile of exposure). There were no statistically significant associations between serum PFOA concentration and liver disease, however, including unlagged and 10-year exposure-lagged models. The authors concluded that there was no indication of associations that would translate into an increased risk of liver disease.

7.1.4.3 Thyroid Hormones and Disease

The thyroid is a butterfly-shaped gland at the base of the neck that releases hormones that control a variety of functions in the body, including metabolism, brain development and function, and the function of other organs, such as the skin, heart, and intestines (Johns Hopkins Medicine, 2015). Several tests are commonly used to monitor thyroid function. These include measurements of serum TSH, a protein made in the pituitary gland, which regulates the synthesis of thyroid hormones, T4, T3, free T4 (T4 not bound to protein carriers), thyroid peroxidase antibodies, and radioactive iodine uptake. Small increases in serum T3 and T4 result in the inhibition of the secretion of TSH, while small decreases result in the increase of TSH secretion.

Hypothyroidism (underactive thyroid) is a common condition that is characterized by elevated levels of TSH and decreased levels of T3 and T4 hormones (Mayo Clinic, 2017). Hyperthyroidism, or overactive thyroid, is a condition in which the thyroid produces too much T4, accelerating metabolism. TSH levels are also suppressed in hyperthyroidism (Mayo Clinic, 2015). Both hyper- and hypothyroidism are treatable with thyroid medications (Mayo Clinic, 2015; A.D.A.M. Medical Encyclopedia, 2015b). As noted previously, although substantial and sustained alterations in thyroid hormone levels can be adverse, small, transient changes in thyroid hormone levels are not necessarily adverse.

Shrestha *et al.* (2014) conducted a cross-sectional evaluation of the association between serum PFOS and PFOA and thyroid hormones in 87 older adults (aged 55-74 years) who resided in communities in the upper Hudson River area in New York. The authors reported no statistically significant association between serum PFOA concentration and any of the measured thyroid hormones (TSH, T3, T4, free T4), after adjusting for potential confounders.

Winquist and Steenland (2014b) conducted a longitudinal cohort study of the association between estimated serum PFOA concentration and functional thyroid disease (medical-record-validated cases) in adult residents and workers exposed to PFOA in drinking water near a production plant in the Mid-Ohio Valley (C8 Cohort). The authors conducted both retrospective and prospective analyses of PFOA concentrations measured in serum samples collected in 2005-2006 and modeled (yearly and cumulative) serum PFOA estimates. The retrospective analysis found increasing risk of functional thyroid disease (and hyperthyroidism, specifically) with increasing estimated PFOA quintiles overall and among women

but not among men. The prospective analyses did not find a clear association between functional thyroid disease and modeled PFOA concentrations for men and women combined, but there was a smaller number of cases compared to the retrospective analyses. Among men, there was an increased risk of hypothyroidism with increasing quintile, especially when exposure was characterized cumulatively (hazard ratios [HRs] for quintiles 2-5 vs. quintile 1 = 1.12, 1.32, 1.45, 2.02), but CIs did not exclude the null. The results were similar in analyses restricted to the community cohort.

Webster *et al.* (2016) conducted a cross-sectional analysis of serum PFC concentrations and thyroid hormone in 1,525 US adults, using NHANES data from 2007 and 2008. The authors assessed associations between PFOA and free T3, free T4, the ratio of free T3/free T4, total T3, total T4, and TSH. They also stratified people into four groups by indicators of thyroid "stress," including higher thyroid peroxidase antibody (TPOAb), a marker of the autoimmune condition Hashimoto's disease,⁷⁶ and low iodine status. There were no statistically significant associations between PFOA and any of the thyroid hormones in those with normal TPOAb and iodine levels, low iodine only, or high TPOAb only. Those with high TPOAb and low iodine had a significant increase in TSH, but not no significant changes in any of the other hormone measures. Only 26 participants had high TPOAb and low iodine (2% of the study size). In addition to its small sample size, this study was limited by its cross-sectional design and thus, the possibility of reverse causation.

7.1.4.4 Serum Lipids and Cardiovascular Disease

Children and Adolescents

Many of the serum lipid studies were conducted with cohorts of children aged 1-18 years (Frisbee *et al.*, 2010; Geiger *et al.*, 2014; Timmermann *et al.*, 2014; Zeng *et al.*, 2015). Frisbee *et al.* (2010) conducted a cross-sectional analysis of 12,476 children and adolescents (1-17.9 years old) enrolled in the C8 Cohort. Mean serum PFOA was 69.2 ng/mL, which was well above the national average from this time. The authors reported that total cholesterol and LDL significantly increased with increasing serum PFOA concentration. There were no associations between PFOA and HDL, and only girls in the 1-11.9 years old group showed associations between PFOA and fasting triglycerides. In analyses dichotomized by abnormal cutoff values, some associations remained significant, but only at specific exposure levels. For example, PFOA was only significantly associated with total cholesterol in children in the fourth and fifth quintiles of exposure. Associations with LDL only remained significant for the fifth quintile of PFOA, and for fasting triglycerides, only the fifth quintile of PFOA exposure.

Among children from the general population, results were somewhat inconsistent across studies. In a cross-sectional study, Geiger *et al.* (2014) reported that serum PFOA concentration was statistically significantly positively associated with total cholesterol and LDL, but not HDL or triglycerides, in a group of 815 12- to 18-year-old children enrolled in NHANES. Timmermann *et al.* (2014) reported that there was no significant increase in serum cholesterol per 10 ng/mL increase in serum PFOA concentration in a cross-sectional analysis of 499 8- to 10-year-old children enrolled in the Danish portion of the European Youth Heart Study. Serum PFOA concentrations ranged from 0.8-35.2 ng/mL. There were also no associations between other markers of adiposity, including BMI, waist circumference, and serum adiponectin and leptin. In stratified analyses, however, overweight children had increased triglycerides (76.2% increase), plasma insulin, insulin resistance, and β -cell function for each 10 ng/mL increase in serum PFOA. Zeng *et al.* (2015) also reported that PFOA was positively associated with total cholesterol, triglycerides, and LDL in a cross-sectional analysis of a group of 225 healthy 12- to 15-year-

⁷⁶ Hashimoto's disease occurs when the immune system attacks the thyroid, which often leads to hypothyroidism. The disease causes the development of antibodies against thyroid peroxidase, which is an enzyme found in the thyroid gland that regulates thyroid hormone production. Measuring serum TPOAb is used to diagnose Hashimoto's disease (Mayo Clinic, 2016).

old children in Taiwan. There was no association between serum PFOA concentration and HDL. While there were exposure-response relationships for triglycerides, both total cholesterol and LDL had no exposure response; in fact, subjects in the middle quartile of PFOA exposure had significantly decreased total cholesterol.

Finally, the only longitudinal study (*i.e.*, prospective cohort study) of children and adolescents that included serum lipids was conducted by Domazet *et al.* (2016). The authors followed >300 children (aged 9 years) enrolled in the European Youth Heart Study until they turned 21 years old. When the authors examined the associations of PFOA measured at 9 years of age and outcomes at 15 and 21 years old, there was no association between increasing serum PFOA concentration and triglyceride levels. There was also no association between serum PFOA measured at 15 years old and triglyceride levels at 21 years old.

Adult Populations

Several other studies have examined cross-sectional associations between PFOA and serum lipids in adult populations, including pregnant women. Eriksen *et al.* (2013) measured serum PFOA and total cholesterol in a cross-sectional analysis of a group of 753 adults enrolled in the Danish Diet, Cancer, and Health (DCH) Cohort. The authors reported that there was a significant, 4.4 mg/dL increase in total cholesterol per IQR increase of serum PFOA concentration (IQR not reported, but the mean and maximum PFOA concentrations were 7.1 ng/mL and 27 ng/mL, respectively).

In a cross-sectional study, Fu *et al.* (2014) found that serum PFOA concentration was not associated with LDL, HDL, or triglycerides in a population of 133 adult hospital patients in China. Exposure to PFOA in this cohort was very low, with a mean serum PFOA concentration of 2.95 ng/mL (range: 0.32-39.46 ng/mL). Participants in the highest quartile of PFOA exposure had total cholesterol levels that were 0.24 mmol/L higher than those in the lowest quartile. However, when analyses were conducted for abnormal serum lipids (using cutoffs of normal ranges), none of the associations were statistically significant, and the only adjusted OR above 1 was for the third quartile (*i.e.*, no indication of an exposure-response relationship). The population in this study, however, had much lower mean PFOA serum concentrations (overall mean: 1.68 ± 1.20 ng/mL, highest quartile: 3.12 ± 1.52 ng/mL) than in the DCH and C8 Cohorts.

Several studies have also investigated the effect of PFOA on serum lipids in pregnant women. Starling *et al.* (2014b) conducted a cross-sectional analysis of serum lipids in a cohort of 891 pregnant women in the Norwegian Mother and Child Cohort Study. Plasma was collected in mid-pregnancy and analyzed for lipids and concentrations of PFCs. PFOA was not statistically significantly associated with alterations in total cholesterol, HDL, LDL, or triglycerides.

Finally, I identified studies of PFOA and serum lipids, hypertension, and coronary artery disease in adults in the C8 Cohort (Steenland *et al.*, 2009; Winquist and Steenland, 2014a). Steenland *et al.* (2009) investigated cross-sectional measures of PFOA and serum lipids in 42,294 adults enrolled in the C8 Cohort. All endpoints except HDL were significantly increased as serum PFOA concentration increased ($p < 0.05$). The difference in total cholesterol from the lowest to the highest exposure group was about 11-12 mg/dL.

Winquist and Steenland (2014a) conducted a cohort study of coronary artery disease, hypertension, and hypercholesterolemia (diagnosed high cholesterol) in more than 30,000 community residents and workers in the Mid-Ohio Valley (C8 Cohort). The authors conducted both prospective (beginning 1 year after age at the time of C8 Cohort enrollment) and retrospective (beginning the year an individual turned 20 years old or 1952, whichever was later). Serum was either measured (prospective) or modeled (retrospective). In the analyses restricted to the community cohort, risk of hypertension was increased in some quartiles of

PFOA exposure in males and in both sexes between the age of 40 and 59 years; however, the associations did not follow an exposure-response pattern (risk was highest in quartile 3 and lowest in quartile 4, relative to quartile 1). Hypercholesterolemia was also significantly increased for all ages in the community cohort and in the 40-59 years old age group, but not in the 60-79 years old age group. Again, however, there were no exposure-response relationships. There were no statistically significant associations between serum PFOA concentration and coronary artery disease.

Fitz-Simon *et al.* (2013) conducted a longitudinal study of serum PFOA concentration and lipids in the 521 participants in the C8 Cohort over a 4.4-year period. During this period, PFOA levels in public water supplies were reduced due to filtration and consequently, serum PFOA concentrations were also reduced (mean of 74.8 ng/mL prior to the water filtration vs. 30.8 ng/mL during). This change was associated with a decrease in LDL and HDL (statistically significant only in the highest tertile of exposure). There were no statistically significant associations between PFOA and total cholesterol or triglycerides. The authors noted that the magnitude of changes in lipids was small, and it is unclear whether a small decrease in LDL and a small decrease in HDL would result in clinically meaningful effects.

An important limitation of all but one (Fitz-Simon *et al.*, 2013) of these studies is the cross-sectional study design; because the investigators measured PFOA and serum lipids at one point in time, it is impossible to establish temporality (*i.e.*, cause and effect) between the exposure and the outcomes. Further, the mechanism by which PFOA may alter serum lipids and cholesterol is unknown (Steenland *et al.*, 2010a); indeed, the biological plausibility of such effects is questionable, based on the lack of a biologically plausible mechanism. Specifically high-dose animal studies of PFOA provide evidence of reduced serum lipids, in contrast to the elevated associations found in some human studies. Finally, some of these populations, such as the C8 Cohort, have overall mean total cholesterol and a prevalence of high cholesterol that is consistent with or lower than rates in the general US population, leading to questions of the clinical significance of the observed associations and the potential selection bias in the low-dose groups (*e.g.*, if the low dose group inadvertently had inherently lower risk of high cholesterol) (Kerger *et al.*, 2011).

7.1.4.5 Immunological Effects

Several studies have investigated the association between exposure to PFOA and markers of immune status (most often, response to vaccination). In a cohort study, Grandjean *et al.* (2012) reported that maternal and child serum PFOA concentrations (at age 7) were statistically significantly associated with decreases in childhood anti-diphtheria antibody concentrations at age 7 in a cohort study of 656 Faroese mothers and children. Further, PFOA concentrations at age 5 were associated with significantly increased odds of falling below a clinically protective level of diphtheria and tetanus antibodies, according to the authors (0.1 IU/mL). Note, however, that the serum diphtheria antibody titer levels were within the range of what the CDC would consider an indication of at least some immunity (*i.e.*, >0.01 IU/mL) (Tiwari, 2011).

In a follow-up analysis of the Grandjean *et al.* (2012) cohort, Grandjean *et al.* (2017) investigated the association between post-natal PFOA exposure and serum antibodies against childhood vaccines in 561 children at age 13. A number of children were admitted to an emergency room and/or had higher antibodies than at age 7. The authors suggested that these children may have had booster vaccinations. These children were excluded from subgroup analyses. Anti-diphtheria antibodies at age 13 decreased slightly with increasing PFOA exposure at age 7 or 13. The association was not statistically significant, however, except in the subgroup analysis of children that received no booster or had an emergency room visit (but again, was not statistically significant in the subgroup of children who received no booster, had an emergency room visit, and had no antibody increase). Anti-tetanus antibodies *increased* with increasing PFOA exposure, but these results were not statistically significant.

In a prospective cohort study, Granum *et al.* (2013) evaluated PFOA and measured vaccine antibody levels and incidence of common infectious disease in a sub-cohort of 99 mother-child pairs in the Norwegian Mother and Child Cohort Study. Maternal serum PFOA was statistically significantly negatively associated with rubella anti-vaccine antibody levels ($p = 0.001$) but not measles, *Haemophilus influenzae* type b, or tetanus antibody levels. The authors did not measure incidence of rubella to determine if the decreased antibody levels increased risk of disease. Maternal PFOA concentration was also associated with an increased number of episodes of the common cold, but was not associated with increased gastroenteritis episodes.

A number of studies have investigated the association between serum PFOA concentration and allergic and infectious disease. Okada *et al.* (2014) evaluated the association between PFOA and other PFCs and the development of allergic diseases (eczema, asthma symptoms, nasal allergy symptoms) in the first year of life and again between 12 and 24 months of age. The study included 2,063 mother-child pairs enrolled in the Hokkaido Study on Environment and Children's Health, a prospective cohort study. Prenatal PFOA exposure was estimated using maternal blood samples taken between 28 and 32 weeks of pregnancy. Health was assessed through parental report based on a questionnaire (which included questions such as, "Has your child had an itchy rash at any time in the past 12 months?"). Adjusted ORs suggested non-significant, decreasing risk of total allergic disease with increasing PFOA exposure (second quartile: OR = 1.05, 95% CI: 0.81-1.37; fourth quartile: OR = 0.79, 95% CI: 0.59-1.04). Similar results were reported for eczema analyzed alone. The authors suggested that prenatal exposure to PFOA may suppress allergic immune system responses during early life. This study is limited by its use of parent-reported symptoms, rather than physician-confirmed allergic immune conditions, and the lack of measurement of post-natal PFC exposure.

Goudarzi *et al.* (2017) conducted additional study of 1,558 mother-child pairs enrolled in the Hokkaido Study on Environment and Children's Health who responded with information on health status at 4 years of age. Mothers reported the occurrence of four doctor-diagnosed childhood infectious diseases – otitis media (middle ear infection), pneumonia, varicella (chicken pox), and respiratory syncytial virus infection. Prenatal PFOA exposure was estimated using maternal blood samples taken between 28 and 32 weeks of pregnancy. The authors found no associations between PFOA and total infectious diseases. Note that these results suggest that the possible immune suppression observed by Okada *et al.* (2014) in this cohort appears not to have manifested in later risk of infectious disease in these children.

Smit *et al.* (2015) evaluated PFOA exposure and asthma, eczema, and wheeze in 1,024 children aged 5-9 years whose mothers were enrolled in the Biopersistent Organochlorines in Diet and Human Fertility (INUENDO) Cohort between 2002 and 2004. Exposure was assessed using measurements of maternal PFOA serum concentrations at enrollment, and health outcomes were determined based on interviews with pediatricians and local health workers. PFOA was associated with *decreases* in the risk of ever experiencing asthma, eczema, or wheeze, as well as contemporaneous wheeze and eczema.

Dalsager *et al.* (2016) evaluated the association between prenatal exposure to PFOA and parent-reported symptoms of infection over the course of a year (fever [$>38.5^{\circ}\text{C}$], stuffed or runny nose, cough, wheezy or whistling breathing, eye inflammation, ear pain, discharge from ear, feeling unwell, diarrhea, blood in stool, and vomiting) in 649 children 1-4 years old enrolled in the Odense Child Cohort. Children in the highest tertile of PFOA exposure had a statistically significant increase in odds of experiencing days with fever above the median (OR = 2.35, 95% CI: 1.31, 4.11). The median number of days was not reported, but the mean was only 1.6 days (range: 0-10.3). Limitations include a low original cohort participant rate (42%), some non-response issues (86% response in each weekly period), and the likelihood of bias in reporting of symptoms. Finally, some included symptoms may have been associated with non-infectious conditions, such as an allergy.

Zhu *et al.* (2016) conducted a case-control study that included 231 10- to 15-year-old Taiwanese children diagnosed with asthma within the previous year, and 225 non-asthmatic control children. Serum was collected at study initiation and levels of PFCs, T_H cytokines (Interleukin 2, 4, and 5 [IL-2, IL-4, IL-5], and interferon gamma [IFN- γ]), and immunoglobulin E (IgE) were measured. Increasing serum PFOA concentration was associated with an increased OR for asthma ($p = 0.001$); by quartile, the ORs were not statistically significant in the second quartile, but were positive for the fourth quartile. As serum PFOA concentration increased, T_H2 cytokines (IL-4 and IL-5) significantly increased in male children with asthma; however, the other markers were not significantly changed. Among females with asthma, only IgE was statistically significantly associated with PFOA.

In a prospective cohort study, Timmermann *et al.* (2017) assessed prenatal and post-natal PFOA exposure and asthma and allergic diseases at ages 5 and 13 in participants of the Children's Health and Environment in the Faroe Islands (CHEF) Project Cohort. The authors also evaluated the potential impact of measles, mumps, and rubella (MMR) vaccination on the association between PFOA and asthma/allergic diseases. PFOA was measured in maternal blood in gestational weeks 34-36 and in the children's blood at ages 5, 7, and 13. Total IgE concentrations were measured in cord blood and again at age 7. At age 5, investigators asked parents if the child had been diagnosed with asthma, hypersensitivity, or allergy. At age 13, investigators asked parents if the child had "suffered from" asthma or eczema or had experienced rhinoconjunctivitis symptoms in the past 23 months. A doubling of age 5 serum PFOA concentration was associated with an increased risk of asthma at age 5 (OR = 10.37, 95% CI: 1.06-101.93) in all the children. When the authors restricted the analysis to children with the MMR vaccine, the risk of asthma was *decreased* with a doubling of PFOA serum concentration. A doubling of serum PFOA concentration at age 5 was not associated with a risk of asthma, positive skin prick test, rhinoconjunctivitis, or atopic eczema at age 13 (regardless of MMR status, although authors only calculated risk of asthma in MMR-vaccinated children). PFOA measured at age 13 was associated with a non-significant increase in atopic eczema, but non-significant *decreases* in risk of asthma, positive skin prick, and allergic rhinoconjunctivitis. Changes in IgE based on PFOA concentrations were not consistent or statistically significant. Authors noted that very few children in the cohort had not had an MMR vaccine.

Finally, I identified a single study of autoimmune conditions in the Mid-Ohio Valley community-based cohort comprising people who lived in any of the PFOA-contaminated water districts in that area (Steenland *et al.*, 2013). Note, however, that these community residents were combined with workers to form the final population in this study. Using a retrospective cohort design, the authors assessed the risk of ulcerative colitis, Crohn's disease, rheumatoid arthritis, Type 1 diabetes, lupus, multiple sclerosis, and any other conditions identified based on medical-record-validated self report. Past exposures (historical yearly serum PFOA estimates) were estimated based on the amount of PFOA released from the DuPont plant, wind patterns, river flow, groundwater flow, and the residential address history provided by the participant. The only statistically significant trends occurred for PFOA and ulcerative colitis, which were apparent in models for both unlagged and 10-year lags for disease latency. In contrast, *decreases* in disease risk were apparent for Type 1 diabetes (particularly unlagged) and rheumatoid arthritis. The trends were generally not statistically significant, although individual quartiles did achieve significance (*e.g.*, fourth versus first quartile of exposure, for rheumatoid arthritis).

Overall, the available studies of PFOA and immune markers and diseases, conducted predominantly in children, are inconsistent. Some studies measured only antibody titer levels, and the reductions in antibodies may not be associated with later risk of disease. Positive associations with frank disease (including fever and risk of asthma) generally were only present in higher quartiles of exposure and showed no apparent exposure-response relationships. Further, several studies indicated a *decreased* risk of some of the same immune conditions and infections, including asthma and total allergic disease.

7.1.4.6 Kidney Effects

I identified three studies of PFOA and kidney disease in exposed communities (Steenland *et al.*, 2010b; Watkins *et al.*, 2013) and the general population (Shankar *et al.*, 2011). In a cross-sectional study, Steenland *et al.* (2010b) found a modest but significant increase in the odds of hyperuricemia, or excess uric acid in the blood, (>6.0 mg/dL for women, >6.8 mg/dL for men) by quintile of PFOA concentration in adult residents and workers in PFOA-contaminated water districts in Ohio and West Virginia. A linear trend test for this effect was statistically significant ($p < 0.0001$); however, the authors note that the trend appeared to plateau rather than increase in a linear fashion. The authors also found a 0.2-0.3 $\mu\text{g/dL}$ increase in uric acid levels between the highest and lowest decile of PFOA concentrations. It is unclear whether, but unlikely that these changes were clinically significant, because the average level of uric acid for the cohort (men and women) was 5.58 mg/dL (range: 4.5-6.6 mg/dL).

Watkins *et al.* (2013) conducted a cross-sectional evaluation of serum PFOA concentrations and estimated glomerular filtration rate (eGFR), a measure of kidney function, in children and adolescents in the C8 Cohort. The authors reported a decrease in eGFR of 0.75 mL/min/1.73 m² (95% CI: -1.41 to -0.10; $p = 0.02$) per IQR increase of serum PFOA concentration (IQR $\ln[\text{PFOA}] = 1.63$). There was no association between eGFR and predicted PFOA concentrations at birth, during childhood, or at time of enrollment estimated from historical PFOA exposure data. Thus, the authors suggest that the association between eGFR and serum PFOA concentration is a consequence, rather than a cause of, decreased kidney function.

Finally, Shankar *et al.* (2011) conducted a cross-sectional study of PFOA and eGFR and kidney disease in the general population using 1999-2000 and 2003-2006 NHANES data. The authors reported that mean eGFR was decreased by 7.6 mL/min/1.73 m² (95% CI: -9.6 to -5.6) in the fourth quartile of serum PFOA concentration compared to the first quartile in a study of the general population (p for trend < 0.0001). The authors also found significantly increased odds of chronic kidney disease for the fourth quartile of serum PFOA concentration (OR = 1.74, 95% CI: 1.06-2.84). The odds of chronic kidney disease in quartiles two and three were non-significantly increased.

Overall, the limited and inconsistent evidence, which is limited to cross-sectional analyses, indicates that there is no clear association between exposure to PFOA in the general population and exposed communities and kidney disease.

7.1.4.7 Cancer

As noted previously, IARC (2016a) recently classified PFOA as Group 2B, *i.e.*, possibly carcinogenic to humans. This classification was based on "limited evidence" for the carcinogenicity of PFOA in both humans and animals. It should be emphasized that IARC did not identify PFOA as a known human carcinogen. The finding for humans was based primarily on evidence of testicular and kidney cancer. The epidemiological evidence, including the studies of the general population that are described below, does not provide reliable evidence that PFOA is carcinogenic to humans.

General Population

Eriksen *et al.* (2009) conducted a case-cohort study of PFOA exposure and prostate, bladder, pancreatic, and liver cancer in a cohort of 1,140 men and women living in Denmark. Plasma was collected at recruitment; PFOA concentrations averaged 6.8 and 6.9 ng/mL in male patients and controls, respectively, and 6.0 and 5.4 in female patients and controls, respectively. The authors found no

statistically significant associations at any dose level and no dose-response trends between plasma PFOA concentration and any of these four cancer types.

Similarly, Vassiliadou *et al.* (2010) found no significant difference in serum PFOA concentrations in cancer cases (all types) at a hospital in Athens, Greece, compared with patients without cancer at an urban or rural/semi-urban hospital. Serum PFOA concentrations were low in both cases and controls, with mean concentrations ≤ 2.0 ng/mL.

Hardell *et al.* (2014) conducted a case-control study of 201 prostate cancer case and 186 control participants residing in Sweden. Blood was collected between 2007 and 2011 at enrollment, prior to any cancer treatment. Serum PFOA (median concentration of 1.9 ng/mL in controls and 2.0 ng/mL in cases) was not associated with increased risk of prostate cancer. However, in an analysis including hereditary risk (*i.e.*, a family history of this type of cancer), the authors reported that compared to participants with a hereditary risk of prostate cancer but a serum PFOA concentration below the median, those with a hereditary risk of prostate cancer and a serum PFOA concentration above the median had a significantly increased risk of prostate cancer (OR = 2.6, 95% CI: 1.2-6.0). This suggests a possible increase in risk of prostate cancer in individuals with PFOA exposure and a family history of prostate cancer, but only with high PFOA exposure levels. Note that PFOA was measured only at the time of diagnosis, and serum concentrations were likely different at the time of disease initiation (cancer latency is often a decade or more).

Innes *et al.* (2014) investigated the cross-sectional association between serum PFOA concentration and colorectal cancer in the C8 Cohort and found an inverse dose-response relationship (*e.g.*, OR = 0.64, 95% CI: 0.44-0.94, $p = 0.02$, in the highest vs. lowest quartile of exposure). In other words, the risk of this type of cancer was reduced as PFOA exposure increased. As with Hardell *et al.* (2014), this study was limited by its use of single, cross-sectional measures of current PFOA exposure as surrogates for PFOA exposure at the time of cancer initiation. The authors also lacked information on important colorectal cancer risk factors, including inflammatory bowel disease. However, the authors were able to test for the effects of dietary folate, anemia, gastrointestinal symptoms, and other factors that could potentially affect the associations observed and found no significant alterations.

Wielsoe *et al.* (2017) conducted a case-control study of PFOA exposure and breast cancer in Greenland. Cases ($n = 97$) were recruited at diagnosis from a hospital in Nuuk, Greenland, between 2000 and 2003 and again from 2011-2014. Controls ($n = 177$) were recruited from two cross-sectional studies of healthy persons with serum PFOA measurements or from the same hospital as cases (and were patients for non-malignant disease). Breast cancer cases had significantly higher serum PFOA concentrations compared with controls ($p = 0.009$); this corresponded with a statistically significant relationship between increasing serum PFOA concentration and a slightly elevated risk of breast cancer (OR = 1.26, 95% CI: 1.01-1.58). When stratified by exposure tertile, only women in the third tertile of exposure (≥ 2.91 ng/mL; maximum measured: 9.52 ng/mL) had a significantly increased risk of breast cancer (OR = 2.64, 95% CI: 1.17-5.97; $p = 0.019$).

Some limitations in this study include the differing control groups (hospital-based *versus* population controls). Further, the serum PFOA concentrations were measured at the time of diagnosis; it is likely that the concentrations of PFOA were different at the time when cancer development was initiated (one to several decades prior to diagnosis). The etiology of breast cancer differs depending on whether it develops pre- or post-menopause; the authors indicated that similar ORs were calculated when stratified on menopause status, but the data were not shown. Note that the analysis of cases recruited between 2000 and 2003 only (Bonefeld-Jorgensen *et al.*, 2011) found no statistically significant associations between PFOA and breast cancer risk.

Exposed Communities

Barry *et al.* (2013) conducted a retrospective cohort study of cancer incidence in residents exposed to PFOA in drinking water near a production plant in the Mid-Ohio Valley (C8 Cohort). Serum PFOA was measured in 2005 and 2006, and retrospective yearly PFOA serum concentrations were estimated from 1952-2011 based on residential histories, ADME parameters, and historical regional data, including PFOA emissions by the DuPont facility (accounting for wind patterns, river flow, and groundwater flow). Cancer status was assessed in questionnaires through 2011; diagnosis was confirmed through medical chart review or state cancer registry matching.

With no lag for exposure, PFOA was borderline statistically significantly associated with testicular cancer ($p = 0.05$), but the CI for the HR included "1." No association was apparent with a 10-year lag. PFOA was associated with a statistically significant *reduction* in breast cancer risk with a 10-year lag (association with no lag was borderline significant, $p = 0.05$). Overall associations between PFOA and other cancer types (*e.g.*, bladder, kidney, thyroid, prostate) were null. The authors also ran analyses by PFOA exposure quartiles for kidney, testicular, and thyroid cancer. Kidney and thyroid cancer showed no clear dose-response relationships, and associations remained not statistically significant. Testicular cancer showed some suggestion of a dose-response relationship with increasing PFOA quartile (based on increased HRs); however, the CIs were wide and all overlapped "1." An important limitation of this study is the potential for exposure misclassification (*i.e.*, error in the exposure estimates) based on modeled historical serum concentrations.

The weight of evidence does not establish an association between PFOA and any cancer type (*e.g.*, bladder, kidney, thyroid, prostate) in general populations studies or exposed communities.

7.1.5 C8 Science Panel

The C8 Science Panel was formed as part of a class action settlement of a lawsuit involving releases of PFOA (also known as C8) from an industrial site in West Virginia. The Panel was tasked with determining whether there was a probable link between PFOA and any human disease. In this legal context, a probable link is defined as, given the scientific evidence, it is "more likely than not that a connection exists" (C8 Science Panel, 2017). As noted by the C8 Science Panel (Steenland *et al.*, 2014), "The criterion for probable link comes from the common law concept of a cause being more likely than not, as distinct from the criminal law burden of 'proof beyond reasonable doubt.'" It is important to note that a "probable link" in a legal context is not the same as a statistically significant association in a scientific context. In the scientific literature, an association is generally accepted as statistically significant if there is $\geq 95\%$ confidence that the association is not a random occurrence. In this legal context, the probable link only needs to be "more likely than not," *i.e.* there must be only a $>50\%$ chance that the association is not random. This standard would not be acceptable in the scientific literature as evidence of an association. In addition, a "probable link" designation does not address whether or not the link is causal.

In making determinations for each disease endpoint, the Panel was required to: (1) conduct community studies in the community of Mid-Ohio Valley residents who lived in the area surrounding the industrial site and were exposed to PFOA in their drinking water, and (2) consider an ongoing study of site workers conducted by the company that owned the site. The Panel was also permitted to consider any other epidemiology evidence it believed to be relevant. The Panel listed the following criteria it used to evaluate the evidence for a probable link: strength and consistency of reported associations, evidence of a dose-response relationship, the potential for associations to occur as a result of chance, adequacy of

control for biases and other causes, and plausibility based on experiments in laboratory animals. The investigations and analyses were carried out from 2005-2013 (C8 Science Panel, 2017).

The C8 Science Panel determined that there is a probable link between PFOA and the following six diseases: high cholesterol, thyroid disease, pregnancy-induced hypertension/preeclampsia, ulcerative colitis, kidney cancer, and testicular cancer. The diseases that were determined by the Panel to have no probable link to PFOA included any other CVD; any other adverse pregnancy condition or outcome (including developmental effects); any neurological, respiratory, infectious, kidney, or liver disease; Type 2 diabetes; osteoarthritis; any autoimmune disease other than colitis; and any other cancers (C8 Science Panel, 2012a).

In this section, I will discuss the C8 Science Panel's conclusions for the six diseases that it determined had a "probable link" with PFOA exposure and the evidence upon which each determination was based. I will also discuss the lack of consistency between the Panel's findings and the critical effects in the animal studies upon which the MDH and US EPA guidance for PFOA are based.

7.1.5.1 High Cholesterol

The C8 Science Panel determined that there was a probable link between PFOA exposure and high cholesterol, but no probable link between PFOA exposure and any coronary artery disease or disease manifestation, including myocardial infarction, angina, and coronary bypass surgery (C8 Science Panel, 2012b). In other words, there was no link to any clinical manifestations of high cholesterol. This brings into the question the toxicological significance of any link between PFOA exposure and high cholesterol.

In its review of published epidemiology studies of PFOA and cholesterol (also reviewed in Sections 7.1.2 and 7.1.4 of this report), the Panel noted that while 6 of 10 studies reported statistically significant associations between PFOA and LDL, the magnitude of effect was greatest in the populations with the lowest exposure levels, and the smallest effects were seen in the most highly exposed populations (C8 Science Panel, 2012b). This is contrary to a basic tenet of toxicology that for chemicals that cause health effects, higher doses lead to stronger effects (see Section 3.2). The Panel also conducted its own studies of the Mid-Ohio Valley residents and site workers, with mixed and contradictory results. Cross-sectional and longitudinal studies showed a modest but statistically significant effect of PFOA on LDL, but a prospective analysis showed a modest but statistically significant *decrease* in risk of high cholesterol with increasing cumulative PFOA exposure (C8 Science Panel, 2012b).

While the Panel described the evidence as inconsistent, it judged that there was a probable link between PFOA and high cholesterol (C8 Science Panel, 2012b). In my opinion (as described in Sections 7.1.2 and 7.1.4), based on the inconsistent and contradictory findings and the lack of evidence of an association between PFOA and clinical CVD, the weight of evidence does not establish an association between PFOA and high cholesterol.

7.1.5.2 Thyroid Disease

The C8 Science Panel determined that there was a probable link between PFOA and thyroid disease (C8 Science Panel, 2012c). This determination was largely based on its own studies of the Mid-Ohio Valley residents, which yielded inconsistent and contradictory results (reviewed in Section 7.1.4.3). For example, in a study of diagnosed thyroid disease among the community residents and workers, PFOA was associated with both hypothyroidism and hyperthyroidism in women (but not men) (Winquist and Steenland, 2014b). In another study of the same community, neither subclinical hypothyroidism nor subclinical hyperthyroidism were associated with PFOA (C8 Science Panel, 2012c). In fact, there was a

reverse association (*i.e.* reduced risk) between subclinical hyperthyroidism and PFOA among women in this study. Both cross-sectional studies by the Panel investigated associations between PFOA and TSH levels; one showed an association and the other did not (C8 Science Panel, 2012c).

The C8 Science Panel studies had results that were inconsistent: "While *each finding in isolation was not compelling, plausibly a result of chance or other errors*, the presence of some independent pieces of evidence indicative of an association was not easily dismissed, *despite a lack of coherence among them*" (C8 Science Panel, 2012c [emphasis added]). In light of the contradictory, weak, or nonexistent associations, or in some cases reverse associations, of PFOA with thyroid disease or thyroid hormone levels in the C8 Science Panel studies, it is surprising that, even using the "more probable than not" criterion, the Panel came to the conclusion of a probable link. In my opinion, these studies and other studies in the literature do not provide evidence of an association between PFOA and thyroid disease.

7.1.5.3 Pregnancy-induced Hypertension and Preeclampsia

The C8 Science Panel determined that there was a probable link between PFOA and pregnancy-induced hypertension and preeclampsia (C8 Science Panel, 2011a). In this evaluation, the Panel grouped these two related conditions together on the basis of difficulty distinguishing them in the records used for verification of the diseases. The Panel based its determination on six studies of the Mid-Ohio Valley community, no other epidemiology studies, and mechanistic and toxicological evidence from animal studies.

Of the six studies the Panel relied upon for this determination (the ones that have been published are also reviewed in Section 7.1.4.1), five were conducted by the C8 Science Panel. The one that was conducted by an independent group (Nolan *et al.*, 2010) was an ecological study (*i.e.*, exposure was assigned based on place of residence rather than actual exposure measurements) that found no association between ZIP Codes that had elevated PFOA concentrations in drinking water and pregnancy-induced hypertension among residents of one of the towns near the industrial site in the Mid-Ohio Valley.

In a study of pregnancy-induced hypertension by the C8 Science Panel, when exposure was estimated based on place of residence either by ZIP Code or exact street address, there was no association between PFOA and pregnancy-induced hypertension. When the authors limited the data to include only exposure estimates based on exact street addresses, they reported a "small association" that was not statistically significant and did not exhibit a dose-response pattern (C8 Science Panel, 2011a). In a companion study, the Panel also reported no association between PFOA and pregnancy-induced hypertension when PFOA exposure was estimated using the residents' comprehensive residential history, rather than just the point-in-time residential addresses (C8 Science Panel, 2011a; Savitz *et al.*, 2012b). When the authors used an alternative method to estimate serum PFOA concentrations by calibrating to serum concentrations measured in 2005-2006, they reported a "strengthened" association that did not exhibit a dose-response and was only statistically significant for quintile 4 compared to quintiles 1 and 2 combined (C8 Science Panel, 2011a).

The fourth study of pregnancy-induced hypertension used by the Panel in its determination was also a C8 Science Panel study. In this study, serum PFOA concentrations were estimated based on values measured in 2005-2006. No statistically significant association was reported for the 25th vs. 75th percentiles of exposure, but when the exposure was divided by quintiles, there were statistically significant associations in quintiles 3, 4, and 5 compared to quintile 1. The results by quintile did not exhibit a dose response.

In a study of preeclampsia conducted by the C8 Science Panel (Stein *et al.*, 2009; C8 Science Panel, 2011a), the authors reported that measured serum PFOA was "weakly and irregularly" associated with preeclampsia. The association, however, was not statistically significant, and there was no dose response.

A second study of preeclampsia by the Panel used historical estimates of PFOA exposure rather than actual serum measurements. The Panel reported a "modest association" between preeclampsia and estimated serum PFOA concentration, but the results were not statistically significant and did not show a dose response. The authors also used an alternative method of estimating serum PFOA concentrations by calibrating to measured values in 2005-2006, and reported a "strengthened" association that was small but statistically significant for the fourth and fifth quintiles compared to the first and second quintiles combined.

Overall, these studies show weak or no associations between PFOA and pregnancy-induced hypertension/preeclampsia. None of the studies showed a robust dose response between PFOA and pregnancy-induced hypertension or preeclampsia. In fact, in some analyses, the highest PFOA exposures resulted in no increased risk. In addition, all of the studies were of the same community, with overlapping populations of study subjects, and most of the studies used estimated rather than measured serum PFOA concentrations, which makes them subject to possible exposure misclassification.

The Panel also discussed evidence from animal studies of reduced fetal growth at high PFOA doses as support for PFOA's association with pregnancy-induced hypertension and preeclampsia, because in humans, these conditions are associated with reduced fetal growth and an increased risk of preterm birth (C8 Science Panel, 2011a). This is incongruous, however, because the Panel determined that there was no probable link between exposure to PFOA and preterm birth or low birth weight (C8 Science Panel, 2011b). In my opinion, the science does not support there being an association between PFOA and pregnancy-induced hypertension and preeclampsia.

7.1.5.4 Ulcerative Colitis

The C8 Science Panel determined that there was a probable link between PFOA and ulcerative colitis (C8 Science Panel, 2012d). This determination was based on a single study conducted by the C8 Science Panel, because there were no other epidemiology or animal studies that investigated this association. The C8 Science Panel study was based on 161 cases of ulcerative colitis that were validated with medical records. The Panel reported a statistically significant increased risk of the disease with increasing quartiles of cumulative PFOA exposure. PFOA exposure values were derived from historical estimates rather than actual serum measurements. A prospective analysis based on 30 cases and actual serum measurements taken in 2005-2006 also showed a positive trend with increasing quartiles, but was not statistically significant.

The positive trend of higher risk of ulcerative colitis with increasing PFOA concentration merits further investigation. However, it is not possible to draw conclusions based on one study that includes two analyses with very limited numbers of cases, with the analysis based on actual serum measurements showing no statistically significant association. In my opinion, the weight of evidence does not establish an association between PFOA and ulcerative colitis.

7.1.5.5 Kidney Cancer

The C8 Science Panel determined that there was a probable link between PFOA and kidney cancer (C8 Science Panel, 2012e). This determination was based on three epidemiology studies conducted by non-Panel authors and four studies conducted by the Panel. The non-Panel studies consisted of two worker studies (one from the Mid-Ohio Valley site [Leonard *et al.*, 2008]) and one general population study, none of which reported associations between PFOA and kidney cancer (C8 Science Panel, 2012e).

In a Panel cohort mortality study of 5,791 workers at the Mid-Ohio industrial site (Steenland and Woskie, 2012, also reviewed in Section 7.1.2.6), the authors conducted a 6-year follow-up study to the Leonard *et al.* (2008) study and found no new cases of kidney cancer and no statistically significant association between kidney cancer and estimated PFOA exposures when compared to unexposed workers at the same company. They did report that there was a statistically significant positive trend for kidney cancer with increasing quartiles of PFOA exposure. This trend was based on only 12 cases, however, and there were no cases in the third quartile of exposure.

A second Panel study (Vieira *et al.*, 2013) was a geographical analysis comparing cancer rates in exposed vs. unexposed areas of the Mid-Ohio Valley, using either the residents' water districts or modelled exposure based on residents' individual addresses as measures of exposure. The trends for kidney cancer risk with increasing PFOA exposure were inconsistent depending on the measure of exposure used. A statistically significant trend was reported for estimated individual exposures, but not for exposure by water district.

A third Panel study of 49,082 exposed community residents compared cancer incidence with measured serum PFOA concentrations and found no evidence of an association between PFOA and kidney cancer (C8 Science Panel, 2012e).

The fourth Panel study compared cancer incidence to modeled individual estimates of cumulative PFOA exposure in the community residents and workers (C8 Science Panel, 2012e). The authors reported a statistically significant trend across quartiles of exposure in an unlagged analysis but not in a lagged analysis (*i.e.*, only cancers that were diagnosed ≥ 10 years after first exposure were counted). In the same study, there were no statistically significant trends in kidney cancer risk vs. exposure for cases that were diagnosed between the 2005-2006 cohort enrollment and the 2009-2011 resident and worker interviews conducted by the Panel.

The C8 Science Panel determined that these studies provided evidence for a probable link (C8 Science Panel, 2012e). It is important to note, however, that the only study that analyzed the association between PFOA and kidney cancer that employed actual serum PFOA measurements (rather than estimates based on work categories, geographical location, or modeled exposure) showed no association. Furthermore, all of these studies are of the population of residents and workers from the exposed Mid-Ohio Valley community, with overlapping subjects. For these reasons, it is my opinion that the evidence does not support there being an association between PFOA and kidney cancer.

7.1.5.6 Testicular Cancer

The C8 Science Panel determined that there was a probable link between PFOA and testicular cancer (C8 Science Panel, 2012e). This determination was based on the same set of studies as noted above for the kidney cancer evaluation. The three non-Panel studies all showed no association between PFOA and testicular cancer.

In the C8 Science Panel mortality study (Steenland and Woskie, 2012), there was only one death from testicular cancer among the cohort of 5,791 workers, so an association between PFOA exposure and mortality from testicular cancer could not be measured. The Panel reported an elevated risk of testicular cancer in the geographical analysis conducted by Vieira *et al.* (2013). However, the trends for risk with increasing PFOA exposure were not statistically significant when the authors used the most accurate measure of exposure, based on individual residence data. In the study that used actual serum PFOA concentrations for exposure measurements, based on 49,082 total participants (the numbers of males/females were not given), there were too few cases of testicular cancer to perform an analysis (C8 Science Panel, 2012e).

In the final study, the Panel compared cancer incidence to modeled individual estimates of cumulative PFOA exposure in the community residents and workers (C8 Science Panel, 2012e). A positive trend was reported for certain analyses (no-lag analysis with log exposure and lagged analysis with linear exposure) but not for others (no-lag analysis with linear exposure and lagged analysis with log exposure). There was no increased risk of cancer in this population compared to the general US population. In addition, there were no new cases of testicular cancer in this cohort after 2005-2006 (compared to about five expected cases), so the Panel could not conduct a prospective analysis of testicular cancer (C8 Science Panel, 2012e).

Based on these inconsistent findings, the Panel determined that there was a probable link between PFOA and testicular cancer. Again, it is important to note that in the only study that analyzed the association between PFOA and testicular cancer that employed actual serum PFOA measurements, there were too few cases for analysis, and there were no new cases after 2005-2006. For these reasons, it is my opinion that the science does not support there being an association between PFOA and testicular cancer.

7.1.5.7 Overall Conclusions for C8 Science Panel

The C8 Science Panel findings are not held to the same rigorous standard as statistically significant associations that are used for drawing conclusions from epidemiology studies. Therefore, I do not rely on the Panel's findings in my analysis regarding the evidence for associations between PFOA exposure and health effects.

In all of the six C8 Science Panel determinations that reported probable links between PFOA exposure and a disease, the Panel's conclusions were based on inconsistent and contradictory results, positive trends that were not statistically significant, modeled and estimated PFOA exposures rather than actual serum measurements, and/or data that were sorted and organized in several different ways such that for any given set of data, associations were found by some methods and not by others. None of the probable link determinations are based on a robust set of results that clearly showed an association between the disease in question and PFOA exposure.

The Panel's determinations are also not consistent with other epidemiological evidence. For example, the Panel determined that there was a probable link between PFOA exposure and pregnancy-induced hypertension and preeclampsia, conditions that are associated with low birth weight and preterm births. However, the Panel did not find a probable link between PFOA and low birth weight or preterm births. While the Panel found a probable link between PFOA exposure and high cholesterol, there is no evidence of an association between PFOA and CVDs that may be caused by high cholesterol (discussed in Section 7.1). For kidney and testicular cancer, three non-Panel studies found no association with PFOA, but the Panel put more weight on its own studies, despite the limitations of those studies outlined above.

Both MDH and US EPA based their PFOA guidelines on animal studies of developmental outcomes. The C8 Science Panel, however, did not find a probable link between PFOA and adverse birth outcomes. This underscores the overall uncertainty and inconsistency regarding the potential health effects of PFOA upon which both legal and regulatory decisions have been made.

7.1.6 Overall Conclusions for Human Studies of PFOA

The weight of evidence does not establish associations between PFOA exposure and health effects across studies of occupational, community, and general population cohorts. For example, changes in thyroid hormones have been associated with PFOA exposure, but the direction of these changes has been

discordant, and changes among males and females are inconsistent. Further, many studies that report effects have important methodological limitations, such as a cross-sectional study design.

7.2 PFOS

The following subsections discuss studies of PFOS and the most commonly assessed health endpoints in humans, including reproductive and developmental effects, thyroid hormone levels, serum lipid levels and CVD, immunological effects, and cancer. These endpoints have been the focus of recent agency reviews by the US EPA and NTP. Human studies are important to consider to fully understand the toxicity associated with PFOS, because these studies require no between-species extrapolation and represent a range of environmentally relevant exposure levels. In general, PFOS exposure is substantially higher in occupationally exposed individuals relative to those living in communities with a nearby point source of PFOS (e.g., a production plant) and the general population with no nearby sources of exposure (who are exposed to PFOS mainly through low levels in food, water, and consumer products). In contrast, most animal studies are conducted at dose levels that are far higher than would be expected in any human population. Human studies can be used to inform the relevance of the animal toxicity studies and also to determine the likelihood of adverse health effects in populations exposed to PFOS.

Human studies have been conducted in both workers and non-occupational populations to evaluate the association between PFOS exposure and causes of mortality, episodes of care (*i.e.*, health problems under care of a health provider), and various biochemical endpoints. These studies have focused on exposure to PFOS and PFOA. As part of these assessments, 3M has monitored workers' serum PFOS concentrations for several decades. 3M has conducted studies in plants in Cottage Grove, Minnesota; Decatur, Alabama; and Antwerp, Belgium. In addition, 3M and DuPont have conducted epidemiology studies in residents living in areas where PFCs, including PFOS, were found in drinking water. Finally, numerous studies have evaluated various health endpoints in both US and non-US populations exposed to environmental background levels of PFOS through water, food, and consumer products. Note that while PFCs have been measured in the serum of people around the world, as stated by the CDC, "[f]inding a measureable amount of PFCs in serum does not mean that the levels of PFCs can cause an adverse health effect" (CDC, 2009a).

Some findings may be a result of reverse causation. Reverse causation occurs when the likelihood of an outcome is causally linked to the exposure of interest. For example, menopause may cause increased PFOS concentrations in the blood of women with menopause relative to women still menstruating, because there is no longer a menstrual blood pathway of excretion in the former group. Thus, it may appear from a study that PFOS contributes to menopause onset, when in fact, menopause causes an increase in serum PFOS concentration.

The weight of evidence does not establish an association between health effects or changes in clinical chemistry parameters and PFOS exposure in workers who have been exposed to high levels of PFOS for long periods of time, in residents with PFOS in their drinking water, or in populations with background exposure to PFOS. In many cases, when health effects were seen, the changes were of unclear clinical significance, or in some cases, were not consistent with animal or mechanistic data for the same endpoints.

7.2.1 Serum Concentrations in Workers

Table 7.4 summarizes the PFOS biomonitoring data for 3M's Antwerp and Decatur plants. As can be seen in the table, the PFOS concentrations at the Decatur plant range from a minimum of 0.015 ppm to a

maximum of 10.6 ppm (or µg/mL, equivalent to 15-10,600 ppb), while the arithmetic means range from 1.621 ppm (or µg/mL, equivalent to 1,621 ppb) in 2002 to 1.290 ppm (or µg/mL, equivalent to 1,290 ppb) in 1995. At the Antwerp plant, the range in PFOS concentrations was smaller, and both the maximum and means were smaller than at the Decatur plant: a minimum of 0.04 ppm to a maximum of 6.24 ppm (or µg/mL, equivalent to 40-6,240 ppb), with arithmetic means ranging from 0.50-0.950 ppm (or µg/mL, equivalent to 500-950 ppb). It should be noted that all the data are cross-sectional (*i.e.*, measured on a single occasion) and, because the serum concentrations provided in these studies are presented as averages across the surveillance groups, they do not provide information about serum concentrations over time on an individual basis. Finally, the serum concentrations of PFOS measured in these working populations are several orders of magnitude higher than expected in the general population or even in communities exposed to PFOS in drinking water.

Table 7.4 Summary of 3M Occupational PFOS Serum Concentrations (ppb or ng/mL)

Plant	N	Range	Arithmetic Mean	Geometric Mean	Reference
Antwerp					
1995	88	0-12,830 ^a	1,930 ^a	NR	Olsen <i>et al.</i> (1999)
1997	65	100-970 ^a	1,480 ^a	NR	
2000	196	40-6,240	950	NR	Olsen and Zobel (2007)
2000	255	40-6,240	800	440	Olsen <i>et al.</i> (2003a)
2001	30	190-1,350	500	430	Olsen <i>et al.</i> (2003c)
Cottage Grove					
2000	122	30-4,790	860	NR	Olsen and Zobel (2007)
2002	38	50-1,170	334	254	Olsen and Mandel (2003a)
Decatur					
1995	90	0-12,830 ^a	2,440 ^a	NR	Olsen <i>et al.</i> (1999)
1997	84	100-970 ^a	1,960 ^a	NR	
1998 (chemical plant)	126	91-10,600	NR	941	Olsen <i>et al.</i> (2003d)
1998 (film plant)	60	15-946	NR	136	
2000	188	60-4,170	1,290	NR	Olsen and Zobel (2007)
2000	263	60-10,060	1,320	910	Olsen <i>et al.</i> (2003a)
2002	54	82-4,258	1,621	1,008	Olsen and Mandel (2003b)

Notes:

N = Number of Participants; NR = Not Reported; ppb = Parts Per Billion.

(a) For the Antwerp and Decatur plants, combined.

(b) Baseline serum concentrations before demolition of manufacturing facility.

7.2.2 Health Endpoint Studies in Workers

Several epidemiology studies have evaluated the relationship between exposure to PFOS and several different disease outcomes, as well as disease biomarkers, in occupationally exposed populations. These populations had much higher PFOS exposures than what would be expected in the general population. Studies conducted at 3M PFC manufacturing facilities have used both retrospective and prospective longitudinal study designs (*i.e.*, the populations were followed over time, often many years, to examine health endpoints) for large cohorts (N = 1,400 to approximately 6,000) to evaluate potential health effects associated with PFOS at exposures that are substantially higher than general population exposures. These studies should be relied on preferentially over cross-sectional (*i.e.*, studies that measure exposure and outcomes at a single point in time) general population studies, which, by study design, are insufficient to determine causation. Moreover, as noted above, because the worker studies involved serum PFOS concentrations at least 20-fold higher than concentrations found in the general public, the occupational studies are generally more useful for establishing associations between exposure to PFOS and health

effects, because any potential health effects from exposure to PFOS are more likely to occur at high exposure levels, relative to low exposure levels. Despite the advantages of the worker studies, there are still some methodological issues in certain studies. For example, the mortality studies that compared the rates of disease in the workers to those in the general population may have been subject to the healthy worker effect.

Overall, I conclude that the weight of evidence from worker studies does not establish an association between PFOS exposure and adverse effects in workers. In particular, there has been no increase in liver disease risk, which US EPA has identified as a key endpoint of concern for PFOS. Changes in serum lipid levels (HDL/triglycerides) have been statistically significantly elevated in some of the worker studies. These changes are inconsistently observed and of limited biological relevance, because there is no evidence that PFOS is associated with CVD or metabolic syndrome.⁷⁷ There is also no evidence that PFOS is associated with any types of cancer in occupational studies. The subsections below summarize studies in workers for these key endpoints in relation to PFOS.

7.2.2.1 Reproductive and Developmental Effects

Partially due to the small female populations exposed to PFOS at the workplace, there are very few occupational studies of reproductive or developmental effects of PFOS in humans. I conclude that the weight of evidence available in these populations does not establish a causal association between PFOS exposure and any developmental or reproductive effects.

Grice *et al.* (2007) conducted a retrospective cohort study of 1,400 workers from the Decatur plant. Job-specific exposure categories were determined based on job titles, departments, dates of employment, and potential for PFOS exposure and grouped by "ever high," "ever low," "low or high ≥ 1 year," or "high > 1 year." Amongst women reporting singleton pregnancies while employed ($n = 439$), 421 resulted in a live birth, 14 in a stillbirth, and 4 had missing data. Because numbers were low, no analysis of stillbirth was conducted. The authors reported no statistically significant association between PFOS exposure and birth weight, regardless of exposure category. In analyses that estimated exposure up to 1 year before pregnancy and those that estimated cumulative exposure the results remained the same (*i.e.*, null).

7.2.2.2 Liver Enzymes

Based on evidence from studies of workers with high exposures to PFOS, I conclude that PFOS is not associated with consistent or adverse changes in liver enzyme parameters in occupational cohorts.

Olsen *et al.* (1999) conducted cross-sectional analyses of the association between PFOS exposure and markers of liver function (including ALP, AST, ALT, GGT, and total liver panel) in the Decatur, Alabama, and Antwerp, Belgium, populations undergoing voluntary routine surveillance examinations in 1995 and 1997 ($n = 178$ and 149 male employees in 1995 and 1997, respectively). The authors reported no statistically significant associations between serum PFOS and any of the parameters measured. In a longitudinal analysis of the Antwerp and Decatur populations participating in the 2000 surveillance, Olsen *et al.* (2003a) reported that while there was a slight increase in ALP with higher serum PFOS in both sexes and a slight increase in ALT in the highest exposure group in males, most individuals' values were within clinical reference ranges. There were no statistically significant associations between PFOS

⁷⁷ Metabolic syndrome consists of a group of risk factors (large waistline, high serum triglycerides, low serum HDL, high blood pressure, and high fasting blood sugar) that together may signify an increased risk for heart disease, diabetes, or stroke (National Heart, Lung, and Blood Institute, 2016).

exposure and the other liver function tests (*i.e.*, GGT, AST, total bilirubin, or direct bilirubin), after adjustment for potential confounders.

In a longitudinal study of 179 workers involved in the demolition of PFC manufacturing facilities, Olsen *et al.* (2012) reported that there was no association between serum PFOS concentrations and the liver enzymes AST and ALT in the entire study population or any subset of it. Serum PFOS concentrations in this study covered a large range (approximately 1-1,000 ng/mL PFOS), and subanalyses considered the effects of lower baseline values (<50 ng/mL PFOS) and those with changes in serum PFOS concentration over the study period. Because this study used a longitudinal design, the results are more reliable than the previous Olsen *et al.* (1999) cross-sectional study.

7.2.2.3 Liver Disease

After reviewing the available studies of workers with high exposures to PFOS, I conclude that the evidence does not support an association between PFOS and liver disease in humans. The available studies (including those investigating mortality incidence and episodes of care studies) conducted at 3M plants demonstrate a lack of an association between liver-related disorders and PFOS exposure.

Mandel and Johnson (1995) conducted a cohort mortality study of 1,957 employees who worked at least 1 year at the 3M plant in Decatur, Alabama; cause of death was followed through December 31, 1991. Deaths from cirrhosis of the liver were not significantly different from the expected deaths from this cause in the general US population. Total mortality however, was lower than expected, indicating there may have been a healthy worker effect (*i.e.*, as noted previously, because severely ill and chronically disabled individuals are generally excluded from employment in certain fields, there are fewer sick individuals relative to the general population).

Alexander *et al.* (2003) conducted a retrospective cohort mortality study of 2,083 workers who worked at least 1 year in the Decatur plant from 1961-1997. The incidence of mortality from cirrhosis of the liver was not significantly increased in this population relative to the general population of Alabama. Total mortality was lower than expected in this study as well, indicating a potential healthy worker effect.

Grice *et al.* (2007) investigated associations between self-reported health conditions, including cancer and non-cancer conditions such as ulcers, liver disease, kidney disease, and prostate conditions, in a retrospective cohort study of 1,400 workers from the Decatur plant. The authors reported that there was no statistically significant association between PFOS exposure and liver disease (including cirrhosis and hepatitis) in any of the exposure groups (*e.g.*, ever high, ever low, low or high ≥ 1 year, or high > 1 year).

There were no statistically significant associations between employment at the Decatur 3M plant and mortality from liver disease (cirrhosis) or self-reported liver disease (cirrhosis and hepatitis).

7.2.2.4 Serum Lipids

Several studies have investigated the association between PFOS exposure and serum lipids in worker populations (Olsen *et al.*, 1999, 2003a, 2012). Overall, I conclude that there is no reliable association between PFOS exposure and clinically meaningful alterations in serum lipids in these populations.

Olsen *et al.* (1999) conducted cross-sectional analyses of the association between PFOS exposure and serum lipids in the Decatur and Antwerp plant populations undergoing voluntary routine surveillance examinations in 1995 and 1997 ($n = 178$ and 149 male employees in 1995 and 1997, respectively). After controlling for potential confounders, such as age, BMI, and smoking, the authors found that HDL

decreased with increasing serum PFOS concentration ($p = 0.04$) in 1995, but not in 1997. LDL and total cholesterol increased significantly with increasing serum PFOS concentration in 1997 ($p = 0.01$), but neither was significantly altered in 1995. Triglycerides were not significantly different among exposure groups in either year. The clinical significance of these changes are questionable. Average total cholesterol was increased in higher relative to lower exposure groups; however, the relative difference in this value between the exposure groups was only about 30 mg/dL. Further, the average LDL and HDL values were well within the definitions of desirable normal levels (A.D.A.M. Medical Encyclopedia, 2014). This study was limited by very low participation rates (35.6% in 1995 and 29.8% in 1997) and a cross-sectional study design.

In the 2000 follow-up cross-sectional analysis of the Decatur and Antwerp plant populations, Olsen *et al.* (2003a) measured serum PFOS concentration and serum lipids in 263 Decatur plant employees (215 males, 48 females) and 255 Antwerp plant employees (206 male and 49 female). In males, cross-sectional measures of serum triglycerides were increased in the highest quartile of exposure (mean: 2.69 ppm) relative to the lowest quartile (mean: 0.27 ppm); however, there were no cross-sectional associations between PFOS exposure and HDL or total cholesterol. None of the serum lipids were significantly different across exposure groups in females. Further, the authors found no statistically significant associations in the 6-year longitudinal analysis for 174 male employees for either total cholesterol and triglycerides.

Olsen *et al.* (2012) conducted a longitudinal assessment of lipids in 179 workers at the Decatur and Cottage Grove plants involved in the demolition and disposal of some of the former manufacturing facilities (3M and non-3M) between 2008 and 2010. The authors ran several models – in model 4 (98 participants whose baseline serum PFOS concentration was <50 ng/mL and did not change over the follow-up period), there was a statistically significant association between increasing PFOS concentration and increasing HDL (1.23 ng/mL increase, $p = 0.04$). No associations were found for the other models, including the model of the entire study population, or any of the other parameters (total cholesterol, non-HDL cholesterol, total cholesterol/HDL ratio).

Overall, I conclude that there is no reliable evidence that PFOS causes adverse changes in serum lipids in workers. While statistically significant associations were reported in worker studies between PFOS serum concentrations and increased measures of some serum lipids, the evidence is inconsistent across studies. In addition, of the two longitudinal analyses, which are more reliable, one showed no associations for any lipids, and the other showed associations only with HDL. When associations were observed in the cross-sectional analyses, changes were modest and within normal values (*i.e.*, not clinically meaningful). It also does not appear that any of the available studies adjusted for all potential confounding factors, such as diet (particularly, saturated fat intake). Finally, in many cases, the changes in lipid concentrations are in the opposite direction of those observed in animal studies; if the association were causal, one would expect the pattern of change(s) to be consistent among animals and humans.

7.2.2.5 Cardiovascular Disease

One of the key health concerns associated with changes in serum lipids is CVD. It is noteworthy that, for workers exposed to high levels of PFCs, most studies show no association with risk of CVD or CVD mortality (Mandel and Johnson, 1995; Alexander *et al.*, 2003).

Mandel and Johnson (1995) conducted a cohort mortality study of 1,957 employees who worked for at least 1 year at the 3M plant in Decatur, Alabama; cause of death was followed through December 31, 1991. The authors calculated the rates of death from "all heart disease" and cerebrovascular disease⁷⁸ and

⁷⁸ Group of diseases related to the blood vessels, particularly those that supply the brain, including stroke.

compared them to expected rates for the US as a whole and the population of Alabama (Mandel and Johnson, 1995). The SMRs for both of these diseases were below expected (though the reduction was not statistically significant).

Alexander *et al.* (2003) conducted a cohort mortality study of 2,083 workers who worked for at least 1 year in the Decatur plant during 1961-1997. As in Mandel and Johnson (1995), the authors found that the SMR for both cerebrovascular disease and "all heart disease" was decreased compared to expected rates; in this study, the decrease for "all heart disease" was statistically significant in the cohort as a whole and in the subanalysis restricted to workers ever employed in a job involving high PFOS exposure. As in the other mortality studies, however, the healthy worker effect was possible in both of these studies, especially given the authors' findings that total mortality for all causes was significantly reduced compared to the US population.

Finally, although not described in detail in this report, diabetes, which is a metabolic syndrome with links to serum lipoproteins, was also not reliably elevated in occupational cohort studies of PFOS (Mandel and Johnson, 1995; Alexander, 2001).

The weight of evidence does not establish an association between occupational exposure to PFOS and increased risk of mortality from CVD. The available studies show no increased mortality from CVD in the Decatur plant worker population, whose serum PFOS concentrations were well above those of the general population (Mandel and Johnson, 1995). I was unable to identify any studies of the incidence of CVD in surviving occupational populations (rather than mortality).

7.2.2.6 Cancer

Several studies have been conducted on the worker population at the Decatur, Alabama, 3M plant (Mandel and Johnson, 1995; Alexander *et al.*, 2003; Alexander and Olson, 2007; Grice *et al.*, 2007). Overall, these studies provide no evidence of consistent increased risk of cancer in populations with high exposure to PFOS in the workplace.

Mandel and Johnson (1995) conducted a retrospective mortality study of 1,957 3M employees (1,639 males and 318 females) at the Decatur plant for at least 1 year after March 1, 1961. The authors collected information on mortality status and cause of death through December 31, 1999, *via* death certificates. SMRs were calculated by comparing the number of deaths in the workers to the death rates in three populations: the US population, the population of the State of Alabama, and the populations of Alabama counties with more than half the county within 100 miles of Decatur (excluding cities >100,000 residents). There were no significant increases in SMRs for all cancers combined and all specific cancer types investigated (including numerous types of digestive, respiratory, reproductive, kidney, nervous system, and hematopoietic tissue cancers) for either sex or either department (chemical or film), as compared to all three reference populations.

Alexander *et al.* (2003) conducted a cohort mortality study of 2,083 members of the Decatur plant cohort followed from 1 year of employment until December 1998 or their death (median: 25.9 person-years of follow-up). Serum samples were collected from a random sample of employees in 1998 (N = 232). Because the investigators knew that serum PFOS concentrations were associated with specific jobs, the authors determined exposure using a matrix based on job, department, and work history, and divided workers into unexposed, low, and high exposure categories. The mortality rates for all cancers combined and individual cancers (including breast, bladder, melanoma, and lymphatic/hematopoietic, among others) were well below the expected rates compared to the general population of Alabama. When deaths in the

high exposure group were selected, the SMR for bladder cancer was significantly higher than the SMR in the general population (0.19 expected, 3 observed; SMR = 16.12, 95% CI: 3.32-47.41).

Grice *et al.* (2007) conducted a cohort study on 1,400 employees at the Decatur plant. The prevalence of diseases were ascertained using self-administered questionnaires, with confirmation of prostate, colon, breast, and melanoma cancer cases through medical records. Incidence of melanoma, prostate, and colon cancer, the most commonly reported cancers, were not statistically significantly associated with any of the PFOS exposure categories. The low number of cases of other cancers (*e.g.*, breast) precluded the calculation of risk estimates for these cancer types.

Alexander and Olson (2007) conducted a cohort study of bladder cancer in 1,895 current and former employees of the Decatur plant. Eleven cases of bladder cancer were identified, including six from self-administered surveys (verified by doctors) and five from death certificates. The standardized incidence ratios (SIRs) showed no significant increase in bladder cancer among the entire cohort or those in any specific exposure group (*e.g.*, ever high, ever low, ≥ 1 year in a high-exposure job), compared to US population-based rates.

The weight of evidence does not establish an association between PFOS exposure and any type of cancer. While one study suggested a possible increase in bladder cancer associated with PFOS exposure, a later follow-up of the same population suggested no increased risk of bladder cancer. Further, these finds are not consistent with the mechanistic and animal evidence that suggest the bladder is not a target of PFOS toxicity. The other available studies indicate no increased risk of any specific cancer type after occupational PFOS exposure.

7.2.3 Serum Concentrations in the General Population and Non-occupational Exposed Cohorts

Exposure to PFOS in the general population is widespread but may vary considerably based on factors such as age, sex, race, diet and other lifestyle factors, and geographical location. Higher serum PFOS concentrations are generally reported for males vs. females (Kato *et al.*, 2015). While some single, short-term analyses have showed very little difference in serum PFOS concentrations between age groups (Kato *et al.*, 2015), an analysis of data from four US NHANES cycles (1999-2008) showed increasing PFOS concentrations with age (Kato *et al.*, 2011). Some of the differences in age groups may be influenced by the patterns of PFOS usage over time; older individuals likely had higher exposures to PFOS in products than children born after the 3M PFOS phase-out. Similarly, mean serum PFOS concentration has shown a gradual decline over time in many populations due to the phase-out, as shown across the NHANES data cycles. Table 7.5 summarizes PFOS biomonitoring data in representative studies of the general population and select cohorts around the world that were exposed to lower levels of PFOS (relative to the occupational cohorts) *via* drinking water (*i.e.*, the C8 Cohort, discussed in Section 7.1.5) or other non-occupational routes, such as food and commercial products.

Table 7.5 Serum PFOS (ng/mL) in Representative Non-occupational Populations

Cohort/Population	N	Range	Arithmetic Mean	Geometric Mean	Reference
NHANES		50 th -95 th Percentile			
1999-2000	1,562	30.2-75.7	NR	30.4 (95% CI: 27.1-33.9)	CDC (2015)
Male	743	34.9-78.3		33.4 (95% CI: 29.6-37.6)	
Female	819	27.8-75.7		28.0 (95% CI: 24.6-31.8)	
2003-2004	2,094	21.2-54.6		20.7 (95% CI: 19.2-22.3)	
Pregnant Women	76	12.0-21.8		12.29 (SD: 1.02)	Woodruff <i>et al.</i> (2011)
Non-pregnant Women	400	15.5-44.0		16.26 (SD: 0.84)	
2005-2006	2,120	17.5-47.5		17.1 (95% CI: 16.0-18.2)	CDC (2015)
2007-2008	2,100	13.6-40.5		13.2 (95% CI: 12.2-14.2)	
2009-2010	2,233	9.70-32.0		9.32 (95% CI: 8.13-10.7)	
Male	1,075	11.8-37.4		11.5 (95% CI: 9.93-13.3)	
Female	819	7.80-28.8		7.65 (95% CI: 6.73-8.71)	CDC (2017a)
2011-2012	1,904	6.53-21.7		6.31 (95% CI: 5.84-6.82)	
Male	966	8.31-24.1		7.91 (95% CI: 7.19-8.70)	
Female	938	5.27-17.5		5.10 (95% CI: 4.70-5.53)	
2013-2014	2,165	5.20-18.5		4.99 (95% CI: 4.50-5.52)	
Male	1,031	6.40-22.1		6.36 (95% CI: 5.62-7.20)	
Female	1,134	4.00-15.1		3.96 (95% CI: 3.60-4.35)	
American Red Cross Donors		50 th -95 th Percentile			
2000-2001	645	35.8-75.1	NR	34.9 (95% CI: 33.3-36.5)	Olsen <i>et al.</i> (2017)
2006	600	14.2-31.5		14.5 (95% CI: 13.9-15.2)	
2010	600	8.6-21.8		8.3 (95% CI: 7.9-8.8)	
2015	616	4.3-8.6		4.3 (95% CI: 4.1-4.6)	
C8 Health Project Cohort (2005-2006)					
Total Cohort (12 to ≥60 years old)	66,899 ^a	NR	23.3	19.2 (SD: 15.6)	Frisbee <i>et al.</i> (2009)
Male	33,240		26.0	21.9 (SD: 16.5)	
Female	35,785		20.7	17.0 (SD: 14.1)	
Pregnant Women	5,262	IQR: 9.0-17.7	NR	14.1 (SD: 7.7)	Stein <i>et al.</i> (2009)
Children (1 to <18 years old)	12,470	NR	NR	22.8 (SD: 12.6) ^b	Frisbee <i>et al.</i> (2010)
Aarhus Birth Cohort (2008-2013)					
Pregnant Women	1,533	IQR: 6.0-10.7	NR	7.90	Bjerregaard-Olesen <i>et al.</i> (2016)
Danish National Birth Cohort (1992-2002)					
Pregnant Women (First Trimester)	1,399	NR	NR	35.3 (SD: 13.0) ^b	Fei <i>et al.</i> (2007)
Pregnant Women (Second Trimester)	200			29.9 (SD: 11.0) ^b	
Infants (Cord Blood)	50			11.0 (SD: 4.7) ^b	
Children (Average Age: 11, Born: 1998-2003)	973	Control IQR: 27.4-35.6	NR	25.40-27.40 ^c	Liew <i>et al.</i> (2015)

Cohort/Population	N	Range	Arithmetic Mean	Geometric Mean	Reference
Danish Diet, Cancer, and Health Cohort (1993-1997)					
Men and Women (50-65 years old)	753	NR	NR	36.1	Eriksen <i>et al.</i> (2013)
Decatur Community Cohort		95 th Percentile			
2010	153	149	NR	39.8 (95% CI: 30.9-48.9)	Worley <i>et al.</i> (2017)
2016	45	70.6	NR	23.4 (95% CI: 18.5-28.4)	
Flemish Environment and Health Study (2007-2015)					
2007-2011 (Infants: Cord Blood)	218	NR	NR	2.66 (95% CI: 2.48-2.85)	Schoeters <i>et al.</i> (2017)
2012-2015 (Infants: Cord Blood)	269	NR	NR	1.10 (95% CI: 1.02-1.18)	
Hokkaido Study (2002-2005)		25 th -75 th Percentile			
Pregnant Women	306	4.0-7.5	6.02 (SD: 2.67)	NR	Kishi <i>et al.</i> (2015)
HOME Study, Cincinnati, Ohio (2003-2006)		25 th -75 th Percentile			
Pregnant Women	204	9.1-18	NR	13 ^d	Braun <i>et al.</i> (2016)
Taiwan Birth Panel Study (2004-2005)					
Pregnant Women	429	NR	NR	5.94 (SD: 1.95)	Chen <i>et al.</i> (2012)
Washington County, Minnesota, Communities (2008)					
Men and Women (20-86 years old)	196	3.2-448	NR	35.9 (95% CI: 32.2-40.1)	Landsteiner <i>et al.</i> (2014)
Men	88	NR		43.9 (95% CI: 38.1-50.7)	
Women	108			30.5 (95% CI: 26.1-35.7)	
Young Taiwanese Cohort Study (2006-2008)					
Males	250	NR	NR	8.97 (95% CI: 3.24-12.72)	Lin <i>et al.</i> (2013b)
Females	394			7.21 (95% CI: 4.41-11.75)	
12-19 years old	231			7.25 (95% CI: 2.44-23.69)	
20-30 years old	413			8.21 (95% CI: 6.27-34.71)	

Notes:

CI = Confidence Interval; HOME = Health Outcomes and Measures of the Environment; IQR = Interquartile Range (25th-75th Percentile); NHANES = National Health and Nutrition Examination Survey; NR = Not Reported; SD = Standard Deviation; PFOS = Perfluorooctane Sulfonate.

(a) Total number of participants is 69,025, but serum data were only available for 66,899 samples.

(b) Unclear whether an arithmetic or geometric mean; because geometric means are more commonly used, it was assumed that this is a geometric mean.

(c) Depending on case or control status.

(d) The authors reported this value as the median in Supplemental Table S2, but as the geometric mean in the text.

7.2.4 Studies in the General Population

Several large cohort studies have investigated the association between serum PFOS concentrations and various health effects in populations with low exposures associated with background environmental sources of PFOS (consumer products, food, water, and ambient air) or low-level contamination events (e.g., exposure *via* drinking water from releases by a PFOS-generating industry). These cohorts are largely the same as those discussed in Section 7.1 for PFOA. However, I repeat that discussion here so

on the discussion of PFOS effects in such populations is complete. The most well-studied cohorts include the C8 Cohort, the Danish National Birth Cohort, the Norwegian Mother and Child Cohort, and NHANES in the US. The C8 Cohort is a cohort of 69,000 residents in Ohio and West Virginia living near a chemical plant who were exposed to PFOA in contaminated drinking water. Participants were recruited in 2005-2006 and investigators intended to study PFOA primarily, though serum PFOS measurements were taken and associations with PFOS were also analyzed. Generally, serum PFOS concentrations in this cohort are lower than PFOA concentrations. The Danish National Birth Cohort is a cohort of 100,000 pregnant women recruited between 2000 and 2002 and followed continuously since that time. Maternal serum and cord blood samples were collected, and interviews were conducted to collect information on factors such as diet and lifestyle. The Norwegian Mother and Child Cohort includes >90,000 pregnant Norwegian women recruited between 1999 and 2008, with collection of biologic samples and birth data through 2009. NHANES is an ongoing program designed to assess the health and nutritional status of a group of representative adults and children in the US. NHANES collects a variety of survey and health data, including serum concentrations of a number of substances, such as heavy metals and PFCs (CDC, 2014).

In general, I relied on these large, well-characterized cohorts for data-rich endpoints (*e.g.*, birth outcomes), unless other cohorts showed distinctly different results from the large cohorts (in which case, I included these divergent results as well). For endpoints for which data were limited, I included discussion of smaller or less well-studied cohorts, in addition to any studies in the larger cohorts. A summary of both the large and small cohorts is presented in Table 7.6. For several endpoints investigated in community and general population studies, only cross-sectional analyses are available, which limits the ability to determine whether a true cause and effect relationship between PFOS exposure and these endpoints exists. The weight of evidence does not establish a causal association between non-occupational PFOS exposure and the endpoints examined.

Table 7.6 Non-occupational PFOS Cohorts

Cohort	Study Population	Location	PFOS Exposure Source	Outcomes Investigated
C8 Health Project Cohort	69,000 adults and children recruited from a community surrounding a PFOA plant	Mid-Ohio Valley, US	Contaminated drinking water	Developmental and reproductive, thyroid hormones and disease, serum lipids, immunotoxicity, kidney effects, cancer
Danish National Birth Cohort	100,000 pregnant women enrolled in 2000-2002	Denmark	Background: Drinking water, food, products	Developmental and reproductive
Hokkaido Birth Cohort Study on the Environment and Child's Health	~500 mother-child pairs studied from 2002-2005	Japan	Background: Drinking water, food, products	Immunotoxicity
Norwegian Mother and Child Cohort	>90,000 pregnant women and their offspring enrolled in 1999-2008	Norway	Background: Drinking water, food, products	Developmental and reproductive, liver enzymes, thyroid hormones, serum lipids, immunotoxicity, kidney effects
NHANES	Ongoing representative sample of general US population	US	Background: Drinking water, food, products	Liver enzymes, thyroid hormones and disease, serum lipids and CVD, kidney effects
INUENDO Cohort	>3,000 pregnant mothers and their children enrolled in 2002-2004	Sweden, Poland, Ukraine, and Greenland	Background: Drinking water, food, products	Developmental and reproductive
ALSPAC Cohort	>14,000 pregnant women and offspring recruited in 1991-1992	UK	Background: Drinking water, food, products	Developmental and reproductive
Danish Fetal Origins 1988 Cohort	965 pregnant women (30 weeks' gestation) enrolled in 1988-1989	Denmark	Background: Drinking water, food, products	Developmental and reproductive
Odense Child Cohort	>2,000 pregnant women and children enrolled in 2010-2012	Denmark	Background: Drinking water, food, products	Developmental and reproductive
CHEF Project Cohort	Ongoing study of >1,000 children born beginning in 1986	Faroe Islands, halfway between Norway and Iceland	Background: Drinking water, food, products; diet high in fish and whale meat	Immunotoxicity
Taiwan Birth Panel Study	486 mother-infant pairs enrolled in 2004-2005	Suburban and Urban Taiwan	Background: Drinking water, food, products	Developmental and reproductive
Danish DCH Cohort	4,769 men and women >50 years old	Denmark	Background: Drinking water, food, products	Serum lipids

Cohort	Study Population	Location	PFOS Exposure Source	Outcomes Investigated
European Youth Heart Survey	>1,700 boys and girls 9-15 years old beginning in 1997 ^a	Denmark, Estonia, Norway, and Portugal	Background: Drinking water, food, products	Serum lipids
Young Taiwanese Cohort Study	>700 students in grades 1-12 enrolled in 2006-2008	Taiwan	Background: Drinking water, food, products	Serum lipids and CVD

Notes:

ALSPAC = Avon Longitudinal Study of Parents and Children; CHEF = Children's Health and the Environment in the Faroes; CVD = Cardiovascular Disease; DCH = Diet, Cancer, and Health; INUENDO = Biopersistent Organochlorines in Diet and Human Fertility; NHANES = National Health and Nutrition Examination Survey; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate; UK = United Kingdom; US = United States.

(a) This is an ongoing prospective study; thus, newer studies (not discussed in this report) likely include additional children recruited after 2000.

7.2.4.1 Reproductive and Developmental Effects

Numerous studies are available that evaluate associations between occupational PFOS exposure and various reproductive and developmental outcomes, including male and female fertility, miscarriage, timing of menopause, birth and growth outcomes, congenital anomalies (*i.e.*, birth defects), neurodevelopmental effects (*e.g.*, hyperactivity), and timing of puberty. Based on this large body of literature, I conclude that the weight of evidence does not establish an association between non-occupational PFOS exposure and developmental and reproductive effects.

Male Fertility

Four studies examined the association between PFOS exposure and markers of male infertility, including semen quality and male hormone levels (Raymer *et al.*, 2012; Joensen *et al.*, 2013; Vested *et al.*, 2013; Buck Louis *et al.*, 2015; Tsai *et al.*, 2015).

In a cross-sectional study of 256 men living in the Durham, North Carolina, area (Raymer *et al.*, 2012), there was no association between plasma and semen PFOS concentrations and measures of semen quality (volume, sperm concentration, motility, semen concentration, and directional motility). While the authors reported an association between LH and plasma PFOS concentration, LH was not associated with semen PFOS concentration. When hormone levels were stratified by clinical definitions of normal, no associations between PFOS and any hormones were found. In another cross-sectional study, Tsai *et al.* (2015) evaluated associations between reproductive hormone levels (including estrogen, testosterone, free testosterone, FSH, SHBG, and LH) and PFOS concentrations in 540 male Taiwanese adolescents and young adults aged 12-30 years. The authors reported statistically significant inverse trends between FSH and PFOS exposure in males aged 12-17 years; no associations were present for the other age group (18-30 years). No associations between PFOS and the other hormones were found, nor were any associations between PFOS and all the hormones found in the other age group. Both Raymer *et al.* (2012) and Tsai *et al.* (2015) were limited by their cross-sectional study design; thus, the authors could not account for natural daily fluctuations in hormones.

Joensen *et al.* (2013) measured serum hormone levels (SHBG, LH, FSH, inhibin-B, estradiol, testosterone, free testosterone, and free androgen index [FAI]) and semen parameters (volume, sperm concentration, total sperm count, and motility) in a cross-sectional study of healthy Danish men. PFOS was not associated with any semen parameter, but was statistically significantly negatively associated with testosterone, free testosterone, FAI, and ratios of testosterone/LH, FAI/LH, and free testosterone/LH. The authors noted, however, that the reproductive hormone levels were within the normal ranges (based on age) in both the highest and lowest PFOS exposure groups. In a cohort study of the effects of prenatal exposure, Vested *et al.* (2013) reported no statistically significant associations between maternal serum PFOS concentration and semen quality or reproductive hormones (LH, FSH, estradiol, and testosterone) in Danish men aged 19-21 years.

Buck Louis *et al.* (2015) assessed various semen parameters (5 general characteristics, such as volume; 8 motility measures; 6 sperm head measures; 12 individual and 2 morphology measures; and 2 sperm chromatin stability measures) in a prospective cohort study of 501 Texas and Michigan couples trying to conceive, who were recruited from the Life Study Cohort. Of the 35 outcomes measured, PFOS exposure was statistically significantly associated with a *lower* percentage of sperm with coiled tails (a morphological change in sperm that may be used as one of several indicators of poor semen quality) ($p < 0.05$).

I conclude that the weight of evidence does not establish an association between PFOS exposure and markers of male fertility. Two cross-sectional studies measuring male hormones found a decrease in some parameters, but none fell outside of the range of normal, indicating the declines may not be associated with clinically significant effects. Two studies reported no effects on various semen parameters, while another found mainly null effects and one inverse association (which may have been spurious, because PFOS would not be expected to improve fertility). The remaining study found one altered hormone (FSH), but only in one age group (men 12-17 years old) but not the other (men 18-30 years old).

Female Fertility

Several studies have evaluated measures of fertility in women, including menstrual cycle disturbances and time to pregnancy (TTP) (Fei *et al.*, 2009; Jorgensen *et al.*, 2014; Lyngso *et al.*, 2014; Velez *et al.*, 2015), the majority of which found no associations between PFOS exposure and female fertility. Jorgensen *et al.* (2014) measured fecundability ratios (probability of conception within 13 months) and infertility (TTP > 13 months) in the INUENDO Cohort, including women from Greenland (n = 448), Poland (n = 203), and Ukraine (n = 287). The authors reported no significant increase in the odds of infertility and no significant decrease in fecundability in any one country or in the pooled analysis. In a subset analysis of male partners of the women (n = 401), there was also no association between male partner serum PFOS concentration and prolonged TTP. Velez *et al.* (2015) conducted a cohort study investigating the association between PFOS concentration in plasma and TTP in a group of 1,743 pregnant Canadian women enrolled in the Maternal-Infant Research on Environmental Chemicals (MIRC) study. The authors reported no statistically significant association between PFOS exposure and increased TTP or odds of infertility (TTP > 12 months).

In a cross-sectional study of the INUENDO Cohort, Lyngso *et al.* (2014) evaluated serum PFOS concentration and regularity and length of menstrual cycle in 1,623 women. The authors found that there were no statistically significant associations between PFOS exposure and irregular, short, or long menstrual cycles; however, a tendency toward an exposure-response relationship was seen for irregular menstrual cycles (OR = 1.7, 95% CI: 0.8-3.5 for highest *versus* lowest tertile of PFOS).

Fei *et al.* (2009) was the only study identified that found an association between PFOS and indices of female fertility. Using a retrospective cohort design, the authors assessed the association between serum PFOS concentration during pregnancy and TTP in a cohort of 1,240 women from the Danish National Birth Cohort and reported that maternal serum PFOS concentration was statistically significantly associated with longer TTP ($p < 0.001$), decreased fecundability, and increased odds of infertility (defined as TTP > 12 months or treatment for infertility) across all exposure groups (OR = 1.77, 95% CI: 1.06-2.95 for PFOS concentration ≥ 43.3 ng/mL). In contrast, in a re-analysis of the same group of women studied in Fei *et al.* (2009), as well as an additional sample of 550 other women from the same cohort, Bach *et al.* (2015) found no association between PFOS exposure and decreased fecundability. Bach *et al.* (2015) suggested that associations between PFOS (and PFOA) and decreased fecundability may be due to reverse causation, because serum PFOS concentration decreases during pregnancy and lactation, such that women who had never been pregnant would have higher serum PFOS concentrations than those that had ever been pregnant.

Supporting the variations based on parity are the studies by Vestergaard *et al.* (2012) and Whitworth *et al.* (2012). Vestergaard *et al.* (2012) conducted a prospective cohort study in females trying to get pregnant for the first time (and were not already pregnant at study initiation); these authors found no association between PFOS exposure and delayed TTP or fecundability ratio. Similarly, Whitworth *et al.* (2012) conducted a case-control study of the Norwegian Mother and Child Cohort and reported a statistically significant association between serum PFOS concentration and subfecundity (TTP > 12 months) in

pregnant parous women (*i.e.*, those that have given birth) but no association among pregnant women who had not previously given birth.

Miscarriage

I identified four studies of PFOS exposure and miscarriage (Stein *et al.*, 2009; Darrow *et al.*, 2014; Jensen *et al.*, 2015; Louis *et al.*, 2016). In a cross-sectional analysis, Stein *et al.* (2009) reported no association between PFOS at any serum concentration and miscarriage among 5,262 pregnancies in the C8 Cohort. Similarly, in a prospective cohort study, Darrow *et al.* (2014) found no increased risk of miscarriage associated with each log ng/mL increase in serum PFOS in 1,128 women from the C8 Cohort (OR = 1.1, 95% CI: 0.94-1.55). However, when analyses were restricted to first-time pregnancies, there was a slight increase in risk of miscarriage (OR = 1.34, 95% CI: 1.02-1.76). Blood samples in this study were collected between 2005 and 2006, and the majority of pregnancies occurred between 2007 and 2010 (1,026/1,438), which introduces possible exposure measurement error.

Jensen *et al.* (2015) conducted a case-cohort study of 51 cases and 204 control women enrolled in the Odense Child Cohort between 2010 and 2012. The authors found no association between serum PFOS concentration (as measured at enrollment) and the risk of miscarriage, regardless of whether the exposure was analyzed by tertile or *via* continuous increments. Finally, Louis *et al.* (2016) conducted a prospective study on a cohort of 501 American couples recruited prior to conception with no history of clinically diagnosed fertility. Pregnancy loss was tracked through home pregnancy tests. The authors found no association between PFOS exposure and increased risk of pregnancy loss.

Menopause and Associated Endpoints

Two studies investigated the association between PFOS exposure and menopausal outcomes (Knox *et al.*, 2011a; Taylor *et al.*, 2014). In a cross-sectional study, Knox *et al.* (2011a) investigated PFOS exposure and serum estradiol concentrations and age of menopause in >25,000 women enrolled in the C8 Cohort. The authors found that PFOS exposure was statistically significantly associated with a greater odds of experiencing menopause at 18 to ≤42 years old and in women >51 to ≤65 years old. PFOS exposure was also statistically significantly associated with decreased serum estradiol concentrations for women >42 to ≤51 and >51 to ≤65 years old. Taylor *et al.* (2014) conducted a cross-sectional study of PFOS exposure and menopause and hysterectomy in 2,732 women in NHANES and found a statistically significant, dose-dependent positive association between PFOS exposure and hysterectomy. PFOS exposure was also statistically significantly associated with earlier menopause, but only in the second tertile of exposure and not in the third tertile of exposure (HR = 1.23, 95% CI: 1.04-1.44, and HR = 1.16, 95% CI: 0.91-1.48, respectively). Because both of these studies were cross-sectional in design, it is impossible to determine whether the measured PFOS exposure affected the timing of menopause (*i.e.*, whether a causal association exists).

Overall, while few studies have examined the association between PFOS exposure and age at menopause and related female reproductive parameters, there is some evidence that such an association may exist. The possibility remains, however, that PFOS concentrations are higher in women who have experienced menopause because they are no longer losing blood through menstruation, which would eliminate some of the PFOS in their blood. Further, both of the identified studies relied on self-reported menopause status, which may lead to outcome misclassification.

Preeclampsia and Pregnancy-induced Hypertension

Several studies have studied the association between exposure to PFOS and pregnancy complications (most often, preeclampsia, an increase in blood pressure during pregnancy that can harm both the mother

and the developing infant, and pregnancy-induced hypertension). Most notably, preeclampsia may cause growth retardation in the fetus.

I identified few studies of PFOS exposure and preeclampsia. In a case-cohort analysis, Starling *et al.* (2014a) investigated the association between PFOS exposure and preeclampsia in 976 women enrolled in the Norwegian Mother and Child Cohort Study. Serum PFOS concentration was not associated with an increased risk of medical-record-validated preeclampsia in first-time mothers. Stein *et al.* (2009) also investigated the association between PFOS exposure and preeclampsia in 5,262 pregnancies within the C8 Cohort. The authors reported a significantly increased risk of preeclampsia with PFOS exposure at or above the median (12.8 ng/mL; OR = 1.3, 95% CI: 1.1-1.7); however, this study was limited by its reliance on self-reported preeclampsia status.

Darrow *et al.* (2013) conducted a prospective cohort study of PFOS exposure and pregnancy outcomes in 1,630 births in women enrolled in the C8 Cohort. The authors reported a statistically significant increase in pregnancy-induced hypertension across all births (OR = 1.47, 95% CI = 1.05-2.04 per log unit increase in PFOS concentration). The association was not statistically significant when calculated per interquartile increase in serum PFOS concentration (10 ng/mL), and the trend was not statistically significant for the analysis stratified by exposure quintile (no exposure-response pattern was seen). An important limitation in this study is possible exposure measurement error, because serum PFOS concentrations may have changed between the collection period (from 2005-2006) and births following this period.

Birth and Growth Outcomes

Numerous studies have investigated the association between gestational exposure to PFOS and birth size (birth weight, length, and other measures) and childhood growth. Based on inconsistent results and likely confounding of observed associations by extraneous factors, I conclude that the findings to date do not demonstrate a reliable association between PFOS exposure and declines in birth weight or other developmental parameters.

Several studies have evaluated PFOS exposure and birth outcomes in children born to women enrolled in the Danish National Birth Cohort. In a cohort study, Fei *et al.* (2007) evaluated the association between plasma concentrations of PFOS and PFOA in 1,400 pregnant women from this cohort and their infants' birth weight and length of gestation. In maternal plasma, the mean concentration of PFOS was 35.3 ppb (based on two samples per mother). No statistically significant association was observed between either PFOS exposure and birth weight or length of gestation. In another analysis of the same cohort, Fei *et al.* (2008a) evaluated PFOS exposure and fetal growth by correlating placental weight, birth length, ponderal index, and head and abdominal circumferences with concentrations of PFOS in maternal blood. Maternal PFOS concentrations were not associated with any one of the five fetal growth indicators.

Some more recent prospective analyses of this cohort of children, followed further into childhood, have found conflicting results. Andersen *et al.* (2010) reported that maternal PFOS concentration was statistically significantly associated with lower childhood weight and BMI at 5 months and 1 year of age (1.1-5.8 g difference) in boys (but not girls), but Andersen *et al.* (2013) reported that there was no association between maternal PFOS concentration and any anthropometric measures (including BMI, waist circumference, and risk of being overweight) at 7 years of age.

Because there were few positive findings reported in the larger cohorts, I expanded my search to smaller or less well-studied cohorts. At least one study of a smaller cohort reported no associations between PFOS exposure and birth outcomes such as birth weight, SGA, and prematurity (Hamm *et al.*, 2010). However, I identified four smaller studies that reported some positive associations between PFOS exposure and fetal growth (Apelberg *et al.*, 2007; Maisonet *et al.*, 2012; Chen *et al.*, 2012; Li *et al.*,

2017a). Apelberg *et al.* (2007) conducted a cross-sectional study of PFOS exposure and singleton births in Baltimore, Maryland, and found that umbilical cord PFOS concentration was statistically significantly inversely associated with both ponderal index and head circumference; however, there were no statistically significant associations between PFOS concentration and birth weight, newborn length, and gestational age. This study was limited by its cross-sectional study design and lack of control for socioeconomic status, which is a potentially important confounder, because it may be associated with both PFOS exposure and birth weight. Further, all differences were also very small (*e.g.*, the 64-g decrement in birth weight), and the authors noted that because of the normal variation in these endpoints within the population, the changes, "would not necessarily have clinical significance" (Apelberg *et al.*, 2007).

A cohort study of 447 mother-child pairs from ALSPAC in the UK reported that increasing maternal serum PFOS concentration was associated with lower birth weight (p for trend < 0.005), shorter birth length (p for trend < 0.01), and increased body weight at 20 months in female children (p for trend < 0.0001) (Maisonet *et al.*, 2012). However, because the actual growth parameter values were not provided for individual exposed groups (only for the population as a whole), it is impossible to determine whether the decrements seen (for example, between 111 and 140 g birth weight) resulted in birth and growth parameters outside of normal population ranges. The authors also failed to adjust for socioeconomic status, which is often strongly associated with birth weight and may also be associated with PFOS exposure (Young *et al.*, 2010; Nelson *et al.*, 2012).

Chen *et al.* (2012) conducted a longitudinal cohort study of 429 mother-infant pairs that were part of the Taiwan Birth Panel Study. They evaluated associations between cord blood PFOS concentration and several developmental effects, including gestational age, birth weight, birth length, head circumference, ponderal index, preterm birth (birth at gestational age < 37 weeks), low birth weight, and SGA at birth. In their analysis, using clinical definitions for prematurity, low birth weight, and SGA, cord blood PFOS concentration was associated with a significantly increased risk for preterm birth and SGA.

Bach *et al.* (2016a) investigated fetal growth in 1,500 first-time mothers enrolled in the Aarhus Birth Cohort in Denmark. The authors indicated that they did not find strong or consistent associations between any of the PFCs measured in maternal serum and birth weight or other indices of fetal growth. While birth weight was slightly lower in the highest vs. lowest quartile of PFOS exposure (50 g and 62 g for all and term births, respectively), the observed associations were not statistically significant. There was no association between PFOS and head circumference.

Li *et al.* (2017a) assessed fetal birth and growth outcomes in a cohort of 321 mother-child pairs in the Guangzhou Birth Cohort Study in China. After adjusting for potential confounders, total PFOS concentration was statistically significantly associated with lower birthweight in all babies (95 g decrease per log-transformed unit increase in PFOS concentration), but when analyzed by sex, birth weight was significantly reduced only in boys. There was also a statistically significant association between PFOS and gestational age (adjusted for birth weight and other potential confounders), with an increase of 0.24 weeks per log-transformed unit increase in PFOS concentration. The association was not statistically significant in girls in the sex-stratified analyses. The authors did not analyze the association between PFOS exposure and the clinical definition of low birth weight ($< 2,500$ g) or preterm status (< 37 weeks). Demographic data indicated that the average birth weight was 3,117 g, with 7.5% of the cohort considered low birth weight, and the average gestational age was 39 weeks, with 6.3% of the babies considered preterm. As with Maisonet *et al.* (2012), it is impossible to tell, based on the analysis by Li *et al.* (2017a), whether PFOS exposure was associated with clinically relevant changes in birth and growth parameters in this study.

Fewer studies are available for communities potentially exposed to higher levels of PFOS in drinking water, relative to the general population. In a cross-sectional study in the C8 Cohort, Stein *et al.* (2009) examined the association between PFOS and low birth weight (live birth <5.5 pounds, approximately equivalent to 2,500 g) and preterm birth (birth at gestational age <37 weeks) and reported a weak, but statistically significant, association with birth weight that followed an exposure-response pattern (OR = 1.5, 95% CI: 1.1-1.9). No association was found for preterm birth per IQR increase in PFOS concentration or for individual exposure groups, except for mothers with high serum PFOS concentrations (120.6-894.4 ng/mL), and even then, the OR was just above 1, indicating a weak effect. Again, however, the incidence of low birth weight and preterm births was derived from maternal self-report, which is subject to bias. Mothers were aware of their PFOS exposure levels, so those who perceived themselves as highly exposed to PFOS may have been more likely to report effects.

Overall, I conclude that the findings to date do not demonstrate a reliable association between PFOS exposure and declines in birth weight or other developmental parameters. The larger, well-characterized cohorts most often found no statistically significant associations between PFOS and fetal and childhood growth, and many of the effects observed in smaller cohort studies were so small that they may not have been clinically significant. Finally, a recent PBPK analysis of the studies on PFOS exposure and birth weight reported that a substantial portion of observed associations between PFOS exposure and birth weight may be attributable to confounding by GFR (Verner *et al.*, 2015). Low GFR is an indicator of reduced kidney function, and lowered kidney function reduces excretion of PFOS (*i.e.*, lower GFR is associated with higher blood concentrations of PFOS). GFR also rises during pregnancy, and pregnant women whose GFR remains low tend to have lower birth weight babies. As such, women with lower birth weight babies (due to low GFR) will also have higher serum concentrations of PFOS.

Finally, because the association between PFOS exposure and birth and growth outcomes is the basis for some of the available drinking water values (see Section 8), and there remains scientific uncertainty about the existence of a potential causal link, I reviewed selected systematic reviews of this endpoint. Recent systematic reviews (*e.g.*, Bach *et al.*, 2015) have reported inconsistent results of unclear clinical significance and thus, are consistent with my findings.

Congenital Anomalies

I reviewed three studies of prenatal exposure to PFOS and congenital anomalies (birth defects), and in these studies, no associations were reported (Stein *et al.*, 2009; Vesterholm Jensen *et al.*, 2014; Toft *et al.*, 2016). Stein *et al.* (2009) examined the incidence of self-reported birth defects in the C8 Cohort. The author reported that there was no association between PFOS exposure and birth defects, regardless of serum PFOS concentration. In addition, one study investigated the association between maternal PFOS exposure and birth effects in male children (Vesterholm Jensen *et al.*, 2014). Vesterholm Jensen *et al.* (2014) reported no statistically significant associations between cord blood PFOS concentration and congenital cryptorchidism (the failure of one or both testes to descend from the abdomen to the scrotum) in a case-control study of Danish and Finnish boys. Similarly, Toft *et al.* (2016) assessed the association between PFOS concentration in amniotic fluid and male genital malformations (cryptorchidism and hypospadias⁷⁹) in a case-control study of Danish boys and found no associations with either malformation.

⁷⁹ Cryptorchidism is undescended testes; hypospadias is a malformation in which the opening of the urethra is not located at the tip of the penis (CDC, 2017b).

Neurodevelopmental Outcomes

Several cohort studies have investigated the association between PFOS exposure and neurodevelopmental effects such as motor skills, hyperactivity, attention deficit/hyperactivity disorder (ADHD), and autism spectrum disorder (ASD). Fei *et al.* (2008b) reported no statistically significant associations between plasma concentrations of PFOS in pregnant women enrolled in the Danish National Birth Cohort and gross, fine motor, and mental developmental milestones in their children. The mental development analysis included the child's attention and cognitive functions, as well as language and social-personal development. Hoyer *et al.* (2015) reported no statistically significant associations between maternal serum PFOS concentrations in women of the INUENDO Cohort and motor skills, hyperactive behavior, and other behavioral difficulties in their children at 5-9 years old. Similarly, Ode *et al.* (2014), Strom *et al.* (2014), Liew *et al.* (2015), and Quaak *et al.* (2016) reported no associations between maternal serum or umbilical cord PFOS concentration and ADHD in children born to mothers enrolled in birth cohorts in Denmark, the Netherlands, and Sweden. Strom *et al.* (2014) also found no association between maternal serum PFOS concentration and depression (up to age 20) or scholastic achievement in the Danish Fetal Origins Cohort. Finally, Lien *et al.* (2016) reported that cord blood PFOS concentration was not associated with ADHD symptoms in 7-year-old children enrolled in the Taiwan Birth Panel Study and the Taiwan Early-Life Cohort.

Timing of Puberty

I identified three studies that investigated age at puberty in girls and boys exposed to PFOS *in utero* and/or childhood (Christensen *et al.*, 2011; Lopez-Espinosa *et al.*, 2011; Kristensen *et al.*, 2013). Christensen *et al.* (2011) conducted a nested case-control study of 218 cases of earlier menarche (*i.e.*, commencement of menstruation before age 11.5 years) and 230 girls with menarche after 11.5 years of age enrolled in the ALSPAC in the UK. Analyses indicated that the risk of early menarche was lowered with increasing gestational PFOS exposure, though the OR was not statistically significant, when PFOS exposure was considered as a continuous measure or above *vs.* below the median exposure level (19.8 ng/mL in maternal serum).

Lopez-Espinosa *et al.* (2011) conducted a cross-sectional analysis of the association between concurrent serum PFOS concentration and age at puberty in boys and girls aged 8-18 years enrolled in the C8 Cohort. Age at puberty was determined using age of menarche and serum estradiol in girls and serum testosterone levels in boys. No statistically significant associations were found between serum PFOS concentration and later puberty in girls; however, there was a statistically significant association between serum PFOS concentration and later puberty in boys in the continuous exposure model and in the upper two quartiles of exposure in the stratified analyses. The median delay of puberty ranged from 82-123 days, but, as noted by authors, a delay in puberty of 3-6 months is of unclear clinical relevance. This study is also limited by its cross-sectional study design; in many cases, the participants had already reached puberty when their serum PFOS concentrations were measured.

Finally, Kristensen *et al.* (2013) assessed the association between prenatal exposure to PFOS and several parameters of female reproductive function around age 20 (age at menarche, menstrual cycle length, hormone levels, and number of follicles/ovary) in a prospective cohort study of the Aarhus Birth Cohort in Denmark. Prenatal PFOS exposure was assessed using maternal serum collected at 30 weeks of gestation. The authors reported that prenatal exposure to PFOS was not associated with any of the reproductive parameters measured.

Overall, I conclude that the weight of evidence does not establish an association between either gestational or childhood exposure to PFOS and age at puberty. Three studies of puberty in girls suggest there is no association between PFOS exposure and early or late puberty. Further, any association

between PFOS exposure and later age at menarche (despite the null results, Lopez-Espinosa *et al.* [2011] suggested that there was a possible association) would likely be confounded by the pharmacokinetics of PFOS, in which case, the association is unlikely to be causal. Specifically, because menstrual blood is one route of removal of PFOS from the body, girls with an earlier first menarche would have lower serum PFOS concentrations than those with later first menarche (Wong *et al.*, 2014; Wu *et al.*, 2015). While one study suggested slightly delayed puberty in boys exposed to PFOS in drinking water, the study's design limitations prevent drawing causal conclusions from its results.

Overall Conclusions

The weight of evidence does not establish an association between non-occupational PFOS exposure and developmental and reproductive effects. In some cases, because of how PFOS is excreted from the body, associations may reflect "reverse causality." This means that the health endpoint being studied may affect serum PFOS concentrations, rather than PFOS affecting the endpoint. For example, because menstrual blood can remove PFOS from the body (Wong *et al.*, 2014), girls with an earlier first menarche would have lower serum PFOS concentrations than those with later first menarche. In other cases, such as birth weight changes, the effects seen in some studies are small enough to be within the range of normal variation, and thus would not be clinically significant.

7.2.4.2 Liver Enzymes and Disease

I identified only two studies of the association between PFOS exposure and liver enzymes in the general population (Lin *et al.*, 2010; Gleason *et al.*, 2015) and one study in an exposed community (Gallo *et al.*, 2012). Lin *et al.* (2010) conducted a cross-sectional analysis of 2,216 adults in NHANES in 1999-2000 and 2003-2004. The authors reported no statistically significant associations between PFOS exposure and ALT or GGT. There was a significant *decrease* in bilirubin (*i.e.*, the opposite of the expected direction of adversity) as PFOA exposure increased. One limitation of this study was the lack of control for medications that may have altered ALT or GGT.

Similarly, Gleason *et al.* (2015) evaluated liver enzymes among participants in the 2006-2008 and 2009-2010 NHANES. Increases in PFOS exposure were associated with increases in total bilirubin but not with changes in ALT, GGT, or ALP.

Gallo *et al.* (2012) conducted a cross-sectional analysis of PFOS and markers of liver function in >47,000 adults enrolled in the C8 Cohort between 2005 and 2006. Increasing PFOS exposure was associated with a small increase in ALT, but was not associated with altered GGT. Bilirubin was significantly *decreased* with increasing PFOS exposure. The authors noted that the small changes in ALT may not lead to diagnosable conditions in the future. The study's cross-sectional design does not allow one to draw conclusions as to whether the observed changes were sustained or reversible.

7.2.4.3 Thyroid Hormones and Disease

Several studies have reported associations between non-occupational exposure to PFOS and alterations in thyroid hormones. However, I was able to identify only a single study of the association between low-level PFOS exposure and thyroid disease. As noted in Section 7.1.4.3, although substantial and sustained alterations in thyroid hormone levels can be adverse, small, transient changes in thyroid hormone levels are often not adverse. Based on my evaluation of the data, I conclude that the weight of evidence does not establish a causal association between PFOS exposure and clinically significant alterations in thyroid hormones or thyroid disease in non-occupational populations. Many of these studies also used a cross-

sectional study design, and thus, they cannot be used to determine whether a causal association between PFOS exposure and these endpoints exists.

Several studies have investigated the association between low-level PFOS exposure and thyroid hormones in children, the general adult population, pregnant women, and older adults (Knox *et al.*, 2011b; Jain, 2013; Wen *et al.*, 2013; Shrestha *et al.*, 2014; Webster *et al.*, 2014, 2016; Wang *et al.*, 2014c; Berg *et al.*, 2015).

Knox *et al.* (2011b) conducted a cross-sectional study of PFOS exposure and TSH, T3 uptake, and T4 in more than 50,000 adults in the C8 Cohort. PFOS exposure was statistically significantly associated with increases in T4 ($p \leq 0.0001$) and decreases in T3 uptake ($p \leq 0.0001$). These changes were not associated with changes in TSH and thus are not consistent with hyper- or hypothyroidism (either clinical or subclinical). As a result, their clinical relevance is unknown. Jain (2013) also reported no statistically significant associations between PFOS exposure and any thyroid hormones in 1,540 adult participants enrolled in NHANES. These null findings were corroborated in another cross-sectional study of PFOS exposure and thyroid hormones among 1,181 NHANES participants >20 years old (Wen *et al.*, 2013).

Shrestha *et al.* (2014) conducted a cross-sectional evaluation of the association between serum PFOS and PFOA concentrations and thyroid hormones in 87 older adults (aged 55-74 years) who resided in communities in the upper Hudson River area in New York. After adjusting for potential confounders (age, sex, education, and total serum polychlorinated biphenyls), serum PFOS concentration was statistically significantly associated with free T4 ($p = 0.044$) and T4 levels ($p = 0.001$). The authors reported a 4% increase in free T4 and a 9% increase in T4 per IQR increase of serum PFOS concentration (IQR = 21.7-45.2 ng/mL). There were no statistically significant associations between serum PFOS concentration and TSH or T3, and thus, the findings are not consistent with hyper- or hypothyroidism (either clinical or subclinical).

In a prospective cohort study, Webster *et al.* (2014) evaluated PFOS exposure and thyroid hormones in pregnant women enrolled in the Chemicals, Health, and Pregnancy (CHirP) Study based in Vancouver, Canada. PFOS was not associated with free T4, total T4, or TSH among women with normal TPOAb levels (TPOAb is a marker of the autoimmune condition called Hashimoto's Disease). Serum PFOS concentration and thyroid hormones levels were each measured twice (at 15 and 18 weeks of gestation). However, among women with high TPOAb, the authors noted a 7% decrease in free T4 per 3.3 ng/mL increase in PFOS concentration, but the association was not statistically significant. Maternal TSH was also significantly increased (69% per 3.3 ng/mL PFOS) in women with high TPOAb levels. There is some uncertainty regarding the clinical relevance of these findings. Maternal T4 is the only source of T4 to the developing fetus before the fetal thyroid begins to function in the middle of gestation; however, it is unclear whether the small decreases in maternal free T4 would have affected the fetus. Women with high TPOAb levels have decreased thyroid capacity to produce T4 and T3 and thus often produce compensatory TSH. However, the PFOS-associated decreases in free T4 were very small and thus inconsistent with the magnitude of change in TSH in the women with high TPOAb levels.

Wang *et al.* (2014c) reported that serum PFOS was statistically significantly positively associated with TSH (0.8% increase, 95% CI: 0.1-1.6% per 1 ng/mL PFOS) in a cross-sectional study of 903 pregnant women in the Norwegian Mother and Child Cohort Study; no other thyroid hormones were measured. TSH levels change during pregnancy (a normal process); thus, a single measurement of thyroid hormones, as was used in this study, is unlikely to accurately characterize thyroid homeostasis during pregnancy. In a longitudinal study in this cohort, Berg *et al.* (2015) found that women with the highest serum PFOS concentrations (11.1-35.9 ng/mL) had a mean TSH value 24% higher than the lowest exposure group at all sampling points (second trimester, 3 days post-partum, and 6 weeks post-partum), but no changes in free T3, free T4, T3, and T4 levels. The proportion of women with subclinical hypothyroidism (in which

TSH levels are slightly elevated [>3.6 mIU/L], but free T4 and free T3 are normal⁸⁰) was also increased, but only in the second trimester visit.

Finally, Webster *et al.* (2016) conducted a cross-sectional analysis of serum PFC concentrations and thyroid hormones in 1,525 US adults, using NHANES data from 2007 and 2008. The authors assessed associations between PFOS exposure and free T3, free T4, the ratio of free T3 to free T4, total T3, total T4, and TSH. They also stratified people into four groups by indicators of thyroid "stress," including higher TPOAb level and low iodine status. There were no statistically significant associations between PFOS exposure and any of the thyroid hormones in those with normal TPOAb and iodine levels, low iodine levels only, or high TPOAb levels only. In the subgroup of people with high TPOAb and low iodine levels, PFOS exposure was associated with a significant increase in TSH but not any of the other hormone measures. Only 26 participants had high TPOAb and low iodine levels (2% of the study size). In addition to its small sample size, this study was limited by its cross-sectional study design and the possibility of reverse causation.

I identified only two studies that considered measures of overt thyroid disease (Melzer *et al.*, 2010; Lopez-Espinosa *et al.*, 2012). Melzer *et al.* (2010) collected serum PFOS data and self-reported disease status from 3,974 individuals that participated in one of three waves of cross-sectional NHANES sampling (1999-2000, 2003-2004, and 2005-2006). Thyroid disease prevalence was ascertained by asking participants if they had ever been told by a doctor/health professional that they had thyroid disease and whether they were currently taking any thyroid disease medications. The authors noted that the data they had did not overlap with thyroid hormone measurement subsamples, so they could not match these data to the reported diagnoses. There were no associations between PFOS exposure and thyroid disease in women; in men, there was a significant increase in the odds of thyroid disease with current medication in the most highly exposed group (quartile 4, mean serum PFOS concentration ≥ 36.6 ng/mL) relative to the low exposure group (mean serum PFOS concentration ≤ 25.5 ng/mL). However, this study suffers from important limitations, most predominantly its reliance on self-reported prevalent (existing) disease (unconfirmed by medical records), which is subject to bias. It is also cross-sectional in design, so it is unclear if whether measured PFOS concentrations accurately represent the participants' exposure at the time of disease diagnosis.

In a cohort study, Lopez-Espinosa *et al.* (2012) investigated the association between PFOS exposure and self-reported thyroid disease, TSH, and total T4 in $>10,000$ children aged 1-17 years enrolled in the C8 Cohort. There were no significant increases in either thyroid hormones in combined and sex-specific analyses. Further, there was no increased risk of thyroid disease or hypothyroidism, whether subclinical (slight changes in hormones that may be associated with disease) or overt disease.

Overall, the findings regarding the association between PFOS exposure and thyroid hormones and disease are generally inconsistent, even among similar populations. The authors of these studies reported that the biological significance of the effects observed were unclear. Small changes in isolated thyroid hormones that remain within normal reference ranges, or do not change in the direction associated with hypo- or hyperthyroidism, are unlikely to result in overt thyroid disease. Further, while thyroid hormones are important for the development of many neurological processes in the fetus, there is no confirmed evidence that the magnitude and timing of the changes observed in these studies were sufficient to alter fetal development (Webster *et al.*, 2014). Considering the lack of consistent, clinically meaningful hormone changes measured at a single point in time (*i.e.*, due to the cross-sectional design of many of

⁸⁰ The clinical significance of subclinical hypothyroidism and the upper limit of "normal" for TSH remains an area of debate. While this condition may progress to overt hypothyroidism, there is insufficient evidence of any other symptoms associated with subclinical hypothyroidism. The upper limit of normal TSH in pregnancy is considered to be 3.5 mIU/L in the second and third trimesters of pregnancy (Fatourehchi, 2009).

these studies) and the availability of only two studies of self-reported thyroid disease (both null), I conclude that the weight of evidence does not establish a causal association between PFOS exposure and alterations in thyroid hormones in non-occupational populations.

7.2.4.4 Serum Lipids and Cardiovascular Disease

Children and Adolescents

Numerous studies have measured serum lipids (*e.g.*, cholesterol, triglycerides) in populations with low-level PFOS exposure, and I identified three studies that have investigated the association between non-occupational PFOS exposure and CVD. It is my opinion that the weight of evidence does not establish an association between low-level PFOS exposure and altered serum lipids or CVD.

In general, many of the PFOS exposure and serum lipid studies were conducted with cohorts of children aged 1-18 years (Frisbee *et al.*, 2010; Timmermann *et al.*, 2014; Geiger *et al.*, 2014; Zeng *et al.*, 2015). Frisbee *et al.* (2010) conducted a cross-sectional analysis of 12,476 children and adolescents (1-17.9 years old) enrolled in the C8 Cohort. The authors reported that total cholesterol, LDL, and HDL significantly increased ($p < 0.001$) with increasing serum PFOS concentration. In analyses dichotomized by abnormal cutoff values, PFOS exposure remained associated with an increased risk of abnormal cholesterol and LDL; however, for HDL, there was a decreased risk of low HDL (*i.e.*, PFOS exposure increased HDL, which is a positive effect, because HDL is considered the "good" cholesterol).

Timmermann *et al.* (2014) reported that there was no significant increase in serum cholesterol per 10 ng/mL increase in serum PFOS concentration in a cross-sectional analysis of 499 8- to 10-year-old children enrolled in the Danish portion of the European Youth Heart Study. There were also no associations observed between PFOS exposure and other markers of adiposity, including BMI, waist circumference, and serum adiponectin and leptin. In stratified analyses, however, overweight children (but not normal weight children) had increased triglycerides (76.2% increase), plasma insulin, insulin resistance, and β -cell function for each 10 ng/mL increase in serum PFOS concentration.

Geiger *et al.* (2014) reported that serum PFOS concentration was statistically significantly positively associated with total cholesterol and LDL, but not HDL or triglycerides, in 12- to 18-year-old children in NHANES. Zeng *et al.* (2015) also reported that PFOS exposure was positively associated with total cholesterol and LDL in a cross-sectional study of group of 225 healthy 12- to 15-year-old children in Taiwan. Unlike the other studies, however, these authors also found a statistically significant positive association between PFOS exposure and triglycerides.

The only longitudinal study of children and adolescents that included serum lipids was conducted by Domazet *et al.* (2016). The authors followed >300 children (age 9) enrolled in the European Heart Study until they turned 21 years old. Serum PFOS concentrations averaged 9 and 9.7 ng/mL at age 9, 3.4 and -3.7 ng/mL in adolescence (15 years of age), and 2.7 and 3.1 ng/mL in young adulthood (21 years of age, in females and males, respectively). There were no associations between serum PFOS concentration measured at 9 years of age and triglyceride levels at 15 and 21 years of age, or between serum PFOS concentration measured at 15 years of age and triglyceride levels at 21 years of age.

Adults

Several other studies have examined associations between PFOS exposure and serum lipids/CVD in adult populations, including pregnant women. Eriksen *et al.* (2013) measured serum PFOS and total cholesterol in a group of 753 adults enrolled in the Danish DCH Cohort. The authors reported that there

was a significant, 4.6 mg/dL, increase in total cholesterol per IQR increase of serum PFOS concentration (IQR not reported, but the mean PFOS concentration was 36.1 ng/mL and the maximum was 132 ng/mL). Steenland *et al.* (2009) investigated cross-sectional measures of PFOS and serum lipids in a population of 42,294 adults enrolled in the C8 Cohort. All the evaluated endpoints except HDL were significantly increased as serum PFOS concentration increased ($p < 0.05$). In contrast, Fu *et al.* (2014) found that serum PFOS concentration was not associated with total cholesterol, LDL, HDL, or triglycerides in a cross-sectional study of a population of 133 adult hospital patients in China. The population in this study, however, had much lower mean serum PFOS concentrations (overall mean: 1.68 ± 1.20 ng/mL; highest quartile: 3.12 ± 1.52 ng/mL) than the Danish and C8 Cohorts. Finally, one study investigated serum lipids in a cohort of 891 pregnant women in the Norwegian Mother and Child Cohort Study (Starling *et al.*, 2014b). The authors reported that each IQR (6.29 ng/mL) increase in maternal serum PFOS concentration was associated with a 4.2 mg/dL (95% CI: 0.8-7.7) increase in total cholesterol and a 2.08 mg/dL (95% CI: 1.12-3.04) increase in HDL; no association was found for LDL or triglycerides.

Fitz-Simon *et al.* (2013) conducted a longitudinal study of serum PFOS concentrations and lipids in the 521 participants in the C8 Cohort over a 4.4-year period. During this period, levels of PFOS in public water were reduced due to filtration of public water supplies and consequently, the population's serum PFOS concentrations were also reduced. In the model that adjusted for the PFOA concentration in public water, the authors reported that a 50% decline in serum PFOS concentration was associated with a 4.6% decrease in LDL (95% CI: 1.97-7.14) and a 3.2% decrease in total cholesterol (95% CI: 1.59-4.81). The authors noted that these changes in lipids were small, though variability in lipids across individuals was high. Without individual data, it is unclear how the overall percentage declines would translate into absolute blood lipid levels and whether these changes would be clinically significant.

An important limitation of all but one (Fitz-Simon *et al.*, 2013) of these studies is the cross-sectional study design; because the investigators measured PFOS and serum lipids at one point in time, it is impossible to establish temporality (*i.e.*, cause and effect) between the exposure and the outcomes assessed. Further, the mechanism by which PFOS may alter serum lipids and cholesterol is unknown; indeed, the biological plausibility of such effects is unclear, due to the lowered serum lipids seen in high-dose animal studies of PFOS (see Section 6.2.1). Finally, some of these populations, such as the C8 Cohort (Steenland *et al.*, 2009) have an overall mean total cholesterol and a prevalence of high cholesterol that is consistent with or lower than rates in the general US population, leading to questions of the clinical significance of the observed associations and the potential selection bias in the low dose groups (*e.g.*, if the low dose group inadvertently had inherently lower risk of high cholesterol) (Kerger *et al.*, 2011).

I identified only three studies of non-occupational PFOS exposure and CVD (Melzer *et al.*, 2010; Lin *et al.*, 2013b; Mattsson *et al.*, 2015). CVD is a set of diseases of the heart and blood vessels, many of which are associated with atherosclerosis, or narrowing of the arteries from plaque (a substance made of calcium fat, cholesterol, fibrin, and other substances) buildup. Melzer *et al.* (2010) collected serum PFOS data and self-reported disease status from 3,974 individuals that participated in one of three waves of NHANES sampling (1999-2000, 2003-2004, and 2005-2006). The authors reported no association between serum PFOS concentrations and self-reported heart disease (coronary heart disease, angina, and/or heart attack). This study was limited by its cross-sectional design and the fact that it used self-reported outcome measures.

Lin *et al.* (2013b) examined the cross-sectional association between serum PFOS concentration and carotid intima media thickness (CIMT)⁸¹ in 644 12- to 30-year-old subjects from the Young Taiwanese Cohort Study. The mean serum PFOS concentration measured was 8.97 ng/mL in males and 7.21 ng/mL

⁸¹ A measurement of the thickness of the carotid artery of the heart, used to detect the presence of atherosclerotic disease (narrowing of the artery).

in females. The authors reported a statistically significant trend in increasing CIMT with increasing serum PFOS concentration ($p < 0.001$). In stratified analyses, the trend was only statistically significant in the 12- to 19-year-old age group and not the 20- to 30-year-old age group. The authors also measured the association between LDL and PFOS exposure and found no statistically significant association in any exposure grouping. Further, there was no association between PFOS exposure and BMI, blood pressure, and other traditional CVD risk factors. The authors suggested that, because PFOS may influence CIMT, it may also affect CVD risk. This study is limited by its cross-sectional design, and due to the young participants, its results cannot be extrapolated to older populations.

Mattsson *et al.* (2015) conducted a case-control study of the association between PFOS exposure and coronary heart disease in a population of 1,782 male farmers and rural residents living in Sweden. Residents were recruited in 1990-1991 and followed for coronary heart disease diagnosis through 2009. The median serum PFOS concentration (measured in 1990-1991) was not significantly different between men who developed coronary heart disease (cases) and those who did not (controls). ORs computed based on PFOS quartile also showed no statistically significant dose-response relationships, and even the fourth quartile of PFOS exposure (>28.5 ng/mL) was not statistically significantly associated with an increased risk of coronary heart disease. Note that although this was a longitudinal study, the authors only had two measures of PFOS, which had to serve as proxies for multiple years of exposure.

Overall, while there are a number of studies linking PFOS exposure and alterations in serum lipids and one study suggesting a possible association between PFOS and CIMT, because of the design of these studies and the lack of a plausible biological mechanism, the weight of evidence does not establish a causal association between low-level PFOS exposure and altered serum lipids or CVD.

7.2.4.5 Immunological Effects

The normal immune response is dynamic and complex. At any one time, an individual's immune response may be suppressed or activated due a number of environmental factors, including stress, diet, medications, or infection (Karol, 1998). This normal intra-individual variability is observed within all organisms and can complicate the interpretation of variable responses in associations with environmental exposures. While immunotoxicity has the potential to contribute to or cause a number of clinical diseases, the natural variability in the immune system necessitates repeated sampling of individuals as well as confirmation of biological plausibility in animal experiments in order to conclude with certainty that an environmental exposure causes biologically relevant immunotoxicity in humans.

The majority of epidemiological studies of PFOS exposure and immune effects involve prenatal exposure and childhood outcomes, predominantly measures of antibodies in blood. Although some individual studies reported reduced antibody levels of certain diseases in specific populations, I conclude that there is no consistent evidence of lowered immunity or overt disease (*i.e.*, a higher risk of infection) in studies of community exposure to PFOS.

Granum *et al.* (2013) measured vaccine antibody levels and incidence of common infectious disease in a sub-cohort of 99 mother-child pairs in the Norwegian Mother and Child Cohort Study. Maternal serum PFOS concentration was statistically significantly negatively associated with rubella anti-vaccine antibody levels ($p = 0.007$), but not measles, *Haemophilus influenzae* type b, or tetanus antibody levels. However, authors did not measure incidence of rubella to determine whether the decreased antibody levels increased risk of disease. Maternal PFOS exposure was not associated with incidence of common cold or gastroenteritis episodes.

Similarly, Grandjean *et al.* (2012) found that maternal and child serum PFOS concentrations (at age 5) were statistically significantly associated with childhood anti-diphtheria antibody concentrations (at ages 5 and 7, respectively) in a cohort study of 656 Faroese mothers and children; a two-fold increase in maternal PFOS concentration was associated with a 39% decrease in titer PFOS concentrations in 5-year-olds. Like Granum *et al.* (2013), the authors did not follow the cohort to determine whether the lowered antibody levels led to a higher risk of the associated diseases. Further, the serum diphtheria antibody titer levels at 5 years of age (pre-booster shot) were within the range of what CDC would consider an indication of at least some immunity (*i.e.*, >0.01 IU/mL) (Tiwari, 2011).

In a follow-up analysis of the Grandjean *et al.* (2012) cohort, Grandjean *et al.* (2017) investigated the association between post-natal PFOS exposure and serum antibodies against childhood vaccines in 561 children at age 13. A number of children were admitted to an emergency room and/or had higher serum antibodies than at age 7. The authors suggested that they may have had booster vaccinations, and these children were excluded from the subgroup analyses. While there was an association between increasing PFOS concentration and decreasing in anti-diphtheria antibodies, particularly for PFOS concentrations measured at age 7, the associations were not statistically significant, and the association was highly attenuated in the subgroup of children who had not received booster vaccinations, had not had emergency room visits, or had increased antibodies (change of -10.8%; 95% CI: -35.5 to 23.5; $p = 0.490$). Anti-tetanus antibodies had a positive association with PFOS exposure (*i.e.*, increased with increasing exposure), but again, these results were not statistically significant.

I reviewed a presentation by Krisko *et al.* (2015), of 3M, at the FLUOROS 2015 meeting in Golden, Colorado. Despite decreases in serum PFOA and PFOS concentrations in the general population since the early 2000s, these investigators found no changes in incidence of diphtheria and tetanus in developed countries with high vaccination rates against these diseases. While not definitive, this suggests that PFOS exposure is not associated with reduced protection from vaccination.

I identified a single study investigating the association between prenatal PFOS exposure and autoimmunity (*i.e.*, when the immune system attacks its own cells) in offspring. Osuna *et al.* (2014) measured maternal and childhood serum PFOS concentrations and several serum autoantibodies for IgG and IgM in a group of 38 mother-child pairs enrolled in the CHEF Project at the National Hospital in Torshavn, Faroe Islands. The authors found that maternal PFOS concentration was statistically significantly negatively associated with antigen-specific IgG (22% decrease per 2-fold increase in prenatal PFOS concentration); however, there was no association between cross-sectional measures of immune markers and childhood serum PFOS concentration at age 7. No associations were found between PFOS exposure and any of the other eight autoantibody types for either IgG or IgM.

I identified seven studies that measured risk of overt immunological health effects in children (Fei *et al.*, 2010; Okada *et al.*, 2012, 2014; Smit *et al.*, 2015; Dalsager *et al.*, 2016; Goudarzi *et al.*, 2017; Timmerman *et al.*, 2017). Fei *et al.* (2010) studied 1,400 pregnant women in the Danish National Birth Cohort and measured the relationship between the mothers' serum PFOS concentrations and hospitalization of their children (as reported in the Danish National Hospital Register) for any type of infectious disease, such as eye, ear, respiratory system, skin, and genitourinary infections. PFOS exposure was not associated with infectious disease hospitalization, regardless of exposure tertile or child's age (0-1, 1 to <2, 2 to <4, or ≥ 4 years old). When stratified by sex, however, there was a slight, statistically significant association between incidence of hospitalization for any disease and maternal PFOS concentration ≥ 33.4 ng/mL in girls only.

In a prospective cohort study, Okada *et al.* (2012) measured the prevalence of self-reported infant allergies and infectious diseases in a cohort of 343 women recruited between 2002-2005 in Sapporo, Japan. Mothers' serum PFOS concentrations were not associated with food allergies, eczema, wheezing,

or otitis media in their children at 18 months of age. The authors also measured cord blood IgE and found that maternal PFOS concentration was also not associated with this endpoint. In a related prospective cohort study, Okada *et al.* (2014) evaluated the association between PFOS and the development of allergic diseases (eczema, asthma symptoms, nasal allergy symptoms) in the first year of life and again between 12 and 24 months of age. The study included 2,063 mother-child pairs enrolled in a prospective cohort study called the Hokkaido Study on Environment and Children's Health. Prenatal PFOS exposure was estimated using maternal blood samples taken between 28 and 32 weeks of pregnancy. Health was assessed through parental report *via* a questionnaire (which included questions such as, "Has your child had an itchy rash at any time in the past 12 months?"). Adjusted ORs suggested a non-significant *decreasing* risk of total allergic disease with increasing PFOS exposure (*e.g.*, OR = 0.86, 95% CI: 0.66-1.13 for the fourth quartile of exposure relative to the first). Similar results were reported for eczema analyzed alone. This study is limited by its use of parent-reported symptoms, rather than physician-confirmed allergic immune conditions, and the lack of measurement of post-natal PFOS exposure.

Goudarzi *et al.* (2017) studied 1,558 mother-child pairs enrolled in the same cohort studied in Okada *et al.* (2014) (the Hokkaido Study on Environment and Children's Health). Mothers reported the occurrence of four doctor-diagnosed childhood infectious diseases – otitis media, pneumonia, varicella, and respiratory syncytial virus infection. Prenatal PFOS exposure was estimated using maternal blood samples taken between 28 and 32 weeks of pregnancy. The odds of all four diseases was slightly, albeit significantly, increased in the second and fourth quartiles of PFOS exposure, but not in the third quartile (*i.e.*, there was no exposure-response relationship). The authors reported that the results were similar for the individual diseases; however, the trends were not statistically significant. There were small sample sizes for individual diseases.

Smit *et al.* (2015) evaluated asthma, eczema, and wheeze in 1,024 children aged 5-9 years old whose mothers were enrolled in the INUENDO Cohort between 2002 and 2004. Exposure was assessed using prenatal PFOS serum concentrations at enrollment, and health outcomes were determined based on interviews with pediatricians and local health workers. The authors grouped PFCs together, but the analyses of PFOS individually indicated that PFOS was associated with *decreases* in the risk of ever experiencing asthma, eczema, or wheeze, in addition to contemporaneous wheeze and eczema.

In a prospective cohort study, Dalsager *et al.* (2016) assessed the association between prenatal exposure to PFOS and other PFCs and parent-reported symptoms of infection over the course of a year (fever [$>38.5^{\circ}\text{C}$], stuffed or runny nose, cough, wheezy or whistling breathing, eye inflammation, ear pain, discharge from ear, feeling unwell, diarrhea, blood in stool, and vomiting) in 359 children 1-4 years old in the Odense Child Cohort. Children in the highest tertile of PFOS exposure had a statistically significant increase in days with a fever (incidence rate ratio [IRR]: 1.65, 95% CI: 1.24-2.18; *p* for trend = 0.001) and an increased odds of experiencing days with fever above the median (OR: 2.35, 95% CI: 1.31-4.11). There were no significant increases in risk of cough, nasal discharge, diarrhea, or vomiting. Limitations include a low original cohort participant rate (42%), non-response in some periods of the study (86% response in each weekly period), and the likelihood of bias in reporting of symptoms.

Timmermann *et al.* (2017) assessed the association between prenatal and post-natal (ages 5 and 13) PFOS exposure and asthma and allergic diseases at ages 5 and 13 in participants of the CHEF prospective birth cohort. The authors also evaluated potential interaction by measles, mumps and rubella (MMR) vaccination. PFOS concentration was measured in maternal blood during gestational weeks 34-36, and the children's blood at ages 5, 7, and 13. Total IgE and immunoglobulin A (IgA) concentrations were measured in cord blood, and IgE was measured again at age 7. At age 5, the investigators asked parents if the child had been diagnosed with asthma, hypersensitivity, or allergy. At age 13, parents were asked if the child had "suffered from" asthma or eczema or had experienced rhinoconjunctivitis symptoms in the past 23 months. A doubling of age-5 serum PFOS concentration was associated with an increased risk of

asthma and allergy at age 5 (ORs = 3.96 and 6.15 for asthma and allergy, respectively) in children without the MMR vaccine, although these risks were not statistically significant. When authors restricted the analysis to children with the MMR vaccine, the risk of these two conditions was decreased with a doubling of PFOS serum concentration. A doubling of serum PFOS concentration at age 5 was associated with a non-significant *decreased* risk of asthma, positive skin prick test, rhinoconjunctivitis, and atopic eczema at age 13 (regardless of MMR vaccination status, although authors only calculated risk of asthma in MMR-vaccinated children). Similarly, PFOS concentration measured at age 13 was also associated with a non-significant *decreases* in risk of asthma, positive skin prick test, rhinoconjunctivitis, and atopic eczema at age 13. Changes in IgE based on PFOS concentrations were not consistent or statistically significant. Prenatal PFOS exposure was not associated with any of these outcomes, regardless of MMR vaccination status. The authors noted that very few children in the cohort had not had an MMR vaccine.

Finally, I identified only two studies of PFOS exposure and immune responses in adult populations. In a cohort study, Looker *et al.* (2014) reported no statistically significant associations between serum PFOS concentrations (regardless of quartile) and antibody titers conferred by influenza vaccines or self-reported cold or flu in 411 healthy adults enrolled in the C8 Cohort. Kielsen *et al.* (2016) conducted a cross-sectional study of the association between PFOS, measured 10 days after vaccination, and diphtheria and tetanus antibody titers 2-30 days after vaccination. Serum PFOS ranged from 5.38-14.3 ng/mL (median: 9.52 ng/mL). There was a significant dose-dependent decrease in diphtheria antibody concentrations between days 4 and 10 (-11.90%, 95% CI: -0.33 to -21.92; $p = 0.044$). There was a slight decrease in tetanus antibody concentrations, but this change was not statistically significant. This study was limited by its very small sample size ($n = 12$).

Overall, I conclude that the weight of evidence does not establish an association between PFOS and immune effects. Many of the available studies measured only antibody titer levels, and the reductions in antibodies may not be associated with later risk of disease. Studies that measured physician-diagnosed infections generally found weak or no association between PFOS and risk of disease. Further, several studies showed decreased risk of disease with increased PFOS. These results indicate that it is unlikely that exposure to PFOS in drinking water would be associated with an increased risk of immune effects.

7.2.4.6 Kidney Effects

I identified three studies of PFOS exposure and kidney disease in exposed communities (Steenland *et al.*, 2010b; Watkins *et al.*, 2013) and the general population (Shankar *et al.*, 2011).

Steenland *et al.* (2010b) conducted a cross-sectional study of serum PFOS concentrations and uric acid levels (a biomarker of kidney function) in adult residents and workers in PFOS-contaminated water districts in Ohio and West Virginia. The authors found an association between PFOS and uric acid levels with an increase in uric acid of 0.2-0.3 $\mu\text{g/dL}$ from the lowest to highest decile for PFOS. The authors did not address the clinical significance of this increase. The odds of hyperuricemia (>6.0 mg/dL for women, >6.8 mg/dL for men) by quintile of PFOS exposure were slightly increased (ORs: 1.00, 1.02, 95% CI: 0.95-1.10; 1.11, 95% CI: 1.04-1.20; 1.19, 95% CI: 1.11-1.27; and 1.26, 95% CI: 1.17-1.35).

Watkins *et al.* (2013), described in a previous section, found a decrease in eGFR of 1.34 mL/min/1.73 m^2 (95% CI: -1.66 to -0.53, $p = 0.0001$) per IQR increase of serum PFOS concentration (IQR $\ln[\text{PFOS}] = 0.64$) in children and adolescents in the C8 Cohort. The authors could not rule out the possibility of reverse causation (that decreased kidney function caused increased serum PFOS concentration) in their analyses.

Finally, in cross-sectional study of the general population, Shankar *et al.* (2011) investigated the association between PFOS exposure and eGFR and kidney function using NHANES data. The authors reported that mean eGFR was decreased by 8.1 mL/min/1.73 m² (95% CI: -10.4 to -5.9) in the fourth quartile of PFOS compared to the first quartile in a study of the general population (p for trend < 0.0001). The authors also found a significantly increased odds of chronic kidney disease for the fourth quartile of serum PFOS concentration (OR = 1.83, 95% CI: 1.02-3.28). The odds of chronic kidney disease in quartiles two and three were non-significantly increased.

Overall, there is some indication that PFOS exposure may be associated with markers of kidney function and kidney disease. However, several of the available studies suffered from possible reverse causation and questions of clinical significance, as well as a lack of exposure-response relationships, limiting confidence in the observed associations.

7.2.4.7 Cancer

There are few studies available that have investigated the association between PFOS exposure and cancer in non-occupational populations, and few of these studies have examined the same cancer type.

Eriksen *et al.* (2009) conducted a case-cohort study of prostate, bladder, pancreatic, and liver cancer in a cohort of 1,140 men and women living in Denmark. The authors found no statistically significant associations between PFOS exposure and these cancers at any PFOS dose level and no dose-response trends between plasma PFOS concentration and any cancer type. Similarly, Vassiliadou *et al.* (2010) found no significant difference in serum PFOS concentrations in cancer cases (all types) at a hospital in Athens, Greece, compared with patients without cancer at an urban or rural/semi-urban hospital.

Bonefeld-Jorgensen *et al.* (2011) conducted a case-control study of 31 breast cancer cases and 115 control Inuit women living in Greenland. The authors reported a statistically significant increase in breast cancer with increasing serum PFOS concentration (OR = 1.03, 95% CI: 1.001-1.07; p < 0.05). While this association was statistically significant, the OR was only just barely greater than 1, indicating that the association is not strong. In a follow-up study to Bonefeld-Jorgensen *et al.* (2011), Wielsoe *et al.* (2017) updated the case-control analysis to include cases recruited from 2011-2014. There was a statistically significant relationship between increasing serum PFOS concentration and slightly elevated risk of breast cancer (OR = 1.02, 95% CI: 1.01-1.03). When stratified by exposure tertile, women in the second and third tertiles of exposure (≥ 2.91 ng/mL, maximum measured: 9.52 ng/mL) had a significantly increased risk of breast cancer (OR = 3.13, 95% CI: 1.20-8.15, and OR = 5.50, 95% CI = 2.19-13.84 for the second and third tertiles, respectively).

Hardell *et al.* (2014) conducted a case-control study of 201 prostate cancer cases and 186 control participants residing in Sweden. Serum PFOS concentration (median concentrations of 8.3 ng/mL in controls and 9.0 ng/mL in cases) was not associated with increased risk of prostate cancer (OR = 1.0, 95% CI: 0.6-1.5). However, in an analysis including hereditary risk (*i.e.*, a family history of this type of cancer), the authors reported that compared to participants with hereditary risk of prostate cancer but a serum PFOS concentration below the median, those with a serum PFOS concentration above the median and a hereditary risk of prostate cancer had a significantly increased risk of prostate cancer (OR = 2.7, 95% CI: 1.04-6.8). This suggests a possible increase in risk of prostate cancer in those with PFOS exposure and a family history of prostate cancer, but only with high PFOS exposures.

In a cross-sectional analysis, Innes *et al.* (2014) investigated the association between serum PFOS concentration and colorectal cancer in the C8 Cohort and found an inverse dose-response relationship (*e.g.*, OR = 0.2, 95% CI: 0.2-0.3; p < 0.00001, in the highest vs. lowest quartiles of exposure). In other

words, the risk of this type of cancer was reduced as PFOS exposure increased. This study was limited by single, cross-sectional measures of current PFOS exposure as surrogates for PFOS exposure at the time of cancer initiation (cancer takes decades to develop). The authors also lacked information on important colorectal cancer risk factors, including having inflammatory bowel disease and diet.

The weight of evidence does not establish an association between PFOS exposure and cancer incidence in community populations. Because of the weak evidence and the availability of no more than two studies for any one specific cancer type, I also conclude that the weight of evidence does not establish an association between PFOS exposure and cancer in non-occupational populations.

7.2.5 Overall Conclusions for Human Studies

Overall, occupational, community, and general population studies of PFOS exposure show inconsistent evidence of effects. Studies that report effects often had important methodological limitations, such as a cross-sectional study design. In other cases, statistically significant effects were not clinically significant (*i.e.*, associated with adverse health outcomes). For example, some studies have observed changes in thyroid hormones after PFOS exposure, but the levels of these hormones were not outside of the range of normal.

I therefore conclude that there is very little evidence of associations between PFOS exposure and health effects in humans, even at the relatively high exposure levels seen in the worker studies. These studies provide evidence that there is low, if any, risk of health effects from PFOS in drinking water.

7.3 PFBA and PFBS

7.3.1 PFBA and PFBS Exposure

Relative to PFOA and PFOS, there is a paucity of data on exposure to PFBA and PFBS in the general population. In available studies and surveys, often a large portion of serum samples do not have detectable concentrations of these compounds (see, for example, Olsen *et al.*, 2017). It is currently thought that the majority of exposure to PFBA is through drinking water (Gebbink *et al.*, 2015), whereas for PFBS, the main route of exposure for non-occupationally exposed person remains to be determined.

7.3.2 Studies in the General Population and Non-occupationally Exposed Populations

I identified very few studies of PFBA and PFBS exposure (one on PFBA, and five on PFBS). Only one category of endpoints (reproductive and developmental outcomes) had more than one study available. Therefore, the weight of evidence does not establish associations between exposure to these two PFCs and any health effects. Below, I discuss the available studies on these two compounds.

7.3.2.1 Reproductive and Developmental Effects

Li *et al.* (2017a) assessed PFBA exposure and fetal birth and growth outcomes in a cohort study of 321 mother-child pairs in the Guangzhou Birth Cohort Study in China. The PFBA concentration was below the limit of quantification in 43% of samples. The mean concentration of PFBA in cord serum was 0.15 ng/mL (standard deviation of 0.26; range: not detected to 3.37 ng/mL). After adjusting for potential confounders, PFBA exposure was not statistically significantly associated with lower birth weight or gestational age. This study benefits from the fact that the authors calculated prenatal estimates of PFBA

exposure, but it is limited by a small sample size and a lack of information on the mothers' GFR (a potential confounder).

In a cross-sectional analysis, Zhou *et al.* (2016) assessed reproductive hormones in 13- to 15-year-old Taiwanese adolescents from the control cohort for the Genetics and Biomarkers Study for Childhood Asthma (GACA) recruited between 2009 and 2010. The authors found no associations between serum PFBS concentration and estradiol or between serum PFBS concentration and testosterone in combined regression analyses or those stratified by sex. Note that this study was limited by its cross-sectional design; the authors also noted that information on menstruation status was missing for the sampling period.

Wang *et al.* (2017) conducted a case-control study of the association between PFBS and endometriosis-related information in a case-control study of 157 cases and 178 controls living in China. Cases were women between 20 and 45 years old who came to a Chinese hospital for treatment of infertility (inability to conceive after at least 12 months of unprotected intercourse) between 2014 and 2015. Median serum PFBS concentrations were 0.091 ng/mL (IQR: 0.088-0.097) in cases and 0.089 ng/mL (IQR: 0.085-0.095) in controls. Authors reported that PFBS exposure was associated with a significantly increased risk of endometriosis-related infertility (highest vs. lowest tertile: OR = 3.04, 95% CI: 1.65-5.57); this association remained even when restricted to nulliparous women. The major limitations of this study are the inability to determine the timing of disease onset and development and how the PFBS exposure related to the disease onset. Another important limitation of this study is that the majority of PFBS measurements were below the areas covered by the calibration curve, so many were extrapolated and thus subject to error.

7.3.2.2 Thyroid Hormones and Disease

In a cross-sectional analysis, Li *et al.* (2017b) evaluated the association between PFBS exposure and TSH, free T4, free T3, thyroglobulin antibody (TGA_b), and thyroid microsomal antibody (TMA_b) in 202 serum samples collected from the general population in three southern Chinese provinces between 2013 and 2014. The authors detected PFBS in only 27% of serum samples, so the authors did not report results for the analyses of the association between PFBS concentrations and thyroid hormones.

7.3.2.3 Serum Lipids

Zeng *et al.* (2015) conducted a cross-sectional study of the association between serum PFBS concentration and serum lipids in a group of 225 healthy 12- to 15-year-old children in Taiwan. There was a significant increase (19.3 mg/dL) in total cholesterol per $\mu\text{g/L}$ increased in PFBS concentration. However, analyses by quartile showed no statistically significant associations between PFBS exposure and total cholesterol, HDL, LDL, or triglycerides in any exposure group.

7.3.2.4 Immunological Effects

Zhu *et al.* (2016) conducted a case-control study that included 231 10- to 15-year-old Taiwanese children diagnosed with asthma within the previous year and 225 non-asthmatic control children, all from GACA. Serum was collected at study initiation and concentrations of PFBS, T_H cytokines (IL-2, IL-4, IL-5, and IFN- γ), and IgE were measured. There was a significant increase in odds of asthma among males in the highest vs. lowest quartiles of serum PFBS concentration (OR = 3.45, 95% CI: 1.15-7.88), but the effect was not observed among females. Among males with asthma, there was a statistically significant trend (p = 0.023) for increasing serum IL-5 with increasing PFBS concentration by quartile. There were no

statistically significant trends between other T_H cytokines or IgE levels and PFBS exposure among males with asthma. Among females with asthma, there were no statistically significant trends between PFBS exposure and any of the T_H cytokines or IgE.

7.4 Overall Conclusions

I identified very few studies of PFBA and PFBS exposures and only one category of endpoints (reproductive and developmental outcomes) had more than one study available. While some studies reported associations between PFBS exposure and infertility and childhood asthma, only a single study was available for each of these endpoints. Further, all the studies of these PFCs were limited by small sample sizes, and many reported that levels of these PFCs a large portion of study samples were below the respective limits of detection. Overall, the weight of evidence does not establish associations between exposure to these two PFCs and any health effects.

8 Agency Guidelines

The US and several state agencies have developed criteria concerning acceptable human exposures to specific PFCs. Agencies may set limits for acceptable total daily intake of PFCs or acceptable concentrations of PFCs in drinking water, food, *etc.* This section describes the daily intake, drinking water guidelines, and soil guidelines set by US EPA and by MDH for PFOA, PFOS, PFBA, and PFBS. It is important to note that exposure limits for chemicals represent levels below which no health effects are anticipated. Exceedance of a health-based guideline does not imply that any exposures above the recommended level will lead to health effects (US EPA, 2000a).

For non-cancer effects, US EPA typically derives an RfD to determine exposure guidelines for a chemical. The RfD is defined as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (US EPA, 2011c). To derive an RfD, US EPA chooses a critical effect from a key study in which humans or animals were exposed to the chemical; determines a NOEL, LOEL, or benchmark dose; and applies UFs to reflect the limitations of the data used.

Once an RfD for a chemical is derived, US EPA may also derive a drinking water Lifetime HA for the same chemical by estimating the proportion of daily intake of the chemical that comes from water intake and calculating a water concentration that is likely to ensure that exposures of the general population are below the RfD. It should be noted that the Lifetime HA is not a drinking water standard, as is the drinking water MCL. The MCL is a health-protective value promulgated by US EPA that also considers other factors such as costs and technical feasibility of compliance (US EPA, 2017c). The MCL represents a mandatory limit for a chemical in drinking water.

MDH also issues drinking water guidelines for non-cancer effects that are based on RfDs and derived using similar procedures to those used by US EPA. The RfDs may be taken from US EPA guidance or may be calculated by MDH. MDH drinking water guidance may be in the form of HRLs or HBVs. An HRL is defined as "the concentration of a groundwater contaminant, or a mixture of contaminants, that can be consumed with little or no risk to health and which has been promulgated under rule" (MDH, 2017d). An HBV is defined as "the concentration of a groundwater contaminant that can be consumed daily with little or no risk to health. HBVs are derived using the same algorithm as HRLs; however, they have not been promulgated as rules, have not undergone peer review, and may be based on less data and/or subject to greater uncertainty than HRLs" (MDH, 2017d).

In 2008 MDH derived RfDs and issued HRLs for PFOA and PFOS (MDH, 2008a,b). In 2016 US EPA issued new RfDs and Lifetime HAs for PFOA and PFOS (US EPA, 2016a,b). MDH then derived new RfDs for PFOA and PFOS, and issued new HBVs (MDH, 2017b,c), based on the animal studies and endpoints identified by US EPA (2016a,b), which were different from the studies and endpoints used by MDH in the derivations of the 2008 HRLs. In this section I describe the basis of the US EPA Lifetime HAs, the 2008 MDH HRLs, the 2017 MDH HBVs, and the respective RfDs from which they were derived. I also describe the US EPA and MDH guidance for PFBA and PFBS.

8.1 PFOA

8.1.1 US EPA

8.1.1.1 RfD

US EPA (2016a) derived an RfD of 0.00002 mg/kg-day for PFOA based on a reproductive and developmental study in mice by Lau *et al.* (2006). In this study, pregnant mice received oral doses of PFOA at 0-20 mg/kg-day from GD 1 to GD 17 or 18. Fetuses from some dams were checked for malformations on GD 18, and pups from the dams that were allowed to give birth were monitored for growth and development. The authors reported delayed skeletal ossification (bone development) and accelerated male puberty in mice, starting at the lowest dose group (1 mg/kg-day). However, neither the delayed skeletal ossification nor the accelerated male puberty in mice showed increased effects in the higher dose groups in a dose-related manner. US EPA concluded that the LOEL from this study was 1 mg/kg-day, corresponding to a predicted serum concentration in the mouse of 38 µg/mL. US EPA calculated an HED of 0.0053 mg/kg-day based on differences between mouse and human PFOA pharmacokinetics. A total UF of 300 (10 for intraspecies differences, 3 for interspecies differences, and 10 for use of a LOEL rather than a NOEL) was applied to the HED to derive an RfD of 0.00002 mg/kg-day for PFOA.

It is my opinion that US EPA's choice of these endpoints as the basis of the RfD for PFOA is not consistent with the underlying science. Specifically, none of the study endpoints used for RfD derivation exhibits a regular dose-response. For example, reduced ossification of the calvaria (part of the skull) has a U-shaped response, with the largest effects in the two lowest dose groups (1 and 3 mg/kg-day). Each of the other two reduced ossification endpoints, for forelimb and hindlimb proximal phalanges (toe bones), also have U-shaped responses. The dose-response for sexual maturation in males is reversed, with the largest effect at the lowest dose and the smallest effect at the highest dose. Irregular dose-response patterns such as these call into question whether the effects are actually related to the agent being tested.

There are also irregularities in the way the endpoints are reported. Ossification effects are typically reported as the number of animals exhibiting the effect, as opposed to the number of affected sites (US EPA, 1986a). This is important because if one animal has multiple affected sites, the effect reported as number of sites appears larger compared to the effect reported as number of animals. Thus it is not possible to tell in this study whether the results would be statistically significant if they were reported in the more standard "number of animals exhibiting the effect" format.

In addition, reduced or delayed ossification is not necessarily an adverse effect. Ossification is usually considered a variation, not a malformation, because ossification reduction is mild and reversible. Variations are alterations in the body that are considered to have no adverse effect on health or body conformity, may have a high incidence, and represent slight deviations from normal morphology (Stump *et al.*, 2012). It is also notable that other agencies, including ATSDR (2015), the European Food Safety Authority (EFSA) (2008), the United Kingdom Food Standards Authority (UK FSA) (2006), and the American Conference of Governmental Industrial Hygienists (ACGIH) (2001), did not include the ossification or puberty effects of PFOA reported by Lau *et al.* (2006) in their determinations of relevant endpoints. ATSDR (2015) considered the LOEL to be 3 mg/kg-day based on the "less serious" effect of a 20% body weight reduction in the pups on PND 23, and a LOEL of 5 mg/kg-day based on the "more serious" effect of full litter resorptions. EFSA (2008) considered the developmental LOEL to be 3 mg/kg-day based on growth deficits in the pups. In my opinion, the NOEL for developmental effects in this study is 1 mg/kg-day and the LOEL is 3 mg/kg-day based on reduced pup growth. The use of 1 mg/kg-

day as a NOEL rather than a LOEL allows for elimination of the LOEL-to-NOEL UF of 10, and would result in a 10-fold higher RfD of 0.0002 mg/kg-day.

Finally, the effects reported by Lau *et al.* (2006) may be mediated by PPAR α , as discussed in Section 5.3. To the extent that PPAR α is involved in these effects, and based on the fact that humans are less susceptible to PPAR α -mediated effects than rodents, using the effects observed in the study by Lau *et al.* (2006) as a basis for the MDH drinking water guideline for PFOA results in an overestimation of risk.

8.1.1.2 Drinking Water

Using the RfD of 0.00002 mg/kg-day, US EPA (2016a) derived a Lifetime HA of 0.07 μ g/L for PFOA in drinking water according to these equations:

$$DWEL = RfD \times \frac{BW}{DWI}$$

and

$$Lifetime\ HA = DWEL \times RSC$$

where:

RfD	=	Reference Dose (mg/kg-day)
BW	=	Body Weight (kg)
DWI	=	Drinking Water Intake (L/kg-day)
DWEL	=	Drinking-water-equivalent Level (mg/L)
RSC	=	Relative Source Contribution (unitless)

The drinking water intake (DWI) used in the above equation is the 90th percentile value for lactating women, equal to 0.054 L/kg-day (US EPA, 2011d). US EPA chose this parameter because of the potential for increased susceptibility during pregnancy and lactation, based on the study of developmental toxicity that was used for the RfD determination (Lau *et al.*, 2006). The RSC is the proportion of total PFOA exposure that comes from drinking water. US EPA used an RSC value of 20% that is not scientifically supported and is more stringent than is necessary to protect public health. US EPA stated that the default RSC value was justified because there was not enough information about other sources of PFOA exposure to choose a value that is more specific to PFOA. I will discuss this point further in Section 8.6.

Using the above equations, US EPA calculated the drinking-water-equivalent level (DWEL) and Lifetime HA as follows:

$$DWEL \left(0.00037 \frac{mg}{L} \right) = 0.00002 \frac{mg}{kg \cdot day} \div 0.054 \frac{L}{kg \cdot day}$$

and

$$Lifetime\ HA \left(0.07 \frac{\mu g}{L} \right) = DWEL \left(0.00037 \frac{mg}{L} \right) \times RSC (0.2) \times \frac{1,000 \mu g}{1 mg}$$

8.1.2 Minnesota

8.1.2.1 RfD (2008)

MDH derived an RfD of 0.000077 mg/kg-day for PFOA (MDH, 2008c). This value was based on a study of cynomolgus monkeys (Butenhoff *et al.*, 2002) in which monkeys received oral doses of AFPO (an ammonium salt that dissociates to produce PFOA) at 0, 3, 10, or 30 (reduced to 20 on day 22) mg/kg-day for 26 weeks. The monkeys exhibited increased absolute liver weights at oral exposures of 3 mg/kg-day (135% of control liver weight) and at 10 mg/kg-day (138% of controls), although no liver tissue injury, changes in clinical chemistry, or any other health effects were observed at these doses. No effects on liver weight or any other health effects were observed in two monkeys from each of the 3 and 10 mg/kg-day dose groups that were allowed a 13-week recovery period. In contrast, the high dose group showed signs of toxicity early in the study (weight loss and reduced food consumption). MDH chose changes in liver weight as the critical effect and calculated a HED based on the serum concentrations in the monkeys and a 70-fold difference between the half-lives of PFOA in monkeys and humans. MDH estimated a 0.0023 mg/kg-day benchmark dose for humans that represents the value at which a 10% response would be expected. A total UF of 30 (10 for intraspecies differences and 3 for interspecies differences) was applied to the benchmark dose to derive the RfD of 0.000077 mg/kg-day.

As previously noted in Section 6, liver weight changes that are reversible and are less than 150% of control, in the absence of any other toxicity, may be considered adaptive rather than adverse effects (US EPA, 2002b; Pohl and Chou, 2005; Hall *et al.*, 2012). Thus, it is my opinion that the underlying science supports the 10 mg/kg-day dose as the NOEL and 20 mg/kg-day as the LOEL for this study.

8.1.2.2 HRL

Based on the RfD of 0.000077 mg/kg-day, in 2008, MDH derived an HRL for PFOA in drinking water of 0.3 µg/L (MDH, 2008c). The HRL was calculated according to this equation:

$$HRL = \frac{RfD \times RSC}{DWI}$$

where:

DWI = 0.053 L/kg-day, the 95th percentile US population average from ages 0-19 years (the estimated amount of time to reach a steady-state concentration of PFOA in the body)
 RSC = 20%

The HRL for PFOA uses assumptions that overestimate risk, because it is based on a non-adverse effect (increase in liver weight) and incorporates an RSC that overestimates non-drinking-water exposure to PFOA, as discussed below in Section 8.6. To the extent that the increase in liver weight is mediated by PPARα (see Section 5.3), it may also be less relevant for humans than for rodents. It is my opinion that the HRL for PFOA could be higher and still be health-protective.

8.1.2.3 RfD (2017)

MDH recently issued a new RfD for PFOA of 0.000018 mg/kg-day, based on a new analysis of PFOA toxicity (MDH, 2017b). MDH's evaluation relied heavily on US EPA's hazard assessment of PFOA (US

EPA, 2016a), including the identification of Lau *et al.* (2006) as the key study and the developmental endpoints of delayed ossification and accelerated male puberty as the critical effects. Like US EPA, MDH concluded that the LOEL was 1 mg/kg-day, corresponding to a predicted serum PFOA concentration in the mouse of 38 µg/mL, and calculated an HED of 0.0053 mg/kg-day based on differences between mouse and human PFOA pharmacokinetics. While MDH also applied a total UF of 300, the individual UFs were different from those applied by US EPA. MDH applied a UF of 10 for intraspecies differences, 3 for interspecies differences, 3 for the use of the LOEL rather than the NOEL, and 3 for database uncertainty.

The choice of a UF of 3 rather than 10 for interspecies differences was appropriate, because the differences in PFOA half-lives between mice and humans were already accounted for (by the calculation of an HED). However, the UF of 10 for intraspecies differences is excessive in the context of setting the HBV, and could be reduced to 3 to account for toxicodynamic (sensitivity to toxic effects of a chemical) differences among populations. The application of a pharmacokinetic component of 3 is typically included in a risk assessment to account for differences in ADME among sensitive populations (US EPA, 2011e). The inclusion in the assessment of a pharmacokinetic component for population variability for PFOA is not necessary in this particular circumstance. The reason is that, in the exposure model that MDH used to derive an HBV, pharmacokinetics unique to the sensitive subpopulation (infants) are already accounted for (see Section 8.1.2.4).

The inclusion of a UF of 3 for database uncertainty is also unnecessary. MDH included this UF to account for the fact that there is no reliable two-generation study of reproductive effects of PFOA in rodents. However, this type of study is not an absolute requirement in standard risk assessments as long as there is a sufficient quantity of other high-quality studies for a chemical. For example, in contrast to MDH's conclusion that a two-generation reproductive study is required for a sufficient database of evidence, US EPA (2002a) recommends considering a wide range of information, such as route-specific information on multiple health endpoints and pharmacokinetic data across species, for determining the sufficiency of a database. In addition, based on the availability of epidemiological data on PFOA and extensive data on PFOA in animals, US EPA did not consider it necessary to include a database UF in its assessment of PFOA (US EPA, 2016a). Thus, the RfD derived by MDH for PFOA could be 30 times higher,⁸² 0.00054 mg/kg-day, when used in setting the HBV and still be health-protective.

8.1.2.4 HBV

MDH also recently issued a new HBV for PFOA (MDH, 2017b) of 0.035 µg/L, based on its new analysis of PFOA toxicity. MDH chose infants that exclusively breastfeed for 1 year as the target population, as opposed to US EPA's target population of lactating women. MDH devised a model for an infant's exposure to PFOA through the mother's water intake and subsequently through breastmilk up to 1 year of age. The RSC used for this evaluation was 50%. The choice of infants as the target population effectively lowered the HBV, because infants take in a greater amount of fluid relative to their body weights than adults do.

To derive an HBV for PFOA, MDH calculated a maximum acceptable infant serum concentration of PFOA based on the serum concentration in maternal mice (38 µg/L) from the Lau *et al.* (2006) study at the dose that MDH considered a LOEL. MDH then divided this value by the total UF of 300 to derive a maximum acceptable serum PFOA concentration in infants of 130 µg/L. This value was then divided by two to account for an RSC of 50%, resulting in a maximum acceptable serum PFOA concentration for

⁸² Based on the critical effect being a NOEL and using a total UF of 10 (UF of 3 for intraspecies differences, 3 for interspecies differences, and 1 for database uncertainty).

infants of 65 µg/L from drinking water (*i.e.*, from maternal DWI, because the infant is assumed to be exclusively breastfeeding in this scenario).

In order to account for the complex pharmacokinetics of PFOA in humans (including for half-life and placental and breast milk transfer), MDH used a pharmacokinetic model to derive the PFOA concentration in drinking water for mothers that would result in an infant serum concentration of ≤65 µg/L. Based on this model, the maximum concentration of PFOA in drinking water that corresponds to a serum concentration of ≤65 µg/L for infants, exclusively breastfed for 1 year, is 0.035 µg/L.

MDH incorporated various pharmacokinetic parameters that overestimate exposure for most individuals into its model of PFOA exposure in infants from maternal DWI. In addition to using 95th percentile water and breastmilk intake values (US EPA, 2011d), MDH incorporated a placental transfer factor of 87% based on mean values obtained from the primary literature (MDH, 2017a). This value is higher than those used in other published PBPK models of PFOA intake: approximately twice that used by Loccisano *et al.* (2013) (46%) and slightly higher than that used in Verner *et al.* (2016) (78%). MDH also incorporated an age adjustment factor for the V_d , which, while reasonable, results in predictions that overestimate exposure for most individuals more than other published models.

The HBV for PFOA is subject to the same uncertainties and limitations as discussed above for the US EPA RfD for PFOA. Specifically, the endpoints chosen as the critical effects from the key study (Lau *et al.*, 2006) for the derivation of both the US EPA RfD and MDH HBV are not supported by the science. In addition, MDH incorporated UFs that overestimate risk to calculate the HBV and chose upper-end values for each variable in its calculations. Therefore, a higher HBV for PFOA would still be health-protective, even for sensitive sub-populations (*i.e.*, the nursing infant).

8.1.2.5 Soil

MDH guidance values for PFOA in soil are based on the RfD of 0.000077 mg/kg-day (MPCA, 2009). MDH has three different Soil Reference Values (SRVs) for PFOA depending on the potential exposure scenario (MPCA, 2009). The PFOA SRV for residential exposure is 2.1 mg/kg. This is based on a 33-year residence, from ages 0 to 33 years, assuming incidental soil ingestion of 100 mg/day from ages 0-6 years, 75 mg/day from ages 7-18 years, and 50 mg/day from ages 19-33 years (MPCA, 1999, 2009). The PFOA SRV for recreational exposure is 2.5 mg/kg, and assumes exposure 92 days/year for 33 years, from ages 0 to 33 years, assuming incidental soil ingestion of 250 mg/day from ages 0-6 years, 175 mg/day from ages 7-18 years, and 100 mg/day from ages 19-33 years (MPCA, 1999, 2009). For the industrial exposure scenario, the SRV is 13 mg/kg, based on a 70-kg adult working 250 days/year for 25 years and assuming incidental soil ingestion of 80 mg/day (MPCA, 1999, 2009).

As with the drinking water values, the MDH SRVs overestimate risk. In addition to being based on a non-adverse effect (liver enlargement), the equations used to derive the SRVs employ default assumptions that overestimate risk. For example, the calculations for residential soil exposure assume 350 days/year spent outdoors (MPCA, 1999). While it is standard practice to use such default assumptions that overestimate risk (US EPA, 1989), it is not necessarily appropriate for all locations. Given Minnesota's climate in the winter, it is unlikely that a resident would spend 350 days/year outdoors; even if that were the case, the soil would be covered with snow for a substantial part of the year. Even without snow cover, the ground in Washington County is frozen for approximately 4 months of every year, based on mean monthly temperatures (US Climate Data, 2017), and soil would not be in a form that is ingestible during that time.

8.2 PFOS

8.2.1 US EPA

8.2.1.1 RfD

US EPA (2016b) derived an RfD of 0.00002 mg/kg-day for PFOS based on two reproductive and developmental studies in rats by Luebker *et al.* (2005a,b), discussed in Section 6.2. In the first study (Luebker *et al.*, 2005a), male and female rats were dosed with PFOS for 6 weeks prior to mating, during mating, and through gestation and lactation across two generations. US EPA chose reduced weight gain in the F2 pups as the critical effect and cited a NOEL for developmental effects of 0.1 mg/kg-day and a LOEL of 0.4 mg/kg-day. The study authors noted that they did not consider the reduced weight gain in the F2 pups a toxicologically significant effect because it was transient (seen at days 7 and 14 but not at day 21 post-partum), could have been related to litter size (*i.e.*, larger litters tend to have smaller pups), or could have been a random effect of culling. The F1 pups did not exhibit this effect; the NOEL for the F1 pups was 0.4 mg/kg-day (the highest dose tested in the F1 pups). Luebker *et al.* (2005a) considered the NOEL for offspring effects to be 0.4 mg/kg-day.

The second study by Luebker *et al.* (2005b) was a one-generation reproduction and development study in rats of the same strain as those used by Luebker *et al.* (2005a). In this study, females only were dosed for 6 weeks prior to mating through day 4 of lactation, and the lowest dose tested was 0.4 mg/kg-day. At this dose, the F1 pups exhibited lower birth weight and reduced weight gain. This is contrary to the first study, in which the F1 pups did not exhibit effects at this dose. The authors did not address the differences in results between the two studies. US EPA cited a LOEL for this study of 0.4 mg/kg-day and considered it to be support for their choice of a NOEL of 0.1 mg/kg-day for the critical effect of reduced weight gain in the F2 pups in the first study (Luebker *et al.*, 2005a).

US EPA used a pharmacokinetic model to predict a rat serum concentration of 6.26 µg/mL at the NOEL dose of 0.1 mg/kg-day, and calculated an HED of 0.00051 mg/kg-day based on differences between rat and human PFOA pharmacokinetics. A total UF of 30 (10 for intraspecies differences and 3 for interspecies differences) was applied to the HED to derive an RfD of 0.00002 mg/kg-day.

The basis of US EPA's RfD for PFOS is weak. US EPA based the RfD on non-adverse, transient effects (reduced weight gain in the F2 pups) that were not considered toxicologically significant by the study authors (Luebker *et al.*, 2005a). The supporting study that US EPA chose (Luebker *et al.*, 2005b) had results (lower birth weight and reduced weight gain in the F1 pups) that were inconsistent with the first study. Because of these inconsistencies, the toxicological endpoint used to develop the RfD for PFOS is not well-founded. A more scientifically supported NOEL would be four-fold higher, at 0.4 mg/kg-day, which would increase the HED to 0.002 mg/kg-day and the RfD to 0.00008 mg/kg-day.

8.2.1.2 Drinking Water

Using the RfD, US EPA (2016b) derived a Lifetime HA of 0.07 µg/L for PFOS in drinking water as described in Section 8.1.1.2.

The DWI used to derive the Lifetime HA is the 90th percentile value for lactating women, equal to 54 mL/kg-day (US EPA, 2011d). US EPA chose this parameter because of the potential for increased susceptibility during pregnancy and lactation, based on the study of developmental toxicity that was used

for the RfD determination (Luebker *et al.*, 2005b). For the RSC, US EPA used a default value of 20%. US EPA stated that the default value was justified because there was not enough information about other sources of PFOS exposure to choose a value that is more specific to PFOS. This will be discussed further in Section 8.6.

Using the equations described in Section 8.1.1.2, US EPA calculated the DWEL and Lifetime HA as follows:

$$DWEL \left(0.00037 \frac{mg}{L} \right) = 0.00002 \frac{mg}{kg \cdot day} \div 0.054 \frac{L}{kg \cdot day}$$

and

$$Lifetime HA \left(0.07 \frac{\mu g}{L} \right) = DWEL \left(0.00037 \frac{mg}{L} \right) \times RSC (0.2) \times 0.000074 mg/L \text{ (rounded to } 0.00007 mg/L \text{)}$$

8.2.2 Minnesota

8.2.2.1 RfD (2008)

MDH derived an RfD of 0.00008 mg/kg-day for PFOS (MDH, 2008c). This value was based on a 26-week study of cynomolgus monkeys (Seacat *et al.*, 2002) in which the monkeys exhibited decreased HDL and T3 at oral exposures of 0.15 mg/kg-day. MDH considered this to be the LOEL and calculated an HED based on the serum concentrations in the monkeys and differences in pharmacokinetics between monkeys and humans, and estimated a 0.0025 mg/kg-day benchmark dose for humans that represents the value at which a 10% response would be expected. A total UF of 30 (10 for intraspecies differences and 3 for interspecies differences) was applied to the benchmark dose to derive the RfD of 0.00008 mg/kg-day.

The POD chosen by MDH as a LOEL to develop the RfD is highly uncertain, as discussed in Section 6.2.1.2, and is more correctly interpreted as a NOEL. While a decrease in HDL in females was observed at the 0.15 mg/kg-day dose, this change was of questionable toxicological significance because the change was mild (*i.e.*, mean levels were still within normal range) and there was no uniformly dose-dependent decrease in total cholesterol (Seacat *et al.*, 2002). In the recovery animals, no clinical or hepatic effects were seen. The authors of this study considered 0.15 mg/kg-day to be the NOEL because the hormone and lipid changes were not toxicologically significant.

MDH also cited changes in thyroid hormone status as an adverse effect. The biological significance of decreased T3 in the animals dosed with 0.15 mg/kg-day PFOS is questionable, given that decreases in T3 levels were modest (*e.g.*, levels in treated monkeys were 80% and 75% of values in male and female control monkeys, respectively), there was no corresponding decrease in T4 levels, and the T3 levels as reported by the Mayo Clinic in a cross-checking analysis were not significantly reduced at 0.15 mg/kg-day. The method used by Ani Lytics in the first analysis for TSH measurement was not optimized for cynomolgus monkeys and the cross-checking by the Mayo Clinic of a subset of samples produced different results, prompting significant concerns about the original results (see Section 6.2). For these reasons, I consider 0.15 mg/kg-day to be the NOEL for this study, not the LOEL.

Recently, Chang *et al.* (2017) conducted a similar monkey study in order to address some of the uncertainties and limitations associated with the Seacat *et al.* (2002) study, such as a lack of baseline and other measurements for HDL and unreliable methods for thyroid hormone measurement (see discussion in Section 6.2.1.2). Using doses that were calculated to match the serum PFOS concentrations in the previous study but with a dose administration regimen that was designed to be less stressful to the

monkeys, Chang *et al.* (2017) found only slight treatment-related changes to serum HDL and total T4 concentrations that were not toxicologically relevant. The authors calculated serum PFOS BMCL₅ values, based on the small reduction in HDL, to be 74 and 76 µg/mL for male and female monkeys, respectively.

The Chang *et al.* (2017) study illustrates the challenges inherent in using animal experiments to extrapolate to a "safe" level of human exposure. While the Seacat *et al.* (2002) study resulted in some of the monkeys becoming ill or dying, the monkeys used in the Chang *et al.* (2017) study, while reaching equivalent serum concentrations of PFOS, stayed healthy throughout the course of the study and did not show any toxicologically relevant effects. This suggests that the animals in the Seacat *et al.* (2002) study were stressed by conditions that were not related to the PFOS exposure. The small (non-toxicologically relevant) effects Chang *et al.* (2017) did observe for HDL and total T4 were reversed after dosing stopped. Furthermore, the methods that Seacat *et al.* (2002) used to measure thyroid hormones were not reliable and resulted in reported effects that were not verifiable by an outside laboratory (see Section 6.2.1.2). Although Chang *et al.* (2017) did not observe any adverse effects, the BMCL₅ values they calculated are four orders of magnitude higher than the geometric mean PFOS serum concentrations in the US population in 2013-2014 of 0.00499 µg/mL (CDC, 2017a).

8.2.2.2 HRL

Based on the RfD of 0.00008 mg/kg-day, MDH derived an HRL for PFOS in drinking water of 0.3 µg/L (MDH, 2008c). The HRL was calculated according to this equation:

$$HRL = \frac{RfD \times RSC}{DWI}$$

where:

- DWI = 0.049 L/kg-day, the 95th percentile US population average from ages 0-27 (the estimated amount of time to reach a steady-state concentration of PFOS in the body)
- RSC = 20%

The HRL for PFOS uses assumptions that overestimate risk because it is based on a non-adverse effect (small changes in HDL and T3 that are within the normal range) and incorporates an RSC that overestimates non-drinking-water exposure to PFOS, as discussed in Section 8.6. It is my opinion that the HRL for PFOS could be higher and still be health-protective.

8.2.2.3 RfD (2017)

MDH recently issued a new RfD for PFOS of 0.0000051 mg/kg-day, based on a new analysis of PFOS toxicity (MDH, 2017c). MDH's evaluation relied heavily on US EPA's hazard assessment of PFOS (US EPA, 2016b), including the identification of Luebker *et al.* (2005b) as the key study and of developmental endpoints as the critical effects. Like US EPA, MDH concluded that the NOEL for PFOS was 0.1 mg/kg-day, corresponding to a predicted serum PFOS concentration in the mouse of 6.26 µg/mL, and calculated an HED of 0.00051 mg/kg-day based on differences between mouse and human PFOS pharmacokinetics. As discussed above, a value of 0.4 mg/kg-day from the Luebker *et al.* (2005b) study is a more scientifically supported NOEL. MDH applied a total UF of 100, which is higher than the US EPA's total UF of 30. MDH's total UF included a UF of 10 for intraspecies differences, 3 for interspecies differences, and 3 for database uncertainty (based on limited data for possible immune effects at lower doses than those that elicited developmental effects) (MDH, 2017e).

As noted above for PFOA, the choice of a UF of 3 for interspecies differences rather than 10 was appropriate. However, the UF of 10 for intraspecies differences overestimates risk in the context of setting the HBV and could be reduced to 3. The inclusion of a pharmacokinetic component in the intraspecies UF for PFOS is not necessary in this particular circumstance, because in the exposure model that MDH used to derive an HBV, pharmacokinetics unique to the sensitive subpopulation (infants) are already accounted for (see Section 8.2.2.4).

The UF of 3 for database uncertainty is also unnecessary. The single immune study that reported PFOS effects at very low doses (Peden-Adams *et al.*, 2008) was based on only five animals per sex per dose group, did not show a dose-response, was specific to one strain of mice only, and has not been replicated (as discussed in Section 6.2.1.4). Other studies in mice (Keil *et al.*, 2008; Lefebvre *et al.*, 2008; Dong *et al.*, 2009; Qazi *et al.*, 2010) did not find immunotoxicity until doses are 50 or more times higher than the LOEL reported in the Peden-Adams *et al.* (2008) study. It should be noted that, based on the availability of epidemiological data on PFOA and extensive data on PFOA in animals, US EPA did not consider it necessary to include a database uncertainty UF in its assessment of PFOS (US EPA, 2016c). Thus, the PFOS RfD derived by MDH could be 40 times higher, or 0.00020 mg/kg-day, when used in setting the HBV and still be health-protective.⁸³

8.2.2.4 HBV

MDH also recently issued a new HBV for PFOS of 0.027 µg/L, based on its new analysis of PFOS toxicity (MDH, 2017c). MDH chose infants that exclusively breastfed for 1 year as the target population, rather than US EPA's choice of lactating women. MDH devised a model for an infant's exposure to PFOS using the same methodology as described above for the PFOA HBV. The choice of infants as the target population effectively lowered the HBV, because infants take in a greater amount of fluid relative to their body weights than adults do.

To derive an HBV for PFOS, MDH first calculated a maximum acceptable infant serum concentration of PFOS based on the predicted serum concentration in rat pups (6.26 mg/L) from the Luebker *et al.* (2005b) study at the dose that MDH considered a NOEL. MDH then divided this value by the total UF of 100 to derive a maximum acceptable serum PFOS concentration in infants of 0.063 mg/L, or 63 µg/L. This value was then divided by two to account for an RSC of 50%, resulting in a maximum acceptable serum PFOS concentration in infants of 31.5 µg/L from drinking water (*i.e.*, from maternal DWI, because the infant is assumed to be exclusively breastfeeding in this scenario).

In order to account for the complex pharmacokinetics of PFOS in humans (including for half-life and placental and breast milk transfer), MDH used a pharmacokinetic model to derive the PFOS concentration in drinking water for mothers that would result in an infant serum concentration of ≤31.5 µg/L. Based on this model, the maximum concentration of PFOS in drinking water that corresponds to a serum concentration of ≤31.5 µg/L for infants, exclusively breastfed for 1 year, is 0.027 µg/L.

As discussed in in Section 8.1.2.3, MDH incorporated parameters that overestimate exposure for most individuals in their pharmacokinetic model of PFOS exposure from drinking water, such as the 95th percentile water and breastmilk intake values for adults and infants, respectively (US EPA, 2011d).

⁸³ Based on a NOEL of 0.4 mg/kg-day (*vs.* 0.1 mg/kg-day) and using a total UF of 10 (3 for intraspecies differences, 3 for interspecies differences, and 1 for database uncertainty).

The HBV for PFOS is subject to the same uncertainties and limitations as discussed above for the US EPA RfD for PFOS. The endpoint chosen as the critical effect from the key study (Luebker *et al.*, 2005b) is not adverse and is of questionable toxicological significance. In addition, MDH incorporated UFs that overestimate risk to calculate the HBV and chose upper-end maximum values for each variable in its calculations. The HBV for PFOS should therefore not be considered a reliable measure by which to make claims of risk to human health.

8.2.2.5 Soil

MDH guidance for PFOS in soil was derived from the RfD of 0.00008 mg/kg-day using the exposure parameters described in Section 8.1.2.3. The SRVs for residential, recreational, and industrial exposure are 2.1, 2.6, and 14 mg/kg, respectively (MPCA, 2009).

As with the drinking water value, the MDH SRVs overestimate risk. In addition to being based on non-adverse effects, the equations used to derive the SRVs employ assumptions that overestimate risk, as noted above for PFOA.

8.3 PFBA

There is no US EPA guidance for PFBA intake. MDH has derived an RfD for PFBA as well as drinking water and soil guidance for PFBA.

8.3.1 RfD

MDH (2011a) derived an RfD of 0.0038 mg/kg-day for PFBA based on a 28-day study in rats (NOTOX B.V., 2007a). A NOEL of 6 mg/kg-day and a LOEL of 30 mg/kg-day were established for decreased cholesterol and increased relative thyroid weight. MDH chose decreased cholesterol as the critical effect (MDH, 2011a) and identified a POD of 3.01 mg/kg-day (the BMDL₁₀) in rats. An HED of 0.38 mg/kg-day was extrapolated using a factor of 8 to account for half-life differences between humans and male rats (as noted in Table 5.6). The HED was further divided by a total UF of 100 (3 for interspecies toxicodynamic differences, 10 for intraspecies variability, and 3 for database insufficiencies, because of a lack of information on thyroid hormone effects).

NOTOX B.V. (2007b) also conducted a 90-day PFBA study, and MDH calculated a chronic RfD using this study (0.0029 mg/kg-day) that was lower than the RfD derived from the short-term 28-day study. However, the chronic non-cancer HRL that MDH calculated using this value was higher than the short-term HRL calculated using the RfD from the 28-day study (as described below). MDH therefore used the RfD derived from the short-term study to calculate the HRL.

8.3.2 Water

Based on the short-term RfD of 0.0038 mg/kg-day, MDH derived an HRL for PFBA in drinking water of 7 µg/L (MDH, 2011a). The HRL was calculated according to this equation:

$$HRL = \frac{RfD \times RSC}{DWI}$$

where:

$$\begin{aligned}\text{DWI} &= 0.289 \text{ L/kg-day, the 95}^{\text{th}} \text{ percentile US population average at ages 1-3 months, as} \\ &\quad \text{estimated by US EPA (Goeden, 2008)} \\ \text{RSC} &= 50\% \text{ (MDH, 2011a)}\end{aligned}$$

This calculation yielded an HRL of 6.57 µg/L, which was rounded to 7 µg/L. MDH used the short-term intake rate for infants aged 1-3 months because infants are assumed to be the most highly exposed population on an intake rate per kg of body weight in short-term timeframes (Goeden, 2008).

When MDH calculated a chronic non-cancer HRL using the RfD derived from the 90-day study, the resulting HRL was higher than the short-term HRL. The main reason for this discrepancy was that MDH used a lower water intake rate of 0.043 L/kg-day in the chronic value calculation, based on a TWA of the 95th percentile intake from birth to 70 years of age (MDH, 2011a; US EPA, 2011d; Goeden, 2008).

The DWI, based on a short-term intake rate at 1-3 months of age, is unusual, because the intake rate should reflect average intake over a lifetime, unless there is evidence to suggest that infants may be more susceptible to PFBA toxicity. None of the developmental PFBA studies (described in Section 6.3.4) indicate that developmental effects are sensitive endpoints for PFBA.

MDH used the lower HRL derived from the 28-day study as the chronic HRL on the grounds that it would be health-protective for both short- and long-term exposures (MDH, 2011a). The fact that the derivation of a short-term HRL for PFBA yielded a lower allowable intake than the derivation of a chronic HRL underscores the uncertainty involved in these types of calculations. It is not plausible that a short-term exposure would be associated with higher risk than a longer-term exposure to the same dose of PFBA. This illustrates the uncertainties inherent in the HRL for PFBA.

8.3.3 Soil

MDH guidance for PFBA in soil was derived from the RfD of 0.0038 mg/kg-day. The SRVs are 77 mg/kg for residential exposures, 94 mg/kg for recreational exposures, and 500 mg/kg for industrial exposures (MPCA, 2009).

The SRVs for PFBA overestimate risk due to the assumptions used (as explained in Section 8.1.2.3) and due to the likelihood that the endpoint on which they are based (decreased cholesterol) is non-adverse.

8.4 PFBS

US EPA has calculated a provisional RfD for PFBS for the purpose of deriving soil and water guidance for this chemical. MDH has established RfD and drinking water guidance, but not soil guidance, for PFBS.

8.4.1 US EPA

8.4.1.1 RfD

US EPA (2014c) derived a provisional RfD for PFBS of 0.02 mg/kg-day based on a 90-day study in rats (Lieder *et al.*, 2009a). US EPA chose kidney hyperplasia as the critical effect and calculated a BMDL₁₀

in rats of 78.7 mg/kg-day. Because pharmacokinetic data are not available for PFBS, US EPA derived an HED by applying a dosimetric adjustment factor of 0.24, from the following equation, and using a body weight of 0.25 kg for rats and 70 kg for humans.

$$\text{Dosimetric Adjustment Factor} = (\text{Animal Body Weight}^{1/4}) \div (\text{Human Body Weight}^{1/4})$$

The application of the dosimetric adjustment factor is standard risk assessment practice (US EPA, 2011b). The resulting human equivalent BMDL₁₀ was 18.9 mg/kg-day. A total UF of 1,000 was applied to the BMDL₁₀: 3 for interspecies toxicodynamic differences; 10 for intraspecies variability, 3 for a lack of developmental data *via* the oral route, because there is only one such study (Lieder *et al.*, 2009b); and 10 for the use of subchronic data. The resulting value was rounded to 0.02 mg/kg-day.

8.4.1.2 Water

US EPA has a PFBS RSL for tapwater of 380 µg/L (US EPA, 2016e). This value is based on a child resident with a water intake rate of 0.78 L/day, plus dermal exposure (bath) of 0.54 hours/day, for 6 years, 350 days/year, from ages 0-6 years (US EPA, 2014d).

8.4.1.3 Soil

Based on the provisional PFBS RfD of 0.02 mg/kg-day, US EPA derived a PFBS RSL for soil of 1,600 mg/kg for residential exposure (US EPA, 2016e). This value was calculated to be protective for 27 years of exposure: 6 years as a 15-kg child and 21 years as a 70-kg adult exposed for 350 days/year. US EPA does not have PFBS screening values for recreational or industrial exposures.

US EPA also derived an RSL for PFBS in soil based on the protection of groundwater. This value, 0.21 mg/kg, was calculated as the soil level that was necessary to achieve the risk-based tapwater RSL of 380 µg/L (US EPA, 2016e).

8.4.2 Minnesota

8.4.2.1 RfD

MDH (2011b) derived an RfD of 0.0014 mg/kg-day for PFBS based on the same 90-day study in rats (Lieder *et al.*, 2009a) that US EPA used to derive its PFBS RfD, although the MDH RfD is an order of magnitude lower due to differences in the method of derivation. MDH chose a NOEL of 60 mg/kg-day for decreased hemoglobin and hematocrit and histological changes in the kidney, as opposed to the NOEL of 200 mg/kg-day chosen by US EPA based on kidney hyperplasia in the same study. MDH calculated an HED of 0.42 mg/kg-day by applying a half-life adjustment factor of 142 for extrapolation from male rats to humans. The HED was further divided by a total UF of 300 (3 for interspecies toxicodynamic differences, 10 for intraspecies variability, 3 for database insufficiencies due to a lack of studies on neurological and thyroid effects, and 3 for the use of subchronic data).

8.4.2.2 Water

Based on the RfD of 0.0014 mg/kg-day, MDH derived an HRL for PFBS in drinking water of 7 µg/L (MDH, 2011b). The HRL was calculated according to this equation:

$$HRL = \frac{RfD \times RSC}{DWI}$$

where:

DWI = 0.043 L/kg-day, the 95th percentile US population average over a lifetime (US EPA, 2011d)
 RSC = 20%

The overestimation of risk in the HRL is demonstrated by its overestimation of non-drinking-water exposures to PFBS (as noted in Section 8.6) and in the choice of decreased hemoglobin and hematocrit and histological changes in the kidney as the critical effects. Neither the study authors (Lieder *et al.*, 2009a) nor US EPA (2014c) considered the hemoglobin and hematocrit changes to be critical effects, because other hematological parameters were unchanged, and therefore the biological significance of the small hemoglobin and hematocrit changes were unclear. In addition, the histological changes in the kidney that MDH referenced in its choice of critical effects are unclear. There is no mention in Lieder *et al.* (2009a), nor in the report from the contract laboratory (Argus Research, 2003) that first reported this investigation, of any histological changes in the kidneys at doses below the highest dose (600 mg/kg-day). Kidney changes were reported in a two-generation study by Lieder *et al.* (2009b) at 300 mg/kg-day; the NOEL for that study was 100 mg/kg-day. However, changes to blood were not evaluated in the two-generation study. It is unclear whether MDH included consideration of the two-generation study in its choice of critical effects and the NOEL; the HRL documentation does not reference this study in the choice of critical effects (MDH, 2011b). Considering both studies together, the NOEL is 100 mg/kg-day, not 60 mg/kg-day. Considering only the 90-day study, in my opinion, the NOEL is 200 mg/kg-day, based on kidney changes, because the changes that were reported in blood were of uncertain biological or toxicological significance. MDH's RfD and HBV for PFBS could be higher and still be health-protective.

8.5 PFHxS

There is no US EPA guidance for PFHxS intake.

MDH has not derived an RfD for PFHxS, nor issued an HRL or HBV for PFHxS. In its Health Based Guidance for Groundwater documentation for PFHxS, MDH (2009) stated that it identified only a single whole animal study of PFHxS toxicity (Butenhoff *et al.*, 2009b), and, after a review of the available information, they were "unable to recommend risk assessment advice (RAA) for PFHxS based on the limited data currently available" (MDH, 2009).

8.6 Relative Source Contribution

In addition to the concerns with the use of the underlying studies on which several of the guidelines are based, I also conclude that the use of a 20% RSC for deriving drinking water values is not scientifically supported and is more stringent than is necessary to protect public health. There have been several studies of dietary, dust, and inhalation exposure to PFOA (Fromme *et al.*, 2009; Lorber and Egeghy,

2011; Tittlemier *et al.*, 2007; Schechter *et al.*, 2010; Haug *et al.*, 2010; Noorlander *et al.*, 2011; EFSA, 2012; Vestergren *et al.*, 2012) and PFOS (Fromme *et al.*, 2009; Egeghy and Lorber, 2011; Tittlemier *et al.*, 2007; UK FSA, 2009; Clarke *et al.*, 2010; Noorlander *et al.*, 2011; Haug *et al.*, 2011; Domingo *et al.*, 2012; EFSA, 2012; Vestergren *et al.*, 2012; Klenow *et al.*, 2013; Gebbink *et al.*, 2015). None of these studies indicate that exposures other than drinking water are likely to add up to 80% of the allowable daily intakes of PFCs at the MDH RfDs.

In the 2017 HBV derivations for PFOA and PFOS, MDH chose a 50% RSC that was based on serum concentrations in both the local Minnesota and general US populations (MDH, 2017b,c). In these derivations, MDH compared the serum PFC concentrations of southern Washington County residents to those of the general US population, assumed to be unexposed to PFCs *via* drinking water and to receive most of its PFC exposure from other sources. The 95th percentile serum PFOA and PFOS concentrations from the general population were assumed to represent the proportions of serum PFOA and PFOS in the exposed population that were from non-drinking-water sources, and the proportions from drinking water were calculated accordingly. MDH also applied an RSC ceiling of 80% to ensure a margin of safety and derived an RSC for both PFOA and PFOS of 50%. While this value more closely reflects the proportion of PFOA and PFOS in serum that comes from drinking water than the default 20% RSC value, it is still very conservative. It should be noted that these calculations consider only the intake components of the HBVs. As discussed earlier in this section, there are other assumptions that overestimate risk in the RfDs themselves, such as the choice of toxicological endpoints that are highly uncertain while other toxicological endpoints are better supported by the underlying science.

8.7 Comparison of Drinking Water Guidelines to Animal Data

To put into perspective the health-protectiveness of the agency guidelines for PFCs in drinking water, I compared the guideline values with the NOEL and LOEL doses in the animal studies that provided the bases for the guidelines (Table 8.1). I used the agencies' methods to calculate the HEDs. In order to reach the human equivalent PFC doses that were associated with health effects in the animal studies, an adult would have to drink thousands of glasses of water at the guideline concentrations. Considering the impossibility of this and the fact that most of the guideline values are based on uncertain science and/or non-adverse effects, it is clear that both the US EPA and the MDH guideline concentrations for PFCs in drinking water are lower than necessary to be protective of human health and that exposure to levels that exceed the guidelines does not mean that health effects will occur. The guideline concentrations could be higher and still be health-protective.

Table 8.1 Comparative Doses from Animal Studies: Number of 8 oz. Glasses of Water at the Guideline Concentrations an Adult Would Need to Drink to Reach the NOEL or LOEL Used as the Basis for PFC Guidance Values

PFC	Study	Agency Guidance	Species	Critical Effects	LOEL/NOEL	Dose (mg/kg-day)	HED ^a (mg/kg-day)	HED for a 70-kg Human (mg/day)	Intake of Water at Guideline Value Needed to Reach HED ^{b,c}	
									L/Day	8 oz. Glasses/Day
PFOA	Lau <i>et al.</i> (2006)	US EPA HA	Mouse	Delayed skeletal ossification, accelerated male puberty	LOEL	1	0.0053	0.371	5,300	22,000
	Butenhoff <i>et al.</i> (2002)	MDH HRL	Monkey	Increased absolute liver weight	LOEL	3	0.012	0.87	2,900	12,000
	Lau <i>et al.</i> (2006)	MDH HBV	Mouse	Delayed skeletal ossification, accelerated male puberty	LOEL	1	0.0053	0.371	11,000	45,000
PFOS	Luebker <i>et al.</i> (2005a)	US EPA HA	Rat	Reduced weight gain in the F2 pups	NOEL	0.1	0.00051	0.0357	510	2,200
	Seacat <i>et al.</i> (2002)	MDH HRL	Monkey	Decreased HDL and T3	NOEL	0.15	0.0031	0.217	720	3,100
	Luebker <i>et al.</i> (2005a)	MDH HBV	Rat	Reduced weight gain in the F2 pups	NOEL	0.1	0.00051	0.0357	1,300	5,600
PFBA	NOTOX B.V. (2007a)	MDH HRL	Rat	Decreased cholesterol and increased relative thyroid weight	NOEL	6.9	0.86	60.2	8,600	36,000
	Lieder <i>et al.</i> (2009a)	US EPA HA	Rat	Kidney hyperplasia	NOEL	200	48	3,360	8,800	37,000
PFBS	Lieder <i>et al.</i> (2009a)	MDH HRL	Rat	Decreased hemoglobin and hematocrit and histological changes in the kidney	NOEL	60	0.42	29.4	4,200	18,000

Notes:

F2 = Second Generation; HA = Lifetime Health Advisory; HBV = Health-based Value; HDL = High-density Lipoprotein; HED = Human Equivalent Dose; HRL = Health Risk Limit; LOEL = Lowest Observed Effect Level; MDH = Minnesota Department of Health; NOEL = No Observed Effect Level; PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate; T3 = Triiodothyronine; US EPA = United States Environmental Protection Agency.

(a) As calculated by US EPA (2014c, 2016a,b) and MDH (2008a,b, 2011a,b).

(b) Water intake is rounded to two significant digits.

(c) The US EPA Lifetime HA and the MDH HBV for PFOS are based on potential effects in infants. An infant aged 1 to <2 years weighing 11.4 kg (US EPA, 2011d) would have to drink 350 cups of water at the Lifetime HA level and 910 cups of water at the HBV to reach the NOEL used as the basis of the guidance.

8.8 Minnesota's Calculation of Health Risk Indices

MDH's website page entitled "Evaluating Concurrent Exposures to Multiple Chemicals" describes MDH's methodology for calculating an HRI (MDH, 2017f), which is equivalent to a standard HI used by US EPA when determining human health risk from multiple chemicals (US EPA, 1989). The MDH (2017f) website states:

Chemicals that share a common health endpoint are evaluated together. Chemicals for which no health endpoint is specified (e.g., None) are not included in any group... For each chemical sharing a health endpoint, a ratio is calculated by comparing the groundwater concentration of the chemical to the exposure duration-specific health-based guidance for that chemical. The ratios are grouped by duration and summed within each health endpoint group.

In a letter to the City of Cottage Grove dated June 7, 2017 (Hogan, 2017), Mr. Tom Hogan of the MDH reiterated the above guidelines and gave the HRI calculation for PFCs using detected drinking water concentrations:

$$(XX \mu\text{g PFBA/L} / \text{PFBA HBV}) + (XX \mu\text{g PFBS/L} / \text{PFBS HBV}) + (XX \mu\text{g PFHxS/L} / \text{PFHxS HBV}^*) + (XX \mu\text{g PFOA/L} / \text{PFOA HBV}) + (XX \mu\text{g PFOS/L} / \text{PFOS HBV}) = \text{Health Index}$$

*Use PFOS HBV as the interim substitute.

MDH has not followed its own guidelines for calculating HRIs associated with PFCs. It has not followed the stipulation that "chemicals that share a common health endpoint are evaluated together" (MDH, 2017f). The endpoints that MDH chose for the derivation of the PFC HBVs are as follows, developmental for PFOA and PFOS, serum lipids (cholesterol) and thyroid weight for PFBA, and blood and kidney changes for PFBS. By MDH's own guidance, the PFOA and PFOS HRIs should be added together, but the PFBA and PFBS HRIs should each be calculated separately. In addition, PFHxS should not be included in any calculation, because MDH has not specified an endpoint, nor issued an HRL or HBV, for PFHxS. There is no scientific support for MDH using the PFOS HBV as a surrogate for PFHxS, because there is no evidence that developmental effects are the most sensitive endpoint for this chemical. In fact, of the two animal studies I identified that investigated the developmental effects of PFHxS (Butenhoff *et al.*, 2009b; Lee and Viberg, 2013), neither reported developmental effects at doses lower than 9.2 mg/kg, which is 92 times higher than MDH's NOEL for PFOS in the HBV determination (MDH, 2017c). MDH justified its use of the PFOS HBV as a surrogate for PFHxS by noting that the half-life of PFHxS is comparable to, and somewhat longer than, that of PFOS (Hogan, 2017). However, there is no evidence that the two chemicals are comparable in terms of toxicity or most sensitive endpoints.

Furthermore, it is my understanding that Part 4717.7880 of the Minnesota Rules, requires that HRIs be calculated using HRLs (MDH, 2017g). There is no provision for using HBVs in these rules. Thus, by calculating HRIs using the PFOA and PFOS HBVs instead of the HRLs for these PFCs, MDH did not follow its own rules. A correct calculation of HRIs would evaluate PFOA, PFOS, and PFBA together based on their HRLs, which share related endpoints (liver weight for PFOA, cholesterol changes for PFOS and PFBA) and would evaluate PFBS separately, because it has endpoints (blood cell and kidney changes) unrelated to liver/cholesterol effects.

8.9 Characteristics of Hazardous Waste Under Minnesota Rules Part 7045.0131

Under Minnesota Rules Part 7045.0131 (MPCA, 2013), a substance is considered hazardous waste if it exhibits any of the following characteristics: ignitability, corrosivity, reactivity, toxicity, lethality, or is an oxidizer. However, PFCs do not meet MPCA's definition of any of these properties, including lethality.

MPCA considers that:

a waste exhibits the characteristic of lethality if a representative sample of the waste has any one of the following properties:

- (1) an oral median lethal dose less than 500 milligrams of material per kilogram of body weight of test animal;
- (2) a dermal median lethal dose less than 1,000 milligrams of material per kilogram of body weight of test animal;
- (3) an inhalation median lethal concentration of less than 2,000 milligrams of material per cubic meter of air, if the material or a component is in a form that may be inhaled as a dust or mist; or
- (4) an inhalation median lethal concentration of less than 1,000 parts per million of material in air, if the material or component may be inhaled as gas or vapor. (MPCA, 2013)

For lethality study methodology, MPCA stipulates that:

- (1) Oral median lethal dose shall be determined by a test in which the specified time is 14 days, the group of test animals is at least ten white laboratory rats of 200 to 300 grams each, half of which are male and half of which are female, and the route of administration is a single oral dose.
- (2) Dermal median lethal dose shall be determined by a test in which the specified time is 14 days and the group of test animals is ten or more white rabbits, half of which are male and half of which are female, and the route of administration is a 24-hour exposure with continuous contact on bare skin.
- (3) Inhalation median lethal concentration shall be determined by a test in which the specified time is 14 days, the group of the test animals is at least ten white laboratory rats of 200 to 300 grams each, half of which are male and half of which are female, and the route of administration is continuous respiratory exposure for a period of one hour. (MPCA, 2013)

Using the search terms PFOA, PFOS, PFBA, PFBS, lethality, median lethal dose (LD₅₀), and acute toxicity in the PubMed search engine and searching through the 3M fluorochemical submissions to US EPA in 2000-2007, I located several lethality studies of PFOA and PFOS and none of PFBA or PFBS. For PFOA, only two oral studies (Glaza, 1990; Corning Hazleton Inc., 1997), one dermal study (Corning Hazleton Inc., 1995), and one inhalation study (Griffith and Long, 1980) met the MPCA study

methodology criteria, and none qualified PFOA as lethal, using the MPCA definition of lethality. For PFOS, only one oral study (Glaza, 1994), no dermal studies, and one inhalation study (Bio/dynamics, Inc., 1979) met the MPCA study methodology criteria, and none qualified PFOS as lethal, using the MPCA definition of lethality. Other studies either did not meet the minimum weight requirement for rats (see, for example, Primedica Redfield, 2000) or did not test both males and females (see, for example, Kennedy, 1985).

Thus, to my knowledge, there are no studies of PFC lethality that meet the standards for the methodology and/or the results necessary for designation of PFOA, PFOS, PFBA, or PFBS as hazardous waste under Minnesota Rules Part 7045.0131.

9 Site Data

For my analysis, I relied on data received from the client to compile a database. I used public and private well groundwater, sediment, surface water, and fish data, as described below. Appendix C presents the raw site data considered in my analyses.

9.1 Data Processing and Compilation

I received 29 documents containing sampling data that were pertinent to our study area from Brewer, which were used to construct a database for my analysis. A summary of the documents containing sampling information is provided in Table 9.1.

Table 9.1 Summary of Files Containing Sample Results

Bates Number or File Name	Date Received	Total Number of Results	Number of Results by Sample Media				Reference	
			Groundwater	Surface Water	Sediment	Fish		Other Media
2017-02-07_1750	3/7/2017	8,783	7,787	24			972	Weston Solutions, Inc. (2017a)
2017-02-07_1752	3/7/2017	3,952	2,281	263	103		1,305	Weston Solutions, Inc. (2017b)
2017-02-07_1805	3/7/2017	8,323	1,843	1,944	2,105	254	2,177	Weston Solutions, Inc. (2017c)
AnchorQEA_20170207	3/7/2017	63,876	63,876					Anchor QEA (2017)
STATE_02424691	4/21/2017	23,010				23,010		Minnesota (Undated-a)
STATE_02471758	4/21/2017	6,512	6,407	105				Minnesota (Undated-b)
STATE_02496119	4/21/2017	377			377			Minnesota (Undated-c)
STATE_04722967	4/28/2017	130	117				13	Minnesota (2013a)
STATE_04723267	4/28/2017	117	117					Minnesota (2013b)
STATE_04723415	4/28/2017	117	65				52	Minnesota (2013c)
STATE_04752940	4/28/2017	182	182					Minnesota (Undated-d)
STATE_04753551	4/28/2017	533		533				Minnesota (2008)
STATE_02512625	5/8/2017	3,559	3,559					Minnesota (2003-2011)
STATE_05826237	5/8/2017	19,847	4,632	3,859	140	8,067	3,149	Minnesota (Undated-e)
AttachmentA1.xlsx	6/15/2017	245	245					MDH (2017h)
AttachmentH1.pdf	6/15/2017	55	55					Hogan (2017)
STATE_04744448	6/27/2017	790				790		Wisconsin (2006-2011)
STATE_04767657	6/27/2017	14	14					MDH (Undated)
STATE_04783203	6/27/2017	164	164					Minnesota (2009-2015)
STATE_05681717	6/27/2017	7	7					Minnesota (2012)
STATE_06293075 & STATE_04743544	6/27/2017 & 7/10/2017	399				399		Minnesota (2011a, Undated-f)
STATE_04899541	6/28/2017	14	14					Minnesota (2013-2016)
STATE_05001933	7/1/2017	1	1					Minnesota (2011b)
STATE_05079553	7/10/2017	1				1		Wisconsin (2001-2011)
STATE_05395786	7/10/2017	1	1					Minnesota (2005-2013)
STATE_05464928	7/10/2017	21	21					Minnesota (2005-2016)
STATE_06262214	7/10/2017	725		725				Minnesota (1998-2014)
Samples with well advisories since August 2016	7/17/2017	973	973					(MDH, 2017i)

Notes:

Groundwater includes samples called drinking water or groundwater.

Other media includes invertebrates, liver, rain water, stormwater, unknown water, porewater, sludge, soil, and solids.

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I oversaw the data processing and compilation process, setting the parameters and goals of the analysis for staff to follow through. I also oversaw the processing of the sampling data using standard environmental database management techniques prior to including them in the database. This included reviewing and standardizing the data qualifiers from different sources.

If the source file contained sample qualifier information, it was directly included in the database. Additionally, detection flags (yes/no, or "<" reporting limit for non-detects), when available, were incorporated to confirm that the data qualifiers were complete. I used the following main data qualifiers in the database.

- U = Not detected
- J = Detected below a reporting limit
- R = Rejected (null or blank results)
- D = Diluted result
- ND = Not detected
- NQ = Not detected
- B = Detected in the blank quality control (QC) sample

If a result was not detected (and not rejected) and had no result values or detection limit listed, I used a value of zero. Some results were reported in the data sources with a value of zero.

In the data produced by Minnesota, if a result for a sample flagged as a "Raw Result" was less than the reported value, I considered those data detected with a J qualifier. This is typical practice with environmental data risk assessments, because it is a more conservative approach than considering the values to be not detected. A review of laboratory data sheets from MDH's laboratory (Minnesota, 2011b) indicates that J-qualified values are estimated detections. For instances in which we have both the laboratory data sheet and database source files (Anchor QEA, 2017), J qualifiers were confirmed as being used by Minnesota (in the laboratory data sheet), even if qualifiers were not included in the data files received. Therefore, in the exposure assessment, J-qualified results were used as detected values.

After reviewing multiple sample IDs, the most plausible interpretation of the naming convention for samples was as follows. The naming convention reflected the sample year and month, with the first two digits indicating the year and the letter indicating the month of that year (A = January, B = February, C = March, D = April, *etc.*). For example, sample 15J0340-04 was collected on October 10, 2015 (15 = 2015, J = 10th letter of the alphabet, so 10th month of the year [October]). Using this naming convention, sample months and years were assigned to samples in the June 2017 MDH letter, Attachment A (MDH, 2017h).

When possible, the Minnesota Unique Well Number (UN) was used to identify a sample location, and if the location or type was not clear from the data source, the Minnesota Geospatial Information Office's 2011 County Well Index (MnGeo, 2011) or MDH's Minnesota Well Index (MDH, 2017j) was reviewed to confirm well locations or types.

Duplicate samples collected for QC purposes were not frequently identified in the data sources, making it possible to have unmarked field duplicates. In addition, due to compiling data from disparate data sources, it was possible to have duplicated sample results in the database with different sample names

from different sources. To avoid double-counting repeated sample results, and to account for potentially unmarked field duplicates, data were averaged from the same combination of:

- Unique Well Number (or other location name),
- Matrix,
- Sample Date,
- Sample Type (*e.g.*, raw or filtered groundwater),
- Sample Depths (if known), and
- Analyte.

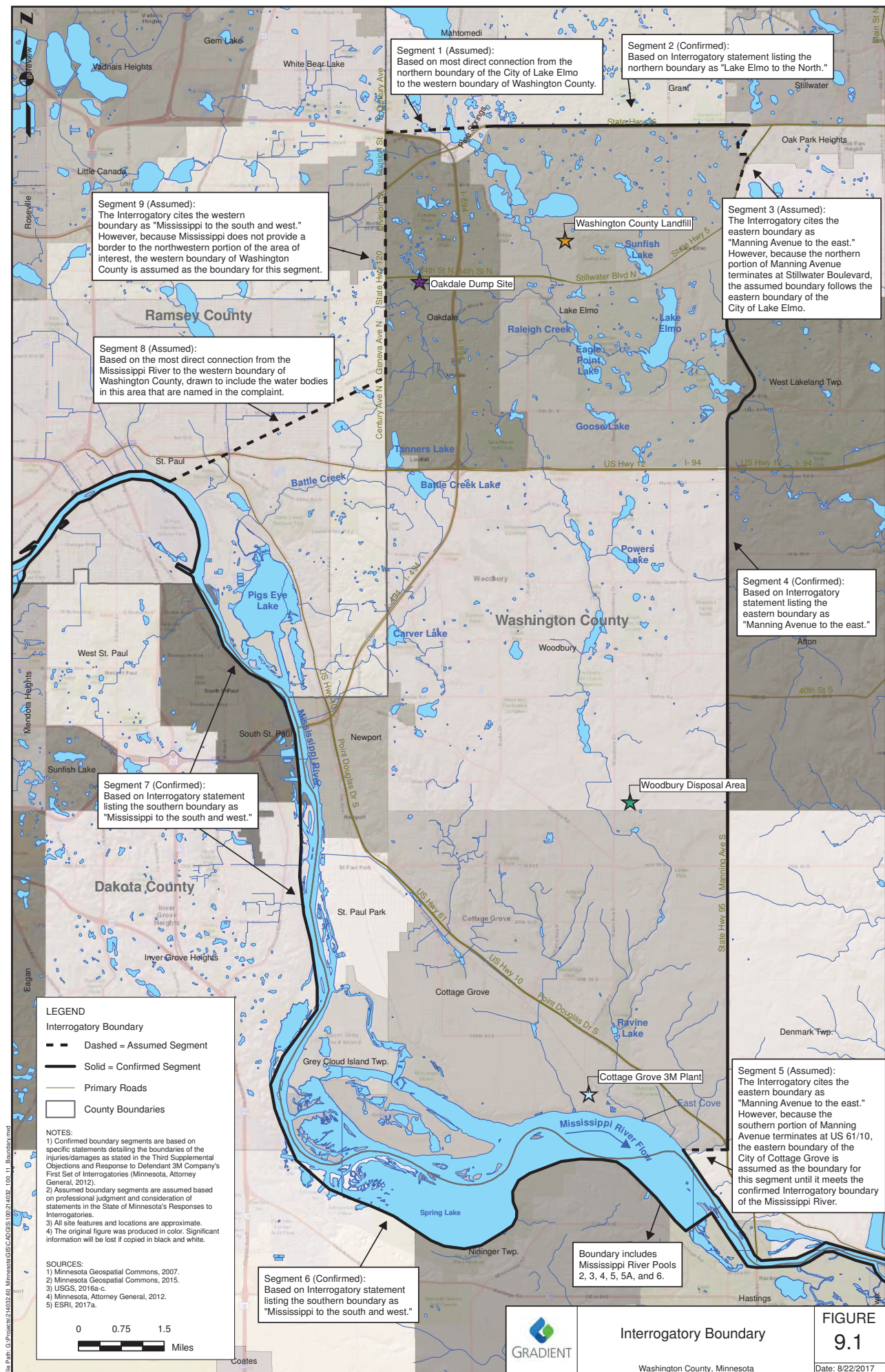
In this average, if one result was detected and the other not detected, the average of half the detection limit and the detected value was used and considered a detected result. All the data used for my analysis were based on this dataset of averaged duplicate results.

Many of the sample results provided in the source files included geographic coordinates to confirm their location. However, some sample locations could be identified only by using the town name or sampling event (Pool 2 sediment sampling, for example) provided with the sampling information. In those cases, only the general town or water body could be determined, and so generalized locations were mapped within a town or water body. Many of the locations in the data sources were assigned a site or group in a town that did not match the coordinates of the location. All towns and locations were confirmed by mapping the provided coordinates *vs.* Minnesota census boundaries (Minnesota Geospatial Commons, 2007, 2015) or the national hydrography dataset (US Geological Survey, 2016a).

9.2 Groundwater

I used data from groundwater sampling locations within southern Washington County, according to the boundaries described in the interrogatory (Minnesota, Attorney General, 2012), shown in Figure 9.1. Any further restrictions to groundwater data selection were made based on specifics in the interrogatories.

I excluded data from sampling locations within this boundary that were located within a 3M site, landfill, or dump (Figure 9.2), because these would not be used for drinking water. I excluded filtered groundwater from my analysis and used only samples categorized as "Raw" or "unknown" in order to evaluate the "worst-case" groundwater and drinking water concentrations. In addition, I had no information on filter efficacy or when the filters were placed. I used only groundwater samples collected from a private well or city well as designated in the data sources and confirmed the well types for Minnesota UNs in the Minnesota Geospatial Information Office's 2011 County Well Index (MnGeo, 2011) and MDH's Minnesota Well Index online search tool (MDH, 2017j). Groundwater sampling locations categorized by type and use within the exposure assessment are summarized in Table 9.2.



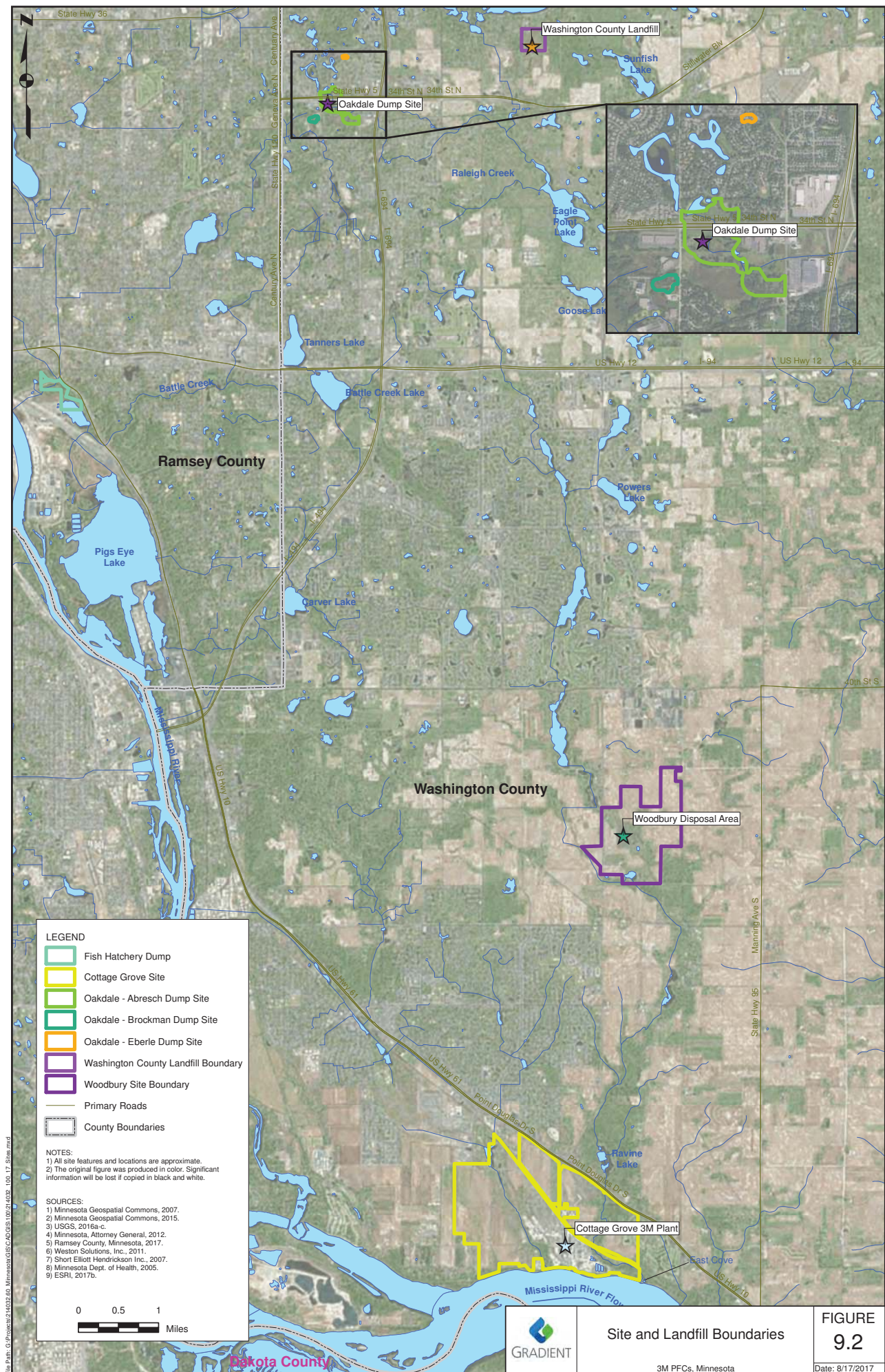


Table 9.2 Groundwater Location Types

Location Type	Use in Groundwater Exposure Assessment?
Private Well	Yes
City Well	Yes
Industrial Well	No, not possible drinking water
Irrigation Well	No, not possible drinking water
Landfill Well	No, not possible drinking water
Monitoring Well	No, not possible drinking water
Non-comm. Well	No, are not regularly used wells
3M Monitoring Well	No, not possible drinking water
Filter Test	No, not possible drinking water
Other DNR Well ^a	No, not possible drinking water
Treatment Plant	No, not possible drinking water
Test Well	No, not possible drinking water

Notes:

DNR = Minnesota Department of Natural Resources.

(a) These wells belong to the DNR and are used for groundwater monitoring (MDH, 2017j).

I evaluated data for private wells and city wells in two ways.

1. **Past: Maximum 12-month moving average.** I calculated the average concentration of a chemical in mg/L over every 1-year period for every private well and city well. I used all the data collected at each private well or group of city wells to identify the maximum 12-month moving average. I then used the data from the maximum 12-month moving average to identify the 95% UCLM for the maximum 12-month moving average at each private well and group of city wells, as discussed in Section 10. If too few samples were available to calculate a 95% UCLM, I used the maximum detected concentration from the group of data that produced the maximum moving average at each private well and group of city wells.
2. **Current: Most-recent two sampling rounds.** I gathered all the samples collected between 2014-2017 to review current groundwater conditions. I limited the dataset to include these years because older data are not representative of current conditions. Based on US EPA (2014a) recommendations, I used data from the latest two rounds of sampling to represent the current conditions at each well. I used the average concentration in mg/L of the most-recent two (or one, if only one was available) sampling rounds between 2014-2017 as the exposure point concentration (EPC), as discussed in Section 10.

The groundwater data considered in my analysis are presented in Appendix C, Table C.1. This table contains the samples available to consider in the past and current groundwater analysis.

9.3 Surface Water

I used surface water data from "named water bodies" based on the Responses to Interrogatories (Minnesota, Attorney General, 2012). Goose Lake was cited in one place within the Responses to Interrogatories, so I included data from Goose Lake. There are multiple Sunfish and Goose Lakes in Minnesota, so I considered the data from the Sunfish Lake located north of Lake Elmo and from the Goose Lake located in Washington County, because these lakes are near the areas described in the Interrogatories.

I was provided a fairly limited amount of surface water data, with the exception of data from Mississippi River Pools 2 and 3. When data were provided to me as being from the Mississippi River with no other identifying information (*i.e.*, pool number or coordinates), they were evaluated as being from the Mississippi River but with an unknown pool number.

I found the average and maximum result within the individual named water bodies and also reviewed the average and maximum results within general water body groups, as shown in Table 9.3.

Table 9.3 Data Availability and Generalized Data Grouping Areas for Named Water Bodies

Water Body	Generalized Group	Surface Water Data	Sediment Data	Fish Data
Raleigh Creek	Lakes	Yes	Yes	
Battle Creek	Lakes	Yes		Yes
Battle Creek Lake	Lakes			
Carver Lake	Lakes			Yes
Powers Lake	Lakes	Yes		Yes
Ravine Lake	Lakes	Yes		Yes
Pigs Eye Lake	Lakes		Yes	
Tanners Lake	Lakes	Yes		Yes
Sunfish Lake	Lakes			
Eagle Point Lake	Lakes			
Lake Elmo	Lakes	Yes		Yes
Goose Lake	Lakes	Yes		
Mississippi River East Cove	Mississippi River Pool 2	Yes	Yes	
Mississippi River Pool 2	Mississippi River Pool 2	Yes	Yes	Yes
Mississippi River Pool 3	Mississippi River Pool 3	Yes	Yes	Yes
Mississippi River Pool 4	Mississippi River Pool 4	Yes	Yes	Yes
Mississippi River Pool 5	Mississippi River Pool 5/5A			Yes
Mississippi River Pool 5A	Mississippi River Pool 5/5A			Yes
Mississippi River Pool 6	Mississippi River Pool 6			
Mississippi Unknown Pool	Mississippi River	Yes		

Notes:

No apparent data provided for: Battle Creek Lake, Sunfish Lake, Eagle Point Lake, or Mississippi River Pool 6.

The Generalized Group "Lakes" also includes the creeks that flow in and out of the lakes.

All surface water sampling data were standardized to mg/L. The surface water data considered in the exposure assessment are presented in Appendix C, Table C.2.

9.4 Soil

Consistent with the groundwater dataset, I excluded soil data from the exposure assessment boundary that were located within a 3M site, landfill, or dump. I only received soil collected from the Oakdale, Cottage Grove, and Woodbury 3M sites or dumps, where exposure would be negligible, if at all. Therefore, soil data were not analyzed in the exposure assessment.

9.5 Sediment

I limited my sediment dataset to the named water bodies listed in Table 9.3 and to shallow sediment only (0-0.5 ft), because direct contact by humans with sediment is most likely to occur in the top couple inches

of sediment (US EPA, 1996). All sediment sampling data from lakes and creeks were considered shallow and accessible enough for human contact based on the depth of the water in which the sediment was collected. Sediment samples collected in the Mississippi River were compared to United States Geological Survey (USGS) aquatic regions, the depths of those regions, and bathymetry information (river depths), when available (USGS, 1989a-c, 1992; WEST Consultants, Inc., 2000). Samples collected in deeper channels of the Mississippi River that would be inaccessible to humans were excluded from the MOE analysis.

All sediment data were assumed to be reported on a dry weight basis (in many data sources, information on whether the data were reported on a wet/dry weight basis was not available), as required by US EPA for evaluating risks (US EPA, 2004b).

All sediment sampling data were standardized to mg/kg. The sediment data considered in the exposure assessment are presented in Appendix C, Table C.3. The sediment samples collected in deeper channels of the Mississippi River that would be inaccessible to humans, and thus were excluded from the exposure assessment, are summarized in Appendix C, Table C.4.

9.6 Fish

I considered only fish data from the named water bodies listed in Table 9.3. To most closely model human fish consumption, I did not use whole organism fish samples and instead used fillet samples (skin on or off). The majority of the fish data I received were incomplete, and the sample type (fillet or whole organism) was not indicated. I used these data of unknown sample type with the fillet samples to have a more complete dataset. Table 9.4 summarizes the number of fish samples from the named water bodies that were fillet samples or of unknown type (excludes whole organism samples).

Table 9.4 Fish Sample Types, by Water Body Type

Sample Type	Lakes	Mississippi River Pool 2	Mississippi River Pool 3	Mississippi River Pool 4	Mississippi River Pool 5/5A
Fillet	85	414	46	44	45
Unknown		296	52		

Only one data source indicated whether the fish results were reported on a wet weight basis. For the other data sources, I found a report presenting some of these data that indicated the results were reported on a wet weight basis (STS Consultants, Ltd., 2007). For the other sources with sample IDs from Axys Analytical Services, Ltd., it was assumed that these data are reported on a wet weight basis, because it is that company's standard protocol to report tissue samples in wet weight (Axys Analytical Services Ltd., 2011). I received no tissue results that were confirmed to be reported on a dry weight basis. I made the reasonable assumption that if the wet/dry weight basis information for the fish data was not reported, the data were reported on a wet weight basis. Two sources contained fish data apparently collected by the State of Wisconsin (Wisconsin, 2001-2011, 2006-2011), and I used any fish data from those files that were collected from the named Mississippi Pools. As shown in Table 9.5, if I excluded fish data reported without a wet/dry weight basis, this would mean excluding all of the fish data collected from the named lakes; all of the fish data collected from Mississippi River Pools 4, 5, and 5A; and approximately half of the fish data collected from Mississippi River Pools 2 and 3. The majority of the fish data I received were incomplete, and the sample type (fillet or whole organism) or wet/dry weight basis was not indicated.

Table 9.5 Summary of Reported Fish Data Units Basis

Water Body	Fish Results Original Reported Units (Wet/Dry Weight Basis, if Available)	Number of Samples
Lakes	µg/kg	85
Mississippi River Pool 2	ng/g (report available confirming wet weight basis)	37
	ng/g (wet weight basis)	296
	µg/kg	377
Mississippi River Pool 3	ng/g (wet weight basis)	52
	µg/kg	46
Mississippi River Pool 4	µg/kg	44
Mississippi River Pool 5/5A	µg/kg	45

All the fish data results were converted to mg/kg and assumed to be on a wet weight basis. The fish data reviewed are presented in Appendix C, Table C.5.

10 Evaluation of Site Data

In this section, I evaluate the significance, in terms of impact to human health, of potential exposures of humans to PFOA, PFOS, PFBA, and PFBS in groundwater, surface water, sediment, and fish in southern Washington County, Minnesota. Specifically, I perform a multipathway exposure assessment for residents in the southern Washington County area, calculating receptor-specific doses, and evaluating the toxicological significance of such exposures, in comparison with the PODs developed by US EPA and MDH to set RfDs and used to develop drinking water guidelines. These comparisons represent MOE values. It should be emphasized that the dose estimates used in the MOE calculations are high-end estimates, intended to overestimate exposure, and should be considered hypothetical values.

I also evaluate the serum concentrations of PFOA and PFOS of individuals residing in the East Metro Minnesota area, where elevated levels of PFCs have been detected in drinking water. For evaluating serum concentrations, I compare the serum concentrations of residents with those of worker populations and with estimated serum concentrations in animals from studies used to develop the PODs for PFOA and PFOS.

10.1 Potentially Exposed Populations

Because the basis for the Minnesota PFOA and PFOS criteria is developmental effects, I focused my exposure evaluation on adult females, the sensitive subpopulation identified by MDH as the basis for its PFOA and PFOS HBVs. I evaluated an adult female resident who lives in southern Washington County and uses groundwater from a private or public well for drinking, showering, and other daily activities, as well as an adult female recreational user who may fish or swim at any of the lakes cited in the Interrogatories (hereafter referred to as the "named water bodies") (Minnesota, Attorney General, 2012). The named water bodies are listed in Table 9.3.

I assumed that the adult resident may use the public water supply in Cottage Grove, Lake Elmo, Newport, Oakdale, St. Paul, St. Paul Park, or Woodbury, or a private well in any of these areas, in addition to Grey Island Township, Maplewood, and West Lakeland Township. These public and private groundwater wells are within the boundaries described in the Interrogatories (Minnesota, Attorney General, 2012) and depicted in Figure 9.1. I evaluated exposure for an adult female ingesting groundwater and using the groundwater for showering or bathing.

I assumed that the adult recreational user may contact sediment and surface water while recreating at any of the water bodies named in the Interrogatories (Minnesota, Attorney General, 2012) and that the adult recreational user may ingest sport-caught fish from any of these named water bodies. Direct contact with sediment and surface water may occur while a recreational user is fishing or boating in the named water bodies. Therefore, I evaluated exposure for an adult female potentially exposed to groundwater *via* ingestion and dermal contact; surface water *via* incidental ingestion; sediment *via* incidental ingestion; and fish *via* ingestion. I did not evaluate dermal contact with PFCs in water for the adult recreational user and the adult resident, because the fraction absorbed (FA) values for PFOA, PFOS, and PFBS⁸⁴ calculated by US EPA (US EPA, 2004b, 2017b,d) were less than 1 and were, therefore, set to zero. Other receptors,

⁸⁴ PFBA is not listed in the US RSL calculator; however, I assumed that the chemical parameters for dermal absorption for this PFC were similar to that of PFOA, PFOS, and PFBS and therefore did not evaluate PFBA for the dermal exposure pathways.

such as adolescent and child swimmers and anglers, are likely to be present at the site, but due to the health endpoint selected for the basis of the agency guidelines, these receptors are not the most sensitive receptors.

My analysis is based on using a reasonable maximum exposure (RME), which US EPA (1989) defines as "the highest exposure that is reasonably expected to occur at a site." An RME is used to estimate a conservative exposure case (*i.e.*, well above the average case) that is still within the range of reasonably possible exposures. An RME is calculated using a combination of high-end and central-tendency values for exposure parameters. RMEs are estimated for individual pathways, and, if a population is exposed *via* more than one pathway, the combination of exposures across pathways also must represent an RME (US EPA, 1989, 2004b). It should be emphasized that the RME estimates represent hypothetical values that are generally intended to yield overestimates of exposure.

10.2 Development of Exposure Point Concentrations

I quantified exposures using media concentrations expressed as EPCs and equations modeling potential daily intake for an adult female in southern Washington County. While individual measured concentrations in private or public well water ranged from 0.0007-3 µg/L for PFOA, 0.003-3.5 µg/L for PFOS, 0.004-22 µg/L for PFBA, and 0.0007-0.3 µg/L for PFBS, it is important to evaluate daily intake estimates to understand the potential health impacts for women consuming this groundwater. EPCs represent an upper bound on the average concentration of a constituent of potential concern (COPC) in a particular media to which an individual may be exposed. Because of the potential uncertainty associated with estimating an exposure concentration, the 95% UCLM is generally used for this estimate (US EPA, 1989). I used the UCLM to provide reasonable confidence that the true average would not be underestimated (US EPA, 1989). This approach is consistent with US EPA exposure assessment methodology.

For groundwater, I calculated EPCs to address both past and current exposures. For current exposures, US EPA (2014a) recommends using data from the latest two rounds of sampling for each well to calculate an EPC representing current conditions. Because older data are not representative of current conditions, I limited the dataset included for current groundwater conditions to the most recent 3 years of data (2014-2017). I used the latest two rounds of groundwater sampling at each well to calculate an average concentration, because the 95% UCLM cannot be calculated for two samples. For past exposures, I used all the data (2003 through 2017) collected at each private well or group of city wells to identify the maximum 12-month moving average. I then used the data from the maximum 12-month moving average to identify the 95% UCLM for the maximum 12-month moving average at each private well and group of city wells. I used a 12-month moving average that approximates the typical 9-month gestational period because of MDH's focus on a woman of reproductive age (and the nursing infant) for its HBV. The choice of the 12-month moving average is consistent with the long half-lives of PFOA and PFOS and is a conservative way to represent groundwater exposure concentrations during the gestational and lactational periods. It is conservative because it results in a higher effective groundwater exposure concentration than would a longer averaging period (*e.g.*, as compared to 21 months for a 9-month gestational exposure and 12-month lactational exposure).

The EPCs for surface water, sediment, and fish were grouped according to the general group categories indicated in Table 9.3. Because all the named lakes are located either within southern Washington County or within close proximity to that area, I assumed that a recreational user could visit any of the named lakes and used the 95% UCLM for all the usable surface water, sediment, and fish samples combined for these lakes. Because Mississippi River Pool 2 through Pool 5A extend approximately 110

miles and it is unlikely that recreational users would travel this distance regularly, I evaluated a recreational user of each pool separately rather than of the combined extent of these pools.

For surface water, I used the 95% UCLM of all samples within the named water bodies as the EPC.

For sediment, I used the 95% UCLM of samples collected from less than 0.5 ft below ground surface and excluded samples collected from the deeper channels of the Mississippi River (*e.g.*, samples in the main navigational channel or impounded areas located beneath >9 ft of water), as discussed in Section 9.5. I averaged the results from shallow sediment samples (0-0.5 ft) collected in the accessible portions of the named water bodies, because direct contact with sediment predominantly occurs in these shallower soils and sediments (US EPA, 1996).

For fish, I used the 95% UCLM of all measured fish samples as the EPC. I excluded data that were analyzed for the whole body of fish, because they included parts that an individual would not consume. US EPA (1989) notes that exposure from fish or shellfish is calculated using the concentration of a chemical in the edible tissues of the organism.

I then used these EPCs to calculate exposure to PFCs in groundwater for an adult female resident and in surface water, sediment, and fish for an adult female recreational user, using the equations described in Section 10.2 (US EPA, 1989).

10.3 Exposure Equations and Assumptions

I used the following equations and exposure assumptions when estimating exposure to adult female residents and recreational users in southern Washington County. The equations and assumptions that I used to estimate exposure for these receptors are based on US EPA exposure assessment guidelines and methodology. When appropriate, I used assumptions that were recommended by either US EPA or the MPCA for use in estimating chemical exposures in the general population. Table D.1 presents the exposure assumptions that I used in my exposure analysis and their basis. I used the typical reproductive age of 15-44 years when determining exposure assumptions for females in my exposure analysis (WHO, 2013).

10.3.1 Ingestion of PFCs in Groundwater

$$Intake = \frac{EPC_{GW} \times IR_{GW} \times EF \times ED \times FS}{BW \times AT}$$

where:

EPC_{GW}	=	Exposure Point Concentration of PFC in Groundwater (mg/L)
IR_{GW}	=	Groundwater Ingestion Rate (L/day)
EF	=	Exposure Frequency (days/year)
ED	=	Exposure Duration (years)
FS	=	Fraction from Source (unitless)
BW	=	Body Weight (kg)
AT	=	Averaging Time (days)

I assumed that women could potentially ingest 2.5 L/day of groundwater for 350 days per year for 29 years (ages 15-44). I conservatively used US EPA's recommended residential groundwater ingestion rate

of 2.5 L/day for the adult female (US EPA, 2017b). This ingestion rate is based on the 90th percentile of drinking water ingestion for adults (US EPA, 2011d).

10.3.2 Ingestion of PFCs in Sediment

$$Intake = \frac{EPC_{sediment} \times IR_{sediment} \times B \times EF \times ED \times FS \times CF}{BW \times AT}$$

where:

$EPC_{sediment}$	=	Exposure Point Concentration of PFC in Sediment (mg/kg)
$IR_{sediment}$	=	Sediment Ingestion rate (mg/day)
B	=	Relative Oral Bioavailability (unitless)
EF	=	Exposure Frequency (days/year)
ED	=	Exposure Duration (years)
FS	=	Fraction from Source (unitless)
CF	=	Conversion Factor (kg/mg)
BW	=	Body Weight (kg)
AT	=	Averaging Time (days)

I assumed that a female recreational user recreates in the named water bodies or the Mississippi River for 4 hours/day, 2 days/week, for the 5 warmer months of the year (May through September) from ages 15-44 years (*i.e.*, 44 days/year for 29 years). These assumptions are highly conservative, because it is unlikely that women who swim and/or fish in these water bodies would visit them every weekend (*e.g.*, due to inclement weather).

I used the mean soil ingestion rate of 50 mg/day for the adult recommended by US EPA (1997) to represent sediment ingestion rate in the named water bodies. This is conservative in that it assumes that all of the soil ingested for the entire day comes from sediment at the lake or river, which is very unlikely. In addition, using the US EPA-recommended soil ingestion rate of 50 mg/day for the recreational user (US EPA, 1997, 2009) is conservative, in light of the findings of two pilot studies of adult soil ingestion rates. Specifically, Stanek *et al.* (1997) reported an average estimate of soil ingestion of 10 mg/day (based on results from 10 adults), while Calabrese *et al.* (1990) reported an average ingestion rate of approximately 40 mg/day (based on results from 6 adults). In addition, Davis and Mirick (2006) studied two adults in the same family and found an overall mean rate of 52 mg/day for those individuals.

I used a body weight of 67.3 kg, which represents the mean body weight for females aged 15-44 years, as reported in US EPA's "Exposure Factors Handbook" (US EPA, 2011d), and an averaging time of 10,585 days (365 days/year × the exposure duration of 29 years) to characterize PFC exposures.

The bioavailability of chemicals in soil or sediment is dependent on a number of factors, including a chemical's form and solubility, the particle size of the ingested soil/sediment, and the soil/sediment type (Richardson *et al.*, 2006). A relative oral bioavailability estimate for a specific compound represents the oral absorption fraction from the exposure route of concern (sediment in this analysis) relative to the oral absorption fraction from food or water (in most toxicity studies, chemicals are administered orally). For the PFCs of concern in this case, I conservatively assumed a relative bioavailability of 100%. The bioavailability values are summarized in Appendix D, Table D.2.

10.3.3 Dermal Contact with PFCs in Sediment

$$Intake = \frac{EPC_{sediment} \times SA \times AF \times DA \times FS \times EF \times ED \times CF}{BW \times AT}$$

where:

$EPC_{sediment}$	=	Exposure Point Concentration of PFC in Sediment (mg/kg)
SA	=	Skin Surface Area Exposed to Sediment (cm ² /day)
AF	=	Soil-Skin Adherence Factor (mg/cm ²)
DA	=	Dermal Absorption Fraction (unitless)
FS	=	Fraction from Source (unitless)
EF	=	Exposure Frequency (days/year)
ED	=	Exposure Duration (years)
CF	=	Conversion Factor (kg/mg)
BW	=	Body Weight (kg)
AT	=	Averaging Time (days)

Absorption of chemicals through the skin is frequently less than 100%. Therefore, a dermal absorption fraction (DA) represents the amount of a chemical that comes into contact with skin that is absorbed through the skin and into the bloodstream. I used DAs of 10% for the PFCs of concern in this case as recommended in the US EPA (2017d) RSL Calculator (summarized in Appendix D, Table D.2).

Dermal contact with sediment was assumed to occur over a 6,615 cm² area of skin for the adult female (aged 21+ years) (US EPA, 2011d). For the recreational user, the skin surface area is the sum of the mean skin surface areas for the head, hands, forearms, lower legs, and feet (US EPA, 2011d, 2014d). An adherence factor for recreational fishing was not available for adults. I used a soil-skin adherence factor of 0.2 mg/cm² based on the geometric mean for children playing in wet soil, an activity for which sediment exposure was deemed similar to that during recreational fishing (US EPA, 2004b). This is a conservative estimate, because most recreational activities that adults participate in have soil-skin adherence factors of 0.2 mg/cm² or less (US EPA, 2004b).

10.3.4 Ingestion of PFCs in Surface Water

$$Intake = \frac{EPC_{SW} \times IR_{SW} \times EF \times ED \times FS}{BW \times AT}$$

where:

EPC_{SW}	=	Exposure Point Concentration of PFC in surface water (mg/L)
IR_{SW}	=	Surface water Ingestion Rate (L/day)
EF	=	Exposure Frequency (days/year)
ED	=	Exposure Duration (years)
FS	=	Fraction from Source (unitless)
BW	=	Body Weight (kg)
AT	=	Averaging Time (days)

I assumed that women could incidentally ingest 0.02 L/day of surface water for 44 days per year, as discussed for the recreational user in Section 10.3.2 (US EPA, 2011d). All other assumptions that I used to evaluate exposure *via* ingestion of surface water are the same as those discussed in Section 10.3.2.

10.3.5 Ingestion of PFCs in Fish

$$Intake = \frac{EPC_{fish} \times IR_{fish} \times EF \times ED \times CF}{BW \times AT}$$

where:

EPC_{fish}	=	Exposure Point Concentration of PFC in Fish (mg/kg)
IR_{fish}	=	Fish Ingestion Rate (mg/day)
EF	=	Exposure Frequency (days/year)
ED	=	Exposure Duration (years)
CF	=	Conversion Factor (kg/mg)
BW	=	Body Weight (kg)
AT	=	Averaging Time (days)

I used a default annualized fish ingestion rate of 30 g/day for adults, which was reported in MPCA's "Human Health-based Water Quality Standards Technical Support Document" (MPCA, 2017). This value represents "the upper percentile freshwater, sport-caught fish consumption and [is] appropriate for use on a statewide default basis" (MPCA 2017). This fish consumption rate is equivalent to approximately one 8-oz. meal of freshwater fish per week (or about 26 lbs/year). MPCA (2017) obtained this fish consumption rate by averaging 80th percentile values for Wisconsin anglers (sport-caught fish) (21.0 g/day) and Ontario (sport-caught fish) (37.5 g/day) to obtain 29 g/day, which MPCA rounded to 30 g/day. The Minnesota fish consumption rates are higher than the 95th percentile fish ingestion rate of 25.3 g/day reported for Minnesota women of child-bearing age (15-44 years), which was reported in US EPA's "Exposure Factors Handbook" (Benson *et al.*, 2001; US EPA, 2011d). The fish consumption rate for sport-caught fish is likely conservative, given that MDH (2012) indicates that only 41% of the female Minnesotan fish consumers they surveyed reported eating sport-caught fish within the past 12 months.

10.4 Comparison of Exposures with the PODs Used to Set Agency Guidelines

I performed an MOE analysis that compares the estimated exposure levels for each PFC to health effect levels used as the basis for agency guidelines.⁸⁵ This comparison helps with the interpretation of the toxicological significance of potential exposures in the southern Washington County area and whether any health effects might be expected from such exposures. Similarly, this analysis helps with the interpretation of the health significance of exceedances of Minnesota health-based guidelines for environmental media. The maximum concentrations of PFOA (3 µg/L), PFOS (3.5 µg/L), and PFBA (22 µg/L) measured in drinking water wells in southern Washington County exceeded their respective MDH HRL⁸⁶ and/or HBV⁸⁷ values. The maximum concentration of PFBS (0.3 µg/L) measured in drinking water wells in southern Washington County did not exceed the MDH HRL of 7 µg/L. I used the MOE analysis to understand the significance of PFOA's, PFOS's, and PFBA's exceedances of their respective MDH regulatory values. It is also important to emphasize that, in contrast to the PFC serum concentrations discussed in Section 10.5, these are high-end hypothetical exposure estimates.

⁸⁵ The MOE approach has been used in regulatory assessments (see, for example Maier *et al.* [2015], in which an MOE approach was used to evaluate the safety of hand sanitizers by healthcare workers).

⁸⁶ The MDH HRL for PFOA and PFOS is 0.3 µg/L and the MDH HRL for PFBA is 7 µg/L. These values are discussed in detail in Section 8.

⁸⁷ The MDH HBV for PFOA is 0.035 µg/L and the MDH HBV for PFOS is 0.027 µg/L. These values are discussed in detail in Section 8.

The MOE is the ratio of the POD to the calculated intake, where the POD and intake are in units of mg/kg-day for oral and dermal exposures:

$$MOE = \frac{POD}{Intake}$$

The PODs used for the MOE analysis correspond to either (a) the highest level at which health effects were not seen in a study, called a NOEL for oral or dermal dose; or (b) the lowest level at which health effects were seen in a study (called a LOEL), as determined by MDH. For the purpose of comparing human exposure to NOELs and LOELs from animal studies, the NOELs and LOELs are converted to HEDs to account for differences in half-lives between animals and humans, as described in Section 8 and in US EPA (2016a,b) and MDH (2011a,b). The MOE is an indication of the magnitude by which the POD for the health effect exceeds the estimated hypothetical exposure estimate for a receptor; thus, an MOE greater than 1 indicates that the exposure estimate is less than the health effect level. I performed a combined MOE analysis for ingestion plus dermal exposures.

10.4.1 Past Adult Female Resident's Exposures to PFCs in Private Wells

For a past adult female resident consuming groundwater from private wells within southern Washington County,⁸⁸ the MOEs for the four PFCs evaluated (PFOA, PFOS, PFBA, PFBS) ranged from 4.3 to 12,000,000 (see Table 10.1 at the end of this section). The highest estimated exposure levels were more than 4-fold lower than the PODs for the individual PFCs.

For past exposure to PFOA in private well water, the MOEs ranged from 50 to about 8,800. The POD for PFOA is the HED that corresponds to the animal dose that MDH chose as the LOEL (1 mg/kg-day) in a study of mice administered PFOA during gestation (Lau *et al.*, 2006). It should be noted that although both US EPA and MDH chose this LOEL, I consider this value to be a NOEL (*i.e.*, a dose at which no health effects were observed; see Section 8.1.1.1). Thus, an MOE of 50 means that the maximum amount of PFOA a past adult female Minnesota resident might be exposed to in this scenario is still 50 times lower than a PFOA exposure level that is not associated with health effects in animals.

For past exposure to PFOS in private well water, the MOEs ranged from 4.3 to 750. The lowest MOE (MOE = 4.3) was calculated for the past adult female resident consuming groundwater from a private well located in Lake Elmo. The POD for PFOS is the HED that corresponds to the animal dose that MDH chose as the NOEL (0.1 mg/kg-day) in a study of rats administered PFOS prior to and during mating, and through gestation and lactation, across two generations (Luebker *et al.*, 2005a). It should be noted that using an alternative and more scientifically supported NOEL of 0.4 mg/kg-day, as described in Section 8.2.1.1, would increase the MOEs for PFOS in this scenario 4-fold, and the minimum MOE would then be 17, with a maximum of about 3,000. Using this alternative NOEL to calculate MOEs is still conservative and health-protective, based on my analysis of the underlying rat study (see Section 8.2.1.1).

For past exposure to PFBA in private well water, the MOEs ranged from 1,100 to 57,000. The POD for PFBA is the HED that corresponds to the animal dose that MDH chose as the NOEL (0.1 mg/kg-day) in a study of rats (NOTOX B.V., 2007a). For past exposure to PFBS in private well water, the MOEs ranged from 39,000 to 12,000,000. The POD for PFBS is the HED that corresponds to the animal dose that MDH chose as the NOEL (0.1 mg/kg-day) in a study of rats (Lieder *et al.*, 2009a).

⁸⁸ Includes groundwater wells within the boundaries described in the Interrogatories (Minnesota, Attorney General, 2012) and depicted in Figure 9.1.

Table 10.1 (at the end of this section) summarizes the hypothetical daily intakes of the PFCs of concern and the associated MOEs for the past adult female resident exposed to those PFCs in private well water.

10.4.2 Past Adult Female Resident's Exposures to PFCs in City Wells

For a past adult female resident consuming groundwater from city wells within southern Washington County, the MOEs for the PFCs evaluated ranged from 12 to 2 million (see Table 10.2 at end of this section).

For past exposure to PFOA in city well water, the MOEs ranged from 190 to 15,000. The POD for PFOA is the HED as described in Section 10.4.1. It should be noted that although both US EPA and MDH chose this LOEL, I consider this value to be a NOEL (*i.e.*, a dose at which no health effects were observed; see Section 8.1.1.1). Thus, an MOE of 190 means that the maximum amount of PFOA a past adult female Minnesota resident might be exposed to in this scenario is still 190 times lower than a PFOA exposure level that is not associated with health effects in animals.

For past exposure to PFOS in city well water, the MOEs ranged from 12 to 2,900. For past exposure to PFBA in city well water, the MOEs ranged from 10,000 to 48,000. For past exposure to PFBS in city well water, the MOEs ranged from 39,000 to 2 million.

The lowest MOE (MOE = 12) was calculated for PFOS exposure for the past adult female resident consuming city well water in Oakdale. The POD for PFOS is the HED as described in Section 10.4.1. Using the more scientifically supported NOEL of 0.4 mg/kg-day, as described in Section 8.2.1.1, would increase the MOEs for PFOS in this scenario 4-fold, and the minimum MOE would then be 47, with a maximum of 11,000. Using this alternative NOEL to calculate MOEs is still conservative and health-protective, based on my analysis of the underlying rat study (see Section 8.2.1.1).

Table 10.2 (at the end of this section) summarizes the daily intakes of the PFCs of concern and the associated MOEs for the past adult female resident exposed to those PFCs in city well water.

10.4.3 Current Adult Female Resident's Exposures to PFCs in Private Wells

For a current adult female resident consuming groundwater from private wells within southern Washington County, the MOEs for all the PFCs evaluated ranged from 31 to 5.9 million⁸⁹ (see Table 10.3, at the end of this section). For exposure to the current levels of the individual PFCs in private well water, the MOEs ranged from 170 to 37,000 for PFOA, 31 to 2,400 for PFOS, 5,000 to 4.8 million for PFBA, and 170,000 to 5.9 million for PFBS. The highest estimated exposure level was more than 31-fold lower than the PODs for the individual PFCs.

The POD for PFOA is the HED as described in Section 10.4.1. It should be noted that although both US EPA and MDH chose this LOEL, I consider this value to be a NOEL (*i.e.*, a dose at which no health effects were observed; see Section 8.1.1.1). Thus, an MOE of 170 means that the maximum amount of PFOA a current adult female Minnesota resident might be exposed to in this scenario is still 170 times lower than a PFOA exposure level that is not associated with health effects in animals.

⁸⁹ Ranges of MOEs in this paragraph include ranges for all 736 private wells that I included in my exposure analysis, rather than just the worst-case scenario wells that I present in Table 10.3. The exposure analysis for all 736 private wells is included in Appendix D.

The lowest MOE (MOE = 31) was calculated for PFOS exposure for the current adult female resident consuming private well water from well 257694, located in Grey Cloud Island Township. The POD for PFOS is the HED as described in Section 10.4.1. Using the more scientifically supported NOEL of 0.4 mg/kg-day, as described in Section 8.2.1.1, would increase the MOEs for PFOS in this scenario 4-fold, and the minimum MOE would then be 120, with a maximum of 9,400 (see Appendix Table D.8 for the full exposure analysis for current private wells). Using this alternative NOEL to calculate MOEs is still conservative and health-protective, based on my analysis of the underlying rat study (see Section 8.2.1.1).

Due to the number of private wells evaluated, I have summarized the daily intakes of the PFCs of concern and the associated MOEs for the current adult female resident exposed to those PFCs in the worst-case scenario private wells (*i.e.*, those with the highest detected PFC concentrations) presented in Table 10.3 at the end of this section.

10.4.4 Current Adult Female Resident's Exposures to PFCs in City Wells

For a current adult female resident consuming groundwater from city wells within southern Washington County, the MOEs for all the PFCs evaluated ranged from 83 to 580,000 (see Table 10.4 at the end of this section). For exposure to the individual PFCs in current city well water, the MOEs ranged from 1,000 to 6,200 for PFOA, 83 to 390 for PFOS, 21,000 to 110,000 for PFBA, and 220,000 to 580,000 for PFBS. The highest estimated exposure level was nearly 83-fold lower than the PODs for the individual PFCs.

The POD for PFOA is the HED as described in Section 10.4.1. It should be noted that although both US EPA and MDH chose this LOEL, I consider this value to be a NOEL (*i.e.*, a dose at which no health effects were observed; see Section 8.1.1.1). Thus, an MOE of 1,000 means that the maximum amount of PFOA a current adult female Minnesota resident might be exposed to in this scenario is still 1,000 times lower than a PFOA exposure level that is not associated with health effects in animals.

The lowest MOE (MOE = 83) was calculated for PFOS exposure for the current adult female resident consuming city well water in Oakdale. The POD for PFOS is the HED as described in Section 10.4.1. Using the more scientifically supported NOEL of 0.4 mg/kg-day, as described in Section 8.2.1.1, would increase the MOEs for PFOS in this scenario 4-fold, and the minimum MOE would then be 330, with a maximum of 1,500. Using this alternative NOEL to calculate MOEs is still conservative and health-protective, based on my analysis of the underlying rat study (see Section 8.2.1.1).

Table 10.4 (at the end of this section) summarizes the daily intakes of the PFCs of concern for the current adult female resident consuming drinking water from city wells and the associated MOEs, including the MOEs calculated using a more scientifically supported POD for PFOS.

10.4.5 Adult Female Recreational User's Exposure to PFCs

I also evaluated the MOEs for a female recreational user who may ingest sediment and surface water and have dermal contact with sediment while recreating at any of the water bodies named in the Interrogatories (Minnesota, Attorney General, 2012) and who may ingest sport-caught fish from any of these named water bodies. For these women, the estimated exposure levels to any of the PFCs were more than 4.6-fold lower than the PODs for the individual PFCs. For exposure to the individual PFCs in sediment, surface water, and fish combined, the MOEs ranged from 3,200 to 3,800,000 for PFOA, 4.6-15 for PFOS, 2,400,000 to 370,000,000 for PFBA, and 1,500,000 to 420,000,000 for PFBS (see Table 10.5 at the end of this section).

The POD for PFOA is the HED as described in Section 10.4.1. It should be noted that although both US EPA and MDH chose this LOEL, I consider this value to be a NOEL (*i.e.*, a dose at which no health effects were observed; see Section 8.1.1.1). Thus, an MOE of 3,200 means that the maximum amount of PFOA an adult female recreational user might be exposed to in this scenario is still 3,200 times lower than a PFOA exposure level that is not associated with health effects in animals.

The lowest MOE (MOE = 4.6) was calculated for PFOS exposure for the adult female recreational user in all the named lakes combined and is primarily due to ingestion of fish. Although my evaluation examined all the named lakes combined, the lowest MOE, which is still 4.6-fold lower than a dose at which no health effects were observed, is predominantly driven by ingestion of fish from Lake Elmo. Exposures of the female recreational user *via* sediment and surface water were negligible. Individuals that consume fish from Lake Elmo are expected to comprise a small fraction of all individuals in the southern Washington County area.

The contribution of the fish exposure pathway to the overall exposure of the recreational user to PFCs is mostly due to the use of the Minnesota state fish consumption rate, which overestimates exposure for most individuals. The fish consumption rate used in my exposure analysis is 30 g/day as recommended by the MPCA (MPCA, 2017). This rate is equivalent to approximately 1 meal of sport-caught fish each week (MPCA, 2017). Two surveys of Minnesotan women indicate that the assumption (30 g/day) used in my exposure analysis is at least 2-4 times higher than actual sport-caught fish consumption rates identified for Minnesotan women (Benson *et al.*, 2001; MDH, 2012). MDH (2012) indicates that the Minnesotan women surveyed eat 1-2 fish meals per month (equivalent to an annualized consumption rate of 7.5-15 g/day), including both commercial and sport-caught fish. Of these women, only 41% reported eating sport-caught fish in the previous 12-month period (MDH, 2012). Benson *et al.* (2001) reported that the Minnesotan women surveyed consume 0.5 to less than 2 meals of sport-caught fish per month, with the higher-end consumption occurring during the warmer months of the year (May-September).

The POD for PFOS is the HED as described in Section 10.4.1. Using the more scientifically supported NOEL of 0.4 mg/kg-day, as described in Section 8.2.1.1, would increase the MOEs for PFOS in this scenario 4-fold, and the minimum MOE would then be 18, with a maximum of 60. Using this alternative NOEL to calculate MOEs is still conservative and health protective, based on my analysis of the underlying rat study (see Section 8.2.1.1).

Table 10.5 (at the end of this section) summarizes the daily intakes of the PFCs of concern for the adult female recreational user across all pathways and the associated MOEs, including the MOEs calculated using a more scientifically supported POD for PFOS.

10.5 Comparison of Resident PFC Serum Concentrations with Worker and Animal PFC Serum Concentrations

For my evaluation of the environmental concentrations of PFCs in southern Washington County, I also considered PFC serum concentrations of residents in the area. Serum concentrations reflect exposures to PFOA and PFOS in media such as food and air, as well as exposures considered in this report (*i.e.*, PFCs in groundwater, surface water, sediment, and fish). Serum concentration comparisons provide a more realistic, but still conservative, evaluation of exposures to PFOA and PFOS in southern Washington County (by virtue of including other sources) than the hypothetical exposure estimates used in the MOE analysis.

MDH measured PFC concentrations in serum samples taken in 2008, 2010, and 2014 from 149 Minnesota residents living in southern Washington County (MDH, 2016e). The results of the serum testing are presented in Tables 10.6 and 10.7 (MDH, 2008-2014).

Table 10.6 shows that the serum PFOA and PFOS concentrations in southern Washington County residents are approximately 3-4 times higher than those in the general US population at all time points, but are declining over time in parallel with the US population values (CDC, 2015, 2017a).

Table 10.6 Serum Concentrations of PFOA and PFOS in Southern Washington County Residents Compared to the US Population

PFC	Year	Serum Concentrations, Geometric Means (µg/L)		Fold Increase
		Southern Washington County ^a GM (Range)	US ^b GM (95% CI)	
PFOA	2008	14.9 (1.6-177.0)	4.12 (4.01-4.24)	3.6
	2010	11.2 (0.94-110.5)	3.07 (2.81-3.36)	3.6
	2014	5.5 (0.24-47.0)	1.94 (1.76-2.14)	2.8
PFOS	2008	35.7 (3.2-448.0)	13.2 (12.2-14.2)	2.7
	2010	24.9 (1.6-234.0)	9.32 (8.13-10.7)	2.7
	2014	18.5 (1.0-180.0)	4.99 (4.50-5.52)	3.7

Notes:

CI = Confidence Interval; GM = Geometric Mean; PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

(a) Population is comprised of 149 people living in southern Washington County (MDH, 2008-2014, 2016e).

(b) US values from CDC (2015, 2017a).

To put the southern Washington County residents' exposure in perspective, in Table 10.7, I compare the residents' geometric mean PFOA and PFOS serum concentrations (as representative of average exposure levels) to PFOA and PFOS serum concentrations in occupationally exposed workers, in the animals from studies that were used as the basis of the MDH HBVs, and in the highest dose group in the Chang *et al.* (2017) monkey study. The comparisons described below are based on the highest average serum concentrations from serum samples collected in 2008. More-current serum concentrations of residents (sampled in 2010 and 2014) are lower than those in sampled 2008.

The worker serum concentrations listed in Table 10.7 are the highest average serum concentrations reported for any PFC workers in the US (Gilliland and Mandel, 1996; Olsen *et al.*, 1999). As discussed in Sections 7.1 and 7.2, the workers did not exhibit any clear and consistent evidence of adverse health effects from these exposures. The highest average measurements in the residents, sampled in 2008, are 220 times lower for PFOA and 68 times lower for PFOS than the corresponding serum concentrations in workers.

Table 10.7 also provides the serum concentrations associated with the LOEL and NOEL that MDH (2017b,c) considered to represent doses at (for PFOA)⁹⁰ or above (for PFOS) which health effects were observed in animal studies. For PFOA, the highest average measurements in southern Washington County residents are approximately 2,600 times lower than the animal serum concentrations for PFOA at the POD. For PFOS, the highest average measurements in southern Washington County residents, sampled in 2008, are 175 times lower than the animal serum concentrations at the POD chosen by MDH. Based on a more scientifically supported NOEL for PFOS (discussed in Section 8.2), the highest average

⁹⁰ As discussed in Section 8.1, the PFOA is more appropriately described as a NOEL.

measurements in southern Washington County residents would be 700 times lower than the animal serum concentrations at the NOEL.⁹¹

Chang *et al.* (2017) administered high doses of PFOS to monkeys to achieve average serum concentrations up to 165,500 µg/L. The highest average PFOS concentration in southern Washington County residents is about 4,600 times lower than this level, at which the only effect observed in the monkeys was a slight reduction in cholesterol, which was not considered by the authors to be toxicologically significant (Chang *et al.*, 2017).

Table 10.7 Serum Concentrations of PFOA and PFOS in Southern Washington County Residents Compared to 3M Workers, MDH LOEL/NOEL, and Chang *et al.* (2017) Study

PFC	Serum Concentrations of PFOA and PFOS (µg/L)					
	Southern Washington County ^a GM (Range)			3M Workers ^b	Serum Concentrations at MDH RfD (LOEL/NOEL ^c)	Highest Value in Chang <i>et al.</i> (2017) Study
	2008	2010	2014			
PFOA	14.9 (1.6-177.0)	11.2 (0.94-110.5)	5.5 (0.24-47.0)	3,300 ^d	38,000 (LOEL)	Not Tested
PFOS	35.7 (3.2-448.0)	24.9 (1.6-234.0)	18.5 (1.0-180.0)	2,440 ^e	6,260 (NOEL)	165,000 ^d

Notes:

GM = Geometric Mean; LOEL = Lowest Observed Effect Level; MDH = Minnesota Department of Health; NOEL = No Observed Effect Level; PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate; RfD = Reference Dose.

(a) Population is comprised of 149 people living in southern Washington County (MDH, 2008-2014, 2016e).

(b) Highest serum concentrations reported in US workers. PFOA values are from Gilliland and Mandel (1996); PFOS values are from Olsen *et al.* (1999).

(c) Using a more scientifically supported POD would change the PFOA LOEL to a NOEL. For PFOS, the serum concentration at the more scientifically supported NOEL would be 25,000 µg/L (as calculated by US EPA, 2016b). The comparison to the Minnesota population would then result in a 700-fold difference.

(d) Authors do not specify whether this is an arithmetic or geometric mean.

(e) Arithmetic mean (geometric mean not reported).

I also performed an MOE analysis comparing geometric mean and 95th percentile serum concentrations in southern Washington County residents to serum concentrations associated with the LOEL for PFOA and the NOEL for PFOS chosen by MDH (MDH, 2017b,c). This analysis is conceptually similar to the MOE analysis based on hypothetical dose estimates as presented in Tables 10.1-10.5.

The results of the serum-based MOE analysis are provided in Table 10.8. For PFOA, the MOEs range from about 2,600 to about 6,900 (based on the geometric mean serum concentrations of southern Washington County residents) and from about 630 to about 1,500 (based on the 95th percentile serum concentrations of the residents). For PFOS, based on the POD selected by MDH, the MOEs range from 180 to 340 (based on the geometric mean serum concentrations of southern Washington County residents) and from 63 to 89 (based on the 95th percentile serum concentrations of the residents). Based on the more scientifically supported POD,⁹² the MOEs range from 700 to 1,400 (based on the geometric mean serum concentrations of southern Washington County residents) and from 250 to 360 (based on the 95th percentile serum concentrations of the residents).

⁹¹ For PFOS, the serum concentration at the more scientifically supported NOEL (see Section 8.2) would be 25,000 µg/L (as calculated by US EPA, 2016b).

⁹² As discussed in Section 8.2.

Table 10.8 Margin of Exposure Comparisons Based on Serum Concentrations in Southern Washington County Residents

PFC	Animal Serum POD (µg/L)	Year	Geometric Mean		95 th Percentile	
			Serum Concentrations in Residents (µg/L)	MOE ^a	Serum Concentrations in Residents (µg/L)	MOE ^a
PFOA	38,000 (LOEL) ^{b, c}	2008	14.9	2,600	60.0	630
		2010	11.2	3,400	48.7	780
		2014	5.5	6,900	26.0	1,500
PFOS	6,260 (NOEL) ^b	2008	35.7	180	100.0	63
		2010	24.9	250	69.5	90
		2014	18.5	340	70.0	89
	25,000 (NOEL) ^d	2008	35.7	700	100.0	250
		2010	24.9	1,000	69.5	360
		2014	18.5	1,400	70.0	360

Notes:

LOEL = Lowest Observed Effect Level; MDH = Minnesota Dept. of Health; MOE = Margin of Exposure; NOEL = No Observed Effect Level; PFC = Perfluorinated Chemical; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate; POD = Point of Departure.

(a) MOE = POD / Serum Concentration. MOEs were rounded to two significant digits.

(b) Serum concentrations at the MDH POD.

(c) Using the more scientifically supported POD would change the PFOA LOEL to a NOEL.

(d) Based on a more scientifically supported NOEL of 0.4 mg/kg-day (see Section 8.2).

10.6 Conclusions

The results of the exposure analysis I conducted using the most current US EPA methodology and assumptions specific to the geographic area and population of interest show that potential exposures of residents of southern Washington County to the PFCs of concern are below POD levels in toxicological studies that agencies have used to develop the RfDs and associated drinking water guidelines for those PFCs. For PFOA, the agencies have considered the POD to be a LOEL, although it is more correctly represented as a NOEL. For PFOS, the POD should be 4-fold higher to more correctly represent the underlying science.

My exposure analysis is conservative and generally employs high-end, plausible assumptions intended to overestimate, rather than underestimate, likely exposures. For example, I used the 95% UCLM concentrations instead of mean concentrations for PFCs in surface water, sediment, and fish, and used the 95% UCLM for the maximum 12-month moving average at each municipality to calculate past groundwater exposures.⁹³ For past groundwater exposures, this means that there were other 12-month periods with lower 95% UCLM concentrations in groundwater. For fish consumption, I used a consumption rate that represents the 80th percentile consumption rate for sport-caught fish, which is at least 2-4 times higher than surveys indicate is typical for Minnesotan women (Benson *et al.*, 2001; MDH, 2012).

For an adult female resident in southern Washington County, the hypothetical dose-based exposure estimates and MOE analysis demonstrated that PFCs in drinking water were below the POD doses that MDH determined to be associated with possible health effects. In addition, conducting the MOE analysis

⁹³ As recommended by US EPA (2014a) guidance, I used the average of the two most recent groundwater sampling rounds to represent current exposures in my analysis.

with the more scientifically supported PFOS NOEL increases the MOEs and results in hypothetical exposure estimates that are further below the levels of toxicological concern for PFOS.

For an adult female recreational user of the named water bodies in and around southern Washington County, the hypothetical exposure estimates and MOE analysis demonstrate that PFCs in surface water, sediment, and fish were below the POD doses that MDH determined to be at or below a dose that may cause health effects in animals. The fish ingestion pathway is the greatest contributor to hypothetical exposure for the adult female recreational user.⁹⁴ The greater contribution of the fish ingestion pathway is likely due to assumptions that overestimate hypothetical exposures from that pathway, as described in Section 10.4.5. Hypothetical exposures to PFCs in sediment and surface water for the adult female recreational user are especially negligible in comparison with levels that have been associated with possible health effects in toxicological studies.

I also evaluated PFC concentrations in human serum from southern Washington County residents compared to serum concentrations reported in occupational and animal studies. I determined that the highest average serum concentrations of PFOA and PFOS in southern Washington County residents (sampled in 2008) were 220 and 68 times, respectively, lower than serum concentrations in workers, and are levels at which there is no evidence of clear and consistent adverse health effects. I determined that the highest average serum concentrations measured in southern Washington County residents are 2,600 times lower for PFOA and 180 times lower for PFOS than the animal serum concentrations corresponding to the PODs chosen by MDH. Furthermore, using the more scientifically supported POD for PFOS, the highest average serum concentrations measured in southern Washington County residents would be 700 times lower than the corresponding PFOS animal serum concentrations at the POD. The highest average PFOS serum concentration is 4,600-fold lower than a concentration that showed no toxicological effects in a recent monkey study. Using more recent serum concentrations from 2010 and 2014 would yield even larger margins between the resident serum concentrations and the comparison values.

I also performed an MOE analysis comparing average and high-end serum concentrations in residents to serum concentrations associated with the PODs that MDH (2017b,c) considered to represent doses at (for PFOA) or above (for PFOS) which health effects were observed in animal studies. This analysis is conceptually similar to the MOE analysis based on hypothetical dose estimates. It is interesting to note that the lowest MOEs based on serum concentrations measured in southern Washington County residents are well above the lowest MOEs based on the hypothetical dose estimates. This observation provides evidence that the dose-based MOE analysis is highly conservative and overestimates plausible exposures for many residents.

The results of my MOE analysis based on hypothetical dose estimates and my MOE analysis based on serum concentrations, as well as consideration of the underlying toxicology and epidemiology of the PFCs at issue, demonstrate that exposure to PFCs in the area of concern are below levels of toxicological concern and that health effects from PFC exposure are not expected in southern Washington County residents.

⁹⁴ While I could have derived better scientifically supported PODs for PFOA, PFBS, and PFBA, the exposure estimates using the current PODs are so far below levels of toxicological concern that it would not affect my conclusions.

Table 10.1 Summary of Hypothetical Daily Intakes and Margins of Exposure for Past Resident's Groundwater Exposure from Private Wells

Exposure Area	Constituent of Potential Concern	Groundwater EPC (mg/L)	Hypothetical Daily Intake from Groundwater (mg/kg-day)	Point of Departure (POD) (mg/kg-day)	Margin of Exposure (MOE = POD/Daily Intake) ^a	Alternative POD (mg/kg-day)	Alternative Margin of Exposure (MOE = POD/Daily Intake) ^a
Cottage Grove Private Wells	PFOA	0.0012	0.000043	0.0053	120		
Cottage Grove Private Wells	PFOS	0.00025	0.0000090	0.00051	57	0.002	220
Cottage Grove Private Wells	PFBA	0.0087	0.00031	0.86	2,800		
Cottage Grove Private Wells	PFBS	0.00030	0.000011	0.42	39,000		
Grey Cloud Island Twp Private Wells	PFOA	0.00044	0.000016	0.0053	340		
Grey Cloud Island Twp Private Wells	PFOS	0.00094	0.000034	0.00051	15	0.002	60
Grey Cloud Island Twp Private Wells	PFBA	0.0021	0.000074	0.86	12,000		
Grey Cloud Island Twp Private Wells	PFBS	0.000058	0.0000021	0.42	200,000		
Lake Elmo Private Wells	PFOA	0.0030	0.00011	0.0053	50		
Lake Elmo Private Wells	PFOS	0.0033	0.00012	0.00051	4.3	0.002	17
Lake Elmo Private Wells	PFBA	0.022	0.00080	0.86	1,100		
Lake Elmo Private Wells	PFBS	0.000050	0.0000018	0.42	240,000		
Maplewood Private Wells	PFOA	0.000022	0.00000077	0.0053	6,900		
Maplewood Private Wells	PFOS	0.000019	0.00000068	0.00051	750	0.002	3,000
Maplewood Private Wells	PFBA	0.00060	0.000021	0.86	40,000		
Maplewood Private Wells	PFBS	0.000090	0.00000032	0.42	1,300,000		
Newport Private Wells	PFOA	0.000017	0.00000061	0.0053	8,800		
Newport Private Wells	PFOS	NA	NA	0.00051	NA	0.002	NA
Newport Private Wells	PFBA	0.0015	0.000054	0.86	16,000		
Newport Private Wells	PFBS	NA	NA	0.42	NA		
Oakdale Private Wells	PFOA	0.00080	0.000028	0.0053	190		
Oakdale Private Wells	PFOS	NA	NA	0.00051	NA	0.002	NA
Oakdale Private Wells	PFBA	0.012	0.00042	0.86	2,100		
Oakdale Private Wells	PFBS	NA	NA	0.42	NA		
St. Paul Private Wells	PFOA	NA	NA	0.0053	NA		
St. Paul Private Wells	PFOS	NA	NA	0.00051	NA	0.002	NA
St. Paul Private Wells	PFBA	0.00064	0.000023	0.86	38,000		
St. Paul Private Wells	PFBS	NA	NA	0.42	NA		
St. Paul Park Private Wells	PFOA	0.000095	0.00000034	0.0053	1,600		
St. Paul Park Private Wells	PFOS	0.000021	0.00000075	0.00051	680	0.002	2,700

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Exposure Area	Constituent of Potential Concern	Groundwater EPC (mg/L)	Hypothetical Daily Intake from Groundwater (mg/kg-day)	Point of Departure (POD) (mg/kg-day)	Margin of Exposure (MOE = POD/Daily Intake) ^a	Alternative POD (mg/kg-day)	Alternative Margin of Exposure (MOE = POD/Daily Intake) ^a
St. Paul Park Private Wells	PFBA	0.0018	0.000064	0.86	13,000		
St. Paul Park Private Wells	PFBS	0.000090	0.00000032	0.42	1,300,000		
West Lakeland Twp Private Wells	PFOA	0.00011	0.0000038	0.0053	1,400		
West Lakeland Twp Private Wells	PFOS	0.00021	0.0000073	0.00051	70	0.002	270
West Lakeland Twp Private Wells	PFBA	0.00042	0.000015	0.86	57,000		
West Lakeland Twp Private Wells	PFBS	0.000010	0.000000036	0.42	12,000,000		
Woodbury Private Wells	PFOA	0.00011	0.0000040	0.0053	1,300		
Woodbury Private Wells	PFOS	0.00016	0.0000057	0.00051	89	0.002	350
Woodbury Private Wells	PFBA	0.0023	0.000082	0.86	10,000		
Woodbury Private Wells	PFBS	0.000090	0.00000032	0.42	1,300,000		

Notes:

EPC = Exposure Point Concentration; NA = Not Available; PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

(a) MOEs were rounded to two significant digits.

Table 10.2 Summary of Hypothetical Daily Intakes and Margins of Exposure for Past Resident's Groundwater Exposure from City Wells

Exposure Area	Constituent of Potential Concern	Groundwater EPC (mg/L)	Hypothetical Daily Intake from Groundwater (mg/kg-day)	Point of Departure (POD) (mg/kg-day)	Margin of Exposure (MOE = POD/Daily Intake) ^a	Alternative POD (mg/kg-day)	Alternative Margin of Exposure (MOE = POD/Daily Intake) ^a
Cottage Grove City Wells	PFOA	0.000040	0.0000014	0.0053	3,700		
Cottage Grove City Wells	PFOS	0.0000050	0.00000018	0.00051	2,900	0.002	11,000
Cottage Grove City Wells	PFBA	0.0018	0.000064	0.86	13,000		
Cottage Grove City Wells	PFBS	0.00030	0.000011	0.42	39,000		
Lake Elmo City Wells	PFOA	0.00020	0.0000071	0.0053	740		
Lake Elmo City Wells	PFOS	0.00020	0.0000071	0.00051	72	0.002	280
Lake Elmo City Wells	PFBA	0.0019	0.000068	0.86	13,000		
Lake Elmo City Wells	PFBS	0.000012	0.00000043	0.42	980,000		
Newport City Wells	PFOA	0.000010	0.00000036	0.0053	15,000		
Newport City Wells	PFOS	NA	NA	0.00051	NA	0.002	NA
Newport City Wells	PFBA	0.00070	0.000025	0.86	34,000		
Newport City Wells	PFBS	NA	NA	0.42	NA		
Oakdale City Wells	PFOA	0.00079	0.000028	0.0053	190		
Oakdale City Wells	PFOS	0.0012	0.000042	0.00051	12	0.002	47
Oakdale City Wells	PFBA	0.0020	0.000071	0.86	12,000		
Oakdale City Wells	PFBS	0.000053	0.00000019	0.42	220,000		
St. Paul City Wells	PFOA	NA	NA	0.0053	NA		
St. Paul City Wells	PFOS	NA	NA	0.00051	NA	0.002	NA
St. Paul City Wells	PFBA	NA	NA	0.86	NA		
St. Paul City Wells	PFBS	NA	NA	0.42	NA		
St. Paul Park City Wells	PFOA	0.000058	0.0000021	0.0053	2,500		
St. Paul Park City Wells	PFOS	0.0000070	0.00000025	0.00051	2,000	0.002	8,000
St. Paul Park City Wells	PFBA	0.0023	0.000082	0.86	10,000		
St. Paul Park City Wells	PFBS	0.0000060	0.00000021	0.42	2,000,000		
Woodbury City Wells	PFOA	0.000052	0.00000019	0.0053	2,900		
Woodbury City Wells	PFOS	0.0000090	0.00000032	0.00051	1,600	0.002	6,200
Woodbury City Wells	PFBA	0.00050	0.000018	0.86	48,000		
Woodbury City Wells	PFBS	0.000010	0.00000036	0.42	1,200,000		

Notes:

EPC = Exposure Point Concentration; NA = Not Available; PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

(a) MOEs were rounded to two significant digits.

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Exposure Area	Constituent of Potential Concern	Groundwater EPC (mg/L)	Hypothetical Daily Intake from Groundwater (mg/kg-day)	Point of Departure (POD) (mg/kg-day)	Margin of Exposure (MOE = POD/ Daily Intake) ^a	Alternative POD (mg/kg-day)	Alternative Margin of Exposure (MOE = POD/ Daily Intake) ^a
Lake Elmo Private Well 730663	PFOA	0.00051	0.000018	0.0053	290		
Lake Elmo Private Well 730663	PFOS	0.00042	0.000015	0.00051	34	0.002	130
Lake Elmo Private Well 730663	PFBA	0.0019	0.000068	0.86	13,000		
Lake Elmo Private Well 730663	PFBS	0.000039	0.0000014	0.42	300,000		
Maplewood Private Well 122019	PFOA	0.000013	0.00000045	0.0053	12,000		
Maplewood Private Well 122019	PFOS	0.000035	0.0000012	0.00051	420	0.002	1,600
Maplewood Private Well 122019	PFBA	0.00026	0.0000091	0.86	95,000		
Maplewood Private Well 122019	PFBS	0.000030	0.0000011	0.42	400,000		
Maplewood Private Well 427872	PFOA	0.000014	0.00000051	0.0053	10,000		
Maplewood Private Well 427872	PFOS	0.000034	0.0000012	0.00051	420	0.002	1,600
Maplewood Private Well 427872	PFBA	0.00039	0.000014	0.86	63,000		
Maplewood Private Well 427872	PFBS	NA	NA	0.42	NA		
Newport Private Well 443910	PFOA	0.000029	0.0000010	0.0053	5,100		
Newport Private Well 443910	PFOS	NA	NA	0.00051	NA	0.002	NA
Newport Private Well 443910	PFBA	0.00076	0.000027	0.86	32,000		
Newport Private Well 443910	PFBS	NA	NA	0.42	NA		
Newport Private Well 551000	PFOA	NA	NA	0.0053	NA		
Newport Private Well 551000	PFOS	NA	NA	0.00051	NA	0.002	NA
Newport Private Well 551000	PFBA	0.00019	0.0000068	0.86	130,000		
Newport Private Well 551000	PFBS	NA	NA	0.42	NA		
St. Paul Private Well 274360	PFOA	NA	NA	0.0053	NA		
St. Paul Private Well 274360	PFOS	NA	NA	0.00051	NA	0.002	NA
St. Paul Private Well 274360	PFBA	0.00035	0.000012	0.86	69,000		
St. Paul Private Well 274360	PFBS	NA	NA	0.42	NA		
St. Paul Park Private Well 257849	PFOA	NA	NA	0.0053	NA		
St. Paul Park Private Well 257849	PFOS	NA	NA	0.00051	NA	0.002	NA
St. Paul Park Private Well 257849	PFBA	0.0016	0.000055	0.86	16,000		
St. Paul Park Private Well 257849	PFBS	NA	NA	0.42	NA		

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Exposure Area	Constituent of Potential Concern	Groundwater EPC (mg/L)	Hypothetical Daily Intake from Groundwater (mg/kg-day)	Point of Departure (POD) (mg/kg-day)	Margin of Exposure (MOE = POD/Daily Intake) ^a	Alternative POD (mg/kg-day)	Alternative Margin of Exposure (MOE = POD/Daily Intake) ^a
West Lakeland Twp Private Well 257205	PFOA	0.00010	0.0000036	0.0053	1,500		
West Lakeland Twp Private Well 257205	PFOS	0.000065	0.0000023	0.00051	220	0.002	860
West Lakeland Twp Private Well 257205	PFBA	0.00042	0.000015	0.86	57,000		
West Lakeland Twp Private Well 257205	PFBS	NA	NA	0.42	NA		
West Lakeland Twp Private Well 274301	PFOA	0.000013	0.00000045	0.0053	12,000		
West Lakeland Twp Private Well 274301	PFOS	0.000015	0.00000053	0.00051	950	0.002	3,700
West Lakeland Twp Private Well 274301	PFBA	0.00020	0.0000069	0.86	120,000		
West Lakeland Twp Private Well 274301	PFBS	0.000026	0.00000091	0.42	460,000		
West Lakeland Twp Private Well 274302	PFOA	0.000056	0.0000020	0.0053	2,700		
West Lakeland Twp Private Well 274302	PFOS	0.000031	0.0000011	0.00051	460	0.002	1,800
West Lakeland Twp Private Well 274302	PFBA	0.00031	0.000011	0.86	78,000		
West Lakeland Twp Private Well 274302	PFBS	NA	NA	0.42	NA		
West Lakeland Twp Private Well 274303	PFOA	0.000057	0.0000020	0.0053	2,600		
West Lakeland Twp Private Well 274303	PFOS	0.000053	0.0000019	0.00051	270	0.002	1,100
West Lakeland Twp Private Well 274303	PFBA	0.00029	0.000010	0.86	83,000		
West Lakeland Twp Private Well 274303	PFBS	NA	NA	0.42	NA		
West Lakeland Twp Private Well 274304	PFOA	NA	NA	0.0053	NA		
West Lakeland Twp Private Well 274304	PFOS	NA	NA	0.00051	NA	0.002	NA
West Lakeland Twp Private Well 274304	PFBA	0.000069	0.0000024	0.86	350,000		
West Lakeland Twp Private Well 274304	PFBS	NA	NA	0.42	NA		
West Lakeland Twp Private Well 409672	PFOA	NA	NA	0.0053	NA		
West Lakeland Twp Private Well 409672	PFOS	NA	NA	0.00051	NA	0.002	NA
West Lakeland Twp Private Well 409672	PFBA	0.00023	0.0000082	0.86	100,000		
West Lakeland Twp Private Well 409672	PFBS	0.0000030	0.00000011	0.42	3,900,000		
West Lakeland Twp Private Well 427898	PFOA	0.00011	0.0000038	0.0053	1,400		
West Lakeland Twp Private Well 427898	PFOS	0.00021	0.0000073	0.00051	70	0.002	270
West Lakeland Twp Private Well 427898	PFBA	0.00033	0.000012	0.86	74,000		
West Lakeland Twp Private Well 427898	PFBS	NA	NA	0.42	NA		
Woodbury Private Well 257241	PFOA	0.000063	0.0000022	0.0053	2,400		
Woodbury Private Well 257241	PFOS	0.000093	0.0000033	0.00051	150	0.002	600
Woodbury Private Well 257241	PFBA	0.00028	0.0000098	0.86	87,000		
Woodbury Private Well 257241	PFBS	NA	NA	0.42	NA		

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Exposure Area	Constituent of Potential Concern	Groundwater EPC (mg/L)	Hypothetical Daily Intake from Groundwater (mg/kg-day)	Point of Departure (POD) (mg/kg-day)	Margin of Exposure (MOE = POD/ Daily Intake) ^a	Alternative POD (mg/kg-day)	Alternative Margin of Exposure (MOE = POD/ Daily Intake) ^a
Woodbury Private Well 507776	PFOA	NA	NA	0.0053	NA		
Woodbury Private Well 507776	PFOS	NA	NA	0.00051	NA	0.002	NA
Woodbury Private Well 507776	PFBA	0.0012	0.000043	0.86	20,000		
Woodbury Private Well 507776	PFBS	NA	NA	0.42	NA		

Notes:

EPC = Exposure Point Concentration; NA = Not Available; PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

For private wells, PFC concentrations are averaged individually by well, as discussed in Section 10.2. The exposure analysis was performed for 736 individual private wells in which PFCs were detected. Exposure estimates and MOEs are displayed in the table above for wells that had the highest exposure estimates for PFOA or PFOS or the lowest overall MOEs. (a) MOEs were rounded to two significant digits.

Table 10.4 Summary of Hypothetical Daily Intakes and Margins of Exposure for Current (2014-2017) Resident's Groundwater Exposure from City Wells

Exposure Area	Constituent of Potential Concern	Groundwater EPC (mg/L)	Hypothetical Daily Intake from Groundwater (mg/kg-day)	Point of Departure (POD) (mg/kg-day)	Margin of Exposure (MOE = POD/Daily Intake) ^a	Alternative POD (mg/kg-day)	Alternative Margin of Exposure (MOE = POD/Daily Intake) ^a
Cottage Grove City Wells	PFOA	0.000029	0.0000010	0.0053	5,200		
Cottage Grove City Wells	PFOS	NA	NA	0.00051	NA	0.002	NA
Cottage Grove City Wells	PFBA	0.00072	0.000026	0.86	33,000		
Cottage Grove City Wells	PFBS	0.000053	0.0000019	0.42	220,000		
Lake Elmo City Wells	PFOA	0.000046	0.0000016	0.0053	3,200		
Lake Elmo City Wells	PFOS	NA	NA	0.00051	NA	0.002	NA
Lake Elmo City Wells	PFBA	0.00022	0.0000077	0.86	110,000		
Lake Elmo City Wells	PFBS	NA	NA	0.42	NA		
Newport City Wells	PFOA	NA	NA	0.0053	NA		
Newport City Wells	PFOS	NA	NA	0.00051	NA	0.002	NA
Newport City Wells	PFBA	0.00033	0.000012	0.86	73,000		
Newport City Wells	PFBS	NA	NA	0.42	NA		
Oakdale City Wells	PFOA	0.00014	0.0000051	0.0053	1,000		
Oakdale City Wells	PFOS	0.00017	0.0000061	0.00051	83	0.002	330
Oakdale City Wells	PFBA	0.00040	0.000014	0.86	61,000		
Oakdale City Wells	PFBS	0.00037	0.0000013	0.42	320,000		
St. Paul Park City Wells	PFOA	0.000024	0.00000086	0.0053	6,200		
St. Paul Park City Wells	PFOS	NA	NA	0.00051	NA	0.002	NA
St. Paul Park City Wells	PFBA	0.0012	0.000041	0.86	21,000		
St. Paul Park City Wells	PFBS	0.00020	0.00000072	0.42	580,000		
Woodbury City Wells	PFOA	0.000034	0.0000012	0.0053	4,300		
Woodbury City Wells	PFOS	0.000036	0.0000013	0.00051	390	0.002	1,500
Woodbury City Wells	PFBA	0.00023	0.0000083	0.86	100,000		
Woodbury City Wells	PFBS	0.000048	0.0000017	0.42	250,000		

Notes:

EPC = Exposure Point Concentration; NA = Not Available; PFOA = Perfluorooctanoic Acid; PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFOS = Perfluorooctane Sulfonate.

For city wells, PFC concentrations are averaged individually by well and then averaged together to provide an average exposure to city well water based on the assumption that water is equally distributed between the wells.

(a) MOEs were rounded to two significant digits.

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Table 10.5 Summary of Hypothetical Daily Intakes and Margins of Exposure for Past and Current Recreational User

Exposure Area	Constituent of Potential Concern	Recreational User Hypothetical Daily Intake, Non-cancer (mg/kg-day)	Point of Departure (POD) (mg/kg-day)	Margin of Exposure (MOE = POD/Daily Intake) ^a	Alternative POD (mg/kg-day)	Alternative Margin of Exposure (MOE = POD/Daily Intake) ^a
Lakes	PFOA	0.0000017	0.0053	3,200		
Lakes	PFOS	0.00011	0.0051	4.6	0.002	18
Lakes	PFBA	0.000000039	0.86	22,000,000		
Lakes	PFBS	0.0000000099	0.42	420,000,000		
Mississippi River Pool 2	PFOA	0.00000047	0.0053	11,000		
Mississippi River Pool 2	PFOS	0.000090	0.0051	5.7	0.002	22
Mississippi River Pool 2	PFBA	0.00000035	0.86	2,400,000		
Mississippi River Pool 2	PFBS	0.00000028	0.42	1,500,000		
Mississippi River Pool 3	PFOA	0.000000014	0.0053	3,800,000		
Mississippi River Pool 3	PFOS	0.000035	0.0051	14	0.002	57
Mississippi River Pool 3	PFBA	0.000000067	0.86	130,000,000		
Mississippi River Pool 3	PFBS	0.000000020	0.42	220,000,000		
Mississippi River Pool 4	PFOA	0.000000027	0.0053	1,900,000		
Mississippi River Pool 4	PFOS	0.000034	0.0051	15	0.002	60
Mississippi River Pool 4	PFBA	0.000000023	0.86	370,000,000		
Mississippi River Pool 4	PFBS	All ND	0.42	All ND		
Mississippi River Pool 5/5A	PFOA	All ND	0.0053	All ND		
Mississippi River Pool 5/5A	PFOS	0.000035	0.0051	15	0.002	57
Mississippi River Pool 5/5A	PFBA	All ND	0.86	All ND		
Mississippi River Pool 5/5A	PFBS	All ND	0.42	All ND		

Notes:

ND = Not Detected; PFBA = Perfluorobutanoic Acid; PFBS = Perfluorobutane Sulfonate; PFOA = Perfluorooctanoic Acid; PFOS = Perfluorooctane Sulfonate.

(a) MOEs were rounded to two significant digits.

11 Development of Scientific Knowledge of PFC Toxicity Over Time

11.1 Introduction

The Plaintiffs' complaint (number 10) states that 3M began production of PFCs in Minnesota in the early 1950s and that during the time that it manufactured PFCs, 3M extensively studied the impact of PFCs on human health and the environment (Minnesota, Attorney General, 2011). The Plaintiffs allege that "3M knew or should have known of the potentially harmful effects that PFCs have on human health and the environment" (number 23; also see number 106) (Minnesota, Attorney General, 2011). While lacking in specifics (*e.g.*, what 3M knew and when), this allegation carries the implication that 3M's historical actions regarding its production and use of PFCs did not appropriately align with its knowledge of the potential consequences of its actions. This section summarizes the development of knowledge of PFC toxicity (in particular, PFOS and PFOA) and how the state of knowledge at various points in time would have informed 3M about the potential harmful effects of PFCs. It includes a decade-by-decade summary of PFC state of knowledge and an overall summary addressing the Plaintiffs' complaint.

In the early 2000s, 3M *et al.* (2003) and the Organisation for Economic Co-operation and Development (OECD), Environment Directorate, Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology (2002) summarized the substantial information as of that date related to PFOS toxicology. Similarly, during this period, US EPA (2002c) summarized the substantial information available as of that date related to PFOA toxicology. The early 2000s also coincided with 3M's phase-out of PFOS and PFOA.

The potential for adverse human health effects from PFOS and PFOA exposure have been informed by animal toxicology and pharmacokinetic studies, 3M worker blood monitoring, medical surveillance and epidemiology studies, and studies of PFOS body burden in the general population. By the early 2000s, it was known from earlier studies that high doses of PFOS and PFOA given to animals causes a variety of adverse health effects, but no such effects were observed in 3M production plant workers, a population reasonably considered to be the highest-exposed humans. It was also known by this time that PFOS and PFOA could be detected in the blood of the general population, but at concentrations approximately 100-1000 times lower than those of occupationally exposed workers. Despite evidence that PFOS and PFOA were widely distributed in humans and the environment, there was no evidence that PFOS and PFOA were causing harm. For example, 3M *et al.* (2003) stated, "[i]n sum, the observed levels of PFOS are not expected to cause adverse effects on human health, wildlife, or the environment." Similarly, Butenhoff *et al.* (2004c), in a risk characterization of PFOA for the general population, calculated MOEs that represented "substantial protection of children, adults, and the elderly."

11.1.1 Analytical Methodology

A discussion of the historical state of knowledge of PFC toxicology should acknowledge the developments in analytical methodology that have played a key role in the overall development of knowledge about PFCs. Most analytical methods are specific to a particular chemical (*e.g.*, PFOS) or a family of closely related chemicals (*e.g.*, total organic fluorine) in a particular sample matrix (*e.g.*, soil,

water, serum, tissues). Modern analytical methodology allows for the detection of PFOS and PFOA at very low levels in a variety of matrices (3M Co., 2000; Lindstrom *et al.*, 2011). This has not always been the case, and for many years was the limiting factor for the eventual understanding that PFOS and PFOA were present at trace concentrations in the general human population and in wildlife. Methodologies and instrumentation have advanced over the years, allowing for greater specificity (*i.e.*, distinguishing between the target compound and other compounds in the sample matrix) and sensitivity (*i.e.*, the smallest detectable amount of the target compound that can be measured with a defined certainty). 3M and outside experts, with efforts requiring decades of active analytical research and development, have developed and published numerous analytical methods for detecting PFCs in many biological and environmental matrices.

The presence of organic fluorine in human blood was first reported in the scientific literature in 1968, when Taves (1968) presented evidence consistent with there being two forms of fluoride in human serum – one of which was the inorganic fluoride ion, and the other having the characteristics consistent with a fluorocarbon molecule (see also, 3M *et al.*, 2003; Butenhoff *et al.*, 2006; Lindstrom *et al.*, 2011). Specific compounds and actual amounts of the fluorocarbons were not (and could not be) analyzed in this study and were not known. Guy *et al.* (1976) analyzed pooled plasma samples collected from a total of 106 individuals living in five different cities with between 0.1-5.6 ppm fluoride in their public water supply. While the average fluoride concentration in plasma was directly related to the fluoride concentration in the water supply, the average organic fluorine concentration in plasma was not. The authors tentatively identified PFOA as a component of the organic fluorine⁹⁵ and also noted that the presence of a sulfonate was a possible interpretation of their findings. The average organic fluorine level in pooled human plasma samples included in the study was approximately 26 ppb (Hansen *et al.*, 2001). Although Guy *et al.* (1976) speculated that there was "widespread contamination of human tissues with trace amounts of organic fluorocompounds derived from commercial products," the source of organic fluorine in blood samples from the general population has been debated and never definitively determined (Hansen *et al.*, 2001). In addition, Guy *et al.* (1976) noted that "other chemicals are usually not toxic in blood concentrations similar to those found here for organic fluorine," and that "there may have been a decrease in the concentration of organic fluorine in human plasma since the late 1950s." In 1978, based on a need for a more-refined fluorine analysis scheme, 3M researchers published a method for the determination of total fluorine in whole blood, serum/plasma, and other biological (*e.g.*, urine, tissue) samples that also allowed for determination of the concentration of both organic and inorganic forms of fluoride (Belisle and Hagen, 1978).

Early analytical methods of measuring organic fluorine in the blood of individual (*i.e.*, not pooled) occupationally exposed workers began to be used in the 1970s and were laborious, non-specific, and had high levels of detection (in the ppm range) (Hansen *et al.*, 2001; Lindstrom *et al.*, 2011). Further advancements in the 1980s to early 1990s allowed for the direct analysis of individual sulfonated PFCs, such as PFOS, with lower sensitivity (high ppb level), but these analyses were difficult to perform and not reliably quantitative. Although total organic fluorine had been identified in human blood for some time, it was not until the mid-to-late 1990s that significant advances in analytical chemistry made it possible to reliably measure individual PFCs (such as PFOS and PFOA) in the low ppb range, allowing background levels of PFCs in biological and environmental matrices to be accurately evaluated for the first time (Lau *et al.*, 2004; Lindstrom *et al.*, 2011).

In summary, the recognition that organic fluorine was present in human blood samples in the late 1960s and 1970s and the ability to detect PFOS and PFOA at low levels in various media by the mid-to-late 1990s were both influential developments in the state of knowledge of PFC toxicity.

⁹⁵ It was later learned that PFOA may have been misidentified as a major component of organic fluorine in 1976 (3M, 1999b).

11.1.2 Bioaccumulation

It is also worthwhile to briefly describe here the development of knowledge of the bioaccumulative properties of PFCs. Based on the available knowledge and technical capabilities, 3M did not establish that PFOS and PFOA were bioaccumulative in the environment until the late 1990s/early 2000s. Bioaccumulation is a process by which a chemical substance is absorbed in an organism by all routes of exposure that occur in the natural environment. It is the net result of competing processes of chemical uptake and elimination (Arnot and Gobas, 2006). These competing processes vary based on individual chemicals. Most bioaccumulative chemicals concentrate in fatty tissues because they are lipophilic, or "fat-loving." PFOS and PFOA, however, are atypical bioaccumulative chemicals in that they do not possess the classic properties for predicting bioaccumulation. In particular, PFOS and PFOA accumulate in proteins rather than fats (3M Co. *et al.*, 2003; Kennedy *et al.*, 2004; Conder *et al.*, 2008).

The evidence shows that 3M carried out testing on PFOS according to contemporaneously available scientific knowledge and, in the late 1970s, reasonably came to the initial conclusion that PFOS would not appreciably bioaccumulate in biota (Welter, 1979). Since then, scientific knowledge and technical capabilities have developed significantly. In particular, the ability to reliably measure trace levels of PFCs in the environment and in biological materials developed in the mid-to-late 1990s. This resulted in the subsequent recognition that PFCs are widely present in the environment and can bioaccumulate in the tissues of wildlife (*e.g.*, Giesy and Kannan, 2001). Until this time, no compound-specific information on the extent of the distribution of fluorinated organic compounds in the environment or in wildlife was available. This was due to the lack of a practical compound-specific method of analysis that could be applied to a wide range of sample types that had sufficient sensitivity to measure ecologically relevant concentrations of specific compounds. The work of Hansen *et al.* (2001) allowed for the survey of four fluorinated organic compounds in liver and blood plasma of wildlife on a global scale.

11.2 Overview of the Chronology of 3M Scientific Knowledge and Actions

11.2.1 1970s

3M began developing scientific knowledge of PFOS and PFOA in the areas of toxicology, occupational health, and analytical chemistry in the 1970s. Importantly, 3M did not wait for this knowledge to be developed elsewhere in the scientific community; during this decade and beyond, 3M initiated the great majority of scientific study, either itself or through its contractors, relevant to the properties of PFOS and PFOA. During this decade, 3M advanced the scientific knowledge of PFOS and PFOA in the areas of acute and non-acute animal toxicology, 3M worker serum concentrations, and analytical methodology.

The first indications of the presence of organic fluorine in human blood occurred in the late 1960s and 1970s (*e.g.*, Taves, 1968; Guy *et al.*, 1976). Although specific PFCs could not be identified in any of the studies in this era, Guy *et al.* (1976) tentatively identified a compound, which the authors stated could have been PFOA, as a major component of the organic fluorine. 3M began investigating the findings of Guy *et al.* (1976) starting in 1976 by evaluating concentrations of organic fluorine in 3M workplace air and worker serum, screening and studying fluorochemical workers, initiating metabolic and toxicological studies of PFOA, and pursuing the development of analytical methods to determine total fluorine and PFOA in biological samples. Based on the findings of Guy *et al.* (1976) and because PFOS was not a major product produced by 3M, the initial focus was on PFOA (*e.g.*, the earliest worker evaluations occurred at the Cottage Grove plant, which produced PFOA). Before the end of the decade, however, similar study of PFOS was underway (*e.g.*, Goldenthal *et al.*, 1978a,b, 1979; 3M, 1979; Johnson and Ober, 1979a,b). 3M dedicated significant manpower and resources in this regard.

In a 1979 report, 3M (1979) noted that PFOS had been detected in serum extract from a Decatur plant employee in 1976. 3M also reported that PFOS contributed 55-80% of the total organic fluorine in the five Decatur plant employees with the highest total organic fluoride concentrations. No actual concentrations were reported, which is consistent with the limited analytical capabilities at the time. There is little evidence that the findings presented in this 1979 report were reliable. Regardless of how these findings were interpreted, 3M took action to study several other potential properties of PFOS before the end of the decade, including acute toxicity, genotoxicity, repeated-dose toxicity, and pharmacokinetics.

Of particular importance were the repeated-dose toxicity studies of PFOS and PFOA that 3M conducted in rats and monkeys in 1977 and 1978 (Goldenthal *et al.*, 1978a,b, 1979) and in 1978 (International Research and Development Corp. [Goldenthal *et al.*], 1978; Goldenthal *et al.*, 1978c), respectively. These studies were informative as to the types of toxicity PFOS and PFOA could cause in animals given sufficient doses. Because the doses were excessive, however (as evidenced by mortality at many of the doses), the studies had limited relevance for assessing potential health effects in humans. These findings serve as an example of the importance of considering multiple lines of evidence for appropriate context. The repeated-dose toxicity studies were informative as to the health endpoints to focus on in medical evaluations of workers, which were already underway. At the time, it was not possible to equate the external doses given to animals to serum and liver concentrations of PFOS and PFOA, nor was it possible to reliably measure PFOS and PFOA in worker serum. Thus, it was not possible to compare the PFOS and PFOA body burdens in experimental animals to those of workers in the Cottage Grove and Decatur plants. Nonetheless, the absence of adverse effects in 3M production plant workers, a population that is reasonably assumed to have the highest exposures to PFOS and PFOA, provided evidence that the adverse effects observed in animals were not occurring in workers.

11.2.2 1980s

Further study by 3M of organic fluorine exposure and PFOS and PFOA toxicity continued in the 1980s, including the first reproductive and developmental toxicity studies. Importantly, 3M reported in 1982 (Roach, 1982a) that it had conducted health evaluations at 3M chemical production sites for several years and had yet to demonstrate evidence of illness or disease patterns among employees exposed to fluorochemicals. Furthermore, a 1981 study by 3M found evidence that the source of the organic fluorine concentrations measured in the blood of the non-fluorochemical workers was primarily natural, rather than from industrial fluorochemicals (Belisle, 1981).

Shortly after the Goldenthal *et al.* (1978a,b, 1979) studies in monkeys and rats, two reproductive and developmental toxicity studies of PFOS were conducted by 3M (Gortner, 1980; Wetzel *et al.*, 1983). These studies indicated that PFOS did not affect reproductive parameters in rats, nor was it a preferential developmental toxicant. The latter finding means that PFOS caused developmental effects only when toxicity to the mother also occurred. Similarly, three reproductive/developmental studies conducted with PFOA (Riker Laboratories, Inc. [Gortner], 1981, 1982; Staples *et al.*, 1984) had unremarkable findings (*i.e.*, there were no reproductive or developmental effects observed at exposure levels lower than those that caused toxicity to the mother).

In the Roach (1982a) report, the results of tests measuring liver enzymes, some hematology parameters, cholesterol, and total organic fluorine concentration were compared between Decatur chemical and film plant employees, two population groups assumed to be similar except for their work histories. No significant differences were found between the two groups. Because fluorochemical workers would be expected to have higher total fluorine concentrations than the general population, the absence of findings

of adverse health effects in workers provided reasonable evidence that fluorochemicals were not posing risks to public health.

Despite evidence that organic fluorine concentrations in blood were not associated with human disease or patterns of adverse effects, 3M pursued the reduction of these exposure levels to the lowest degrees possible (Roach and Sorenson, 1984). A comparison of the results of organic fluorine blood monitoring between 1982 and 1983 in Decatur plant employees demonstrated a downward trend in organic fluorine concentrations in nearly all production areas,⁹⁶ indicating that the improved exposure controls were working (Roach and Sorenson, 1984). This would likely have indicated to 3M that employee serum organic fluorine concentrations were not likely to increase beyond then-current concentrations, as long as measures to control exposure remained in place.

In the field of analytics, Belisle (1981) investigated the suggestion of Guy *et al.* (1976) that organic fluorocompounds in human blood are derived from commercial sources. Belisle (1981) examined serum from humans in a rural area of China, who had little chance for exposure to industrial fluorochemicals, and compared organic fluorine concentrations to reported concentrations for people in urban areas of the US. All of the eight samples from the Chinese subjects contained detectable concentrations of organic fluorine, though these were at the low end of the range in people from urban areas. The authors concluded that naturally occurring organic fluorine, rather than industrial fluorochemicals, may be the main source of organic fluorine in human blood. This would have suggested at the time that 3M products were not the sole source of the organic fluorine measured in pooled general population blood samples.

The 1980s were thus a time in which the prior concerns about organic fluorine measured in human blood, which had motivated 3M to investigate the potential implications of organic fluorine exposure, were somewhat relieved by several findings. Most notably, 3M's own workers, who were most likely the population with the highest blood organic fluorine exposure levels, were not found to be adversely affected by those exposures, and the source of organic fluorine in pooled general population blood was unconfirmed.

In my opinion, in the 1980s, the results of ongoing health evaluations of 3M workers provided reasonable assurances of the safety of PFOS and PFOA, and the continued conduct of those evaluations was an appropriate means to monitor the potential for human health risks from PFOS and PFOA exposure. The medical and scientific communities were not aware of the widespread presence of PFOS and, less so, PFOA in the serum of the general population until the late 1990s, because the analytical methodology for such determinations was not available until then. It is not appropriate to accuse 3M of failing to act on these finding before they were (or could have been) known.

11.2.3 1990s

The 1990s started with 3M continuing to monitor and perform health evaluations of its workers. By the mid-to-late 1990s, investigators were capable of reliably measuring PFOS and PFOA specifically (rather than just total organic fluorine) at increasingly lower concentrations in worker serum. As before, there was continued evidence, *via* worker health evaluations, that workers were not being harmed by their exposures to fluorinated chemicals such as PFOS and PFOA. Further advancements in analytical methodology allowed for measuring PFOS and PFOA at very low levels (low ppb), consistent with those in the general population serum as well as in other biological and environmental matrices. This development led to discoveries, including the widespread detection of trace levels of PFOS and PFOA in

⁹⁶ Most changes, presented by work area and worker type (*i.e.*, supervisor, operator), were on average less than 1 ppm organic fluorine.

the general population and wildlife, that changed and intensified the development of scientific knowledge of these PFCs.

Voluntary blood samples of 3M Decatur and Antwerp plant workers in 1995 and 1997 showed that mean serum concentrations of PFOS were in the low ppm range (95% of employees had PFOS concentrations <6 ppm), with background levels <1 ppm (Mandel and Burris, 1995; Olsen *et al.*, 1999). PFOA blood concentrations were also measured in 3M Decatur and Antwerp workers, who would not have been exposed to PFOA during its actual production (PFOA was only produced at the Cottage Grove plant), but in its use as a surfactant in the production of fluoropolymers. The 1995 and 1997 mean serum PFOA concentrations were 1.2 and 1.8 ppm, respectively, in the Antwerp plant employees and 1.72 and 1.40 ppm, respectively, in the Decatur plant employees.

3M also decided to conduct random sampling of employees in 1998 to address the possibility that their previous results were biased by the voluntary nature of the program (Olsen *et al.*, 2003d). The results of the voluntary and random sampling events were similar. Health evaluations of 3M workers at the Decatur and Antwerp plants, in the form of analyses of medical surveillance data (Mandel and Burris, 1995; Olsen *et al.*, 1999), and a retrospective cohort mortality study (Mandel and Johnson, 1995) did not find any relationships between PFOS concentrations and hematological and clinical chemistry parameters, nor increased mortality among these workers. Importantly, the adverse findings in the animal studies were not observed in 3M employees, further confirming what had been observed since the late 1970s and indicating that the serum concentrations measured in 3M workers were simply lower than the concentrations observed to cause adverse effects in animals.

Olsen *et al.* (1998) reported mean serum PFOA concentrations among Cottage Grove plant workers of 5.0 ppm in 1993 and 6.8 ppm in 1995, while Olsen *et al.* (2000) reported a mean PFOA concentration of 6.4 ppm at the same plant in 1997. Also in the 1990s, updates to earlier mortality studies of employees at the Cottage Grove plant (Schuman and Mandel, 1980; Mandel and Schuman, 1989) continued to find, with a few questionable exceptions (*e.g.*, a significant increase in prostate cancer mortality based on a small number of cases), no significant excess of mortality overall or attributable to any cancer or non-cancer cause (Gilliland, 1992, later published as Gilliland and Mandel, 1993a; Alexander, 2001). Gilliland (1992), later published as Gilliland and Mandel (1996), also found, based on medical surveillance data from Cottage Grove plant employees, that PFOA exerted no significant clinical hepatic toxicity despite the marked hepatic effects observed in animals experiments.

In the late 1990s, 3M began pursuing the identification of PFOS (and other specific fluorochemicals, including PFOA) in the blood of members of the population not exposed occupationally to these chemicals. These investigations led 3M to preliminarily conclude in May 1998 that PFOS, and to a lesser extent PFOA, were present in human serum in trace (ppb) levels.

Also in the late 1990s, 3M initiated a number of new toxicological studies of PFOS and PFOA.

11.4.4 Early 2000s

In May 2000, 3M management announced its decision to voluntarily phase out the manufacture and sale of PFOS and PFOA, with substantial discontinuation of manufacture by the end of 2002. A decision by a manufacturer such as 3M to phase out the manufacture and sale of a substance for precautionary reasons should not be taken to mean that its presence in the environment is harmful or merits remediation. For example, with regard to PFOS chemistry, US EPA stated: "3M data supplied to EPA indicated that these chemicals are very persistent in the environment, have a strong tendency to accumulate in human and animal tissues and *could potentially pose a risk to human health and the environment over the long term*"

(US EPA, 2000b [emphasis added]). In fact, the phase-out of the manufacture of PFOS was not based on actual risks, as indicated in a contemporaneous article: "Since the 1970s, 3M has been monitoring PFOS in the blood of plant workers at a level of about two parts per million, which is about 100 times the level found in samples from the general population. No studies have turned up increased rates of illness or death attributable to PFOS exposure" (Lazaroff, 2000).

As stated by the CDC (2009b), "The measurement of an environmental chemical in a person's blood or urine is an indication of exposure; it does not by itself mean that the chemical causes disease or an adverse effect." Specifically with regard to PFCs, CDC (2009a) states:

Finding a measurable amount of PFCs in serum does not imply that the levels of PFCs cause an adverse health effect. Biomonitoring studies on levels of PFCs provide physicians and public health officials with reference values so that they can determine whether people have been exposed to higher levels of PFCs than are found in the general population. Biomonitoring data can also help scientists plan and conduct research on exposure and health effects.

The phase-out of the manufacture of PFOS and PFOA did not stop 3M from pursuing additional study of these chemicals, including additional toxicity and epidemiology studies (discussed in Sections 6 and 7), biomonitoring of 3M workers, analyses of general population and wildlife samples, and analytical chemistry development.

11.3 Summary

I have provided a description of the development of knowledge of PFOS and PFOA toxicity from the 1970s to the early 2000s. These developments occurred *via* human and animal studies and advances in analytical methodologies, mostly through the efforts of 3M (itself or *via* its contractors). By sponsoring academic and laboratory research, as well as publishing some of its findings, 3M was engaging and informing the scientific community about PFOS and PFOA toxicity. Studies of some of the other PFCs, such as PFBA and PFBS, have been conducted largely since the end of the period addressed in this section (*i.e.*, the early 2000s) and are thus not addressed here.

3M began to study PFOS and PFOA toxicology in animals when it was recognized in the 1970s that organic fluorine could be measured in pooled human blood and correlated with worker exposure. These studies helped 3M gain an understanding of the health effect endpoints, MoA, and dose-response aspects of these chemicals. Starting around the same time, 3M began to monitor worker serum concentrations of total organic fluorine, and, later, PFOS and PFOA specifically, and worker health. Importantly, 3M workers were reasonably anticipated to have experienced the highest human exposures to PFCs, but these exposures were understood to be much lower than those employed in animal studies. 3M gained assurances that the high-dose effects seen in animals were not being seen in lesser-exposed workers. This was affirmed *via* worker epidemiology studies starting in the 1980s. With advances in analytical methodology, studies in humans improved over time to allow for specific PFCs to be measured at low levels in a variety of matrices. With these improved capabilities, it was recognized in the late 1990s/early 2000s that PFCs (particularly PFOS) could be detected in the low ppb levels in the general population and other biota. The recognition of the widespread occurrence of PFCs in biota, including humans, spawned additional study of the toxicology and epidemiology of low levels of PFC exposure and the precautionary phase-out of PFOS and PFOA.

I conclude that 3M's response to the initial findings about the toxicity of PFOA and PFOS in the 1970s, specifically to conduct worker health studies, was scientifically appropriate, especially considering that

such workers would be expected to have much higher exposures to these chemicals than the general population. It is important to note that the weight of evidence from the initial worker studies, as well as from subsequent worker studies to date, does not establish an association between adverse health effects and exposure to PFOS or PFOA.

12 Awareness of Health Risks from Waste Disposal

As described in Section 4, 3M began commercial production of PFCs in the early 1950s (ATSDR and MDH, 2012). For a time, 3M production wastes were disposed of on-site and/or at off-site landfills, leading to discharges to surface waters and migration of PFCs to groundwater and soil (MDH, 2016a). Specifically, the Oakdale Disposal Site was used during the late 1940s-1950s for waste burial, drum reclamation, and open burning of combustible materials (MDH, 2016b) and received liquid and solid industrial waste from 3M from approximately 1956-1960 (ATSDR and MDH, 2012). PFC wastes were also disposed of in the former Washington County Landfill during the 1960s-1970s (MDH, 2016c). The Woodbury Disposal Site was also used as a disposal site for liquid and solid industrial waste from 3M from approximately 1960-1966 (ATSDR and MDH, 2012).

To put the waste disposal actions of 3M into context, this section addresses the historical state of knowledge of environmental contamination by industrial waste, including contamination of groundwater, and public awareness of chemicals in the environment.

12.1 Waste Disposal and Contamination of Groundwater

It was not until the 1970s that the medical and scientific community began to develop an understanding of potential health risks associated with contaminated sites (Shea, 1996). Initial concerns in this regard centered around water and air pollution, while concern for landfilled chemicals came much later (Moya and Fono, 1997). As described in Section 4.5, in the 1970s, 3M no longer disposed of PFC-containing wastes in landfills, which coincided with the medical and scientific communities' initial recognition of potential risks from man-made chemicals in groundwater at contaminated sites.

The Safe Drinking Water Act (SDWA) was passed in 1974, and provides the basis for how drinking water supplies in the US are protected and monitored. The SDWA also established MCLs, regulatory values that represent the highest level of a contaminant that is allowed in drinking water (US EPA, 2017e). Following the passage of the SDWA, US EPA initiated a series of water surveys that focused on different sources of water and chemical pollutants. The Ground Water Supply Survey (GWSS), which the US EPA launched in the early 1980s, was described by US EPA as the first survey of its kind to focus exclusively on drinking water supplies with groundwater sources (US EPA, 1981). Results from the GWSS provided some of the earliest data that led scientists and regulators to recognize that chemicals were entering groundwater throughout the US. Findings of groundwater contamination by organic chemicals in the early 1980s appear to have been unexpected and dramatic, as shown in the following excerpts:

Ground water has generally been viewed as a pristine resource, unspoiled by human activities. The contamination of ground water by synthetic organic chemicals was viewed as a series of isolated problems caused by the accidental "mishandling" of chemicals. The results of EPA surveys and the efforts of many states have changed the perception of ground water quality. (US EPA, 1981)

It seems as though the presence of toxic organic chemicals in groundwater has sprung into the national consciousness almost overnight. (Burmester, 1982)

By the mid-1980s, the broader scientific community began to acknowledge the potential for groundwater contamination and to highlight the deficits in research and regulations that still remained. In its 1984 report, "Protecting the Nation's Groundwater from Contamination," the US Office of Technology Assessment stated:

Contamination of groundwater—by organic and inorganic chemicals, radionuclides, and/or microorganisms—has occurred in every State and is being detected with increasing frequency. For a long time, the land surface and subsurface were considered safe and convenient depositories for many of society's wastes and non-waste products. Only recently has the limited capacity of natural soil processes to change contaminants into harmless substances, before they reach groundwater, become widely recognized. (US Congress, 1984)

In 1984, US EPA stated that, "[i]n the last decade the public has grown increasingly aware of the potential problem of ground-water contamination. Reports of chemicals threatening drinking water supplies have mobilized State, local and Federal governments to respond" (US EPA, 1984). US EPA acknowledged that groundwater contamination was widespread, but also that information on the extent of groundwater contamination was inadequate to assess the severity of the problem (US EPA, 1984).

Also in 1984, the NRC (at the request of US EPA) established a Committee on Ground Water Quality Protection, whose findings were published in 1986 (NRC, 1986). Some of these findings were:

None of the programs studied by the committee was based on explicit evaluation of the health, environmental, economic, social, and political costs and benefits to society associated with the protection of ground water quality. (NRC, 1986)

More scientific and technological information is needed concerning the extent of ground water contamination, its effects on health, the environment, society, and the economy, and strategies and technologies to prevent it. (NRC, 1986)

The federal role appears to be inadequate in both magnitude and expertise in the following areas: (1) determinations of the health effects of ground water contamination and establishing drinking water standards; (2) research on resource reduction, control methods, and contaminant transport; (3) technology transfer, and (4) exchange of information among states. (NRC, 1986)

The first national assessment of a large number of VOCs (55 total) in groundwater did not occur until 1985-2001, when the USGS analyzed about 3,500 water samples for these contaminants (USGS, 2006). These man-made chemicals were selected because of their ubiquitous, widespread, and long-term use, including in industry, households, commerce, and military sites. Like VOCs, some PFCs have been detected in the serum of the general population, indicating that PFCs have a widespread presence in the US. However, the analytical capabilities for detecting PFOS and PFOA in groundwater were only developed in 2003 (and expanded in 2006), and nationwide water sampling for PFCs was not conducted until 2013-2015 (ATSDR and MDH, 2012; US EPA, 2016f). Thus, because analytical methods for detecting PFCs were not available until the mid-to-late 1990s for workers and until 2003 for water, along with the lack of evidence of health effects in highly exposed populations, there was no basis for 3M to conduct analyses of groundwater for PFC contamination.

12.2 Awareness of Chemicals in the Environment

A number of events occurred that brought chemicals in the environment and industrial waste to the forefront of public awareness. In 1962, Rachel Carson's *Silent Spring* was published and widely read by the American public, who developed a new awareness of the environment and the chemicals being used (Lear, 1993; Schaumburg, 1990). Although the book addressed the effects of chemicals, specifically synthetic organic pesticides, on animals and the environment, it did not address the effects of these same chemicals on humans. Following the publication of *Silent Spring*, additional public concern developed over contamination-related fish kills, beach closings, and burning rivers (e.g., the Cuyahoga River in 1969), all of which received media attention (US EPA, 1979, 2016g; Ross and Amter, 2010).

The American public became motivated politically and environmentally to address this concern, and a torrent of major environmental regulations and legislation followed in the 1970s: in 1970, US EPA was established and the National Environmental Protection Act and Clean Air Act Amendments were enacted; in 1972, the Clean Water Act was enacted; in 1974, the SDWA was enacted; and in 1976, the Toxic Substances Control Act (TSCA) and the Resource Conservation and Recovery Act (RCRA) – the primary law governing the disposal of solid and hazardous waste in the US – were enacted (US Congress, 1970, 1976a,b; Ross and Amter, 2010; US EPA, 2010, 2017f).

Perhaps the most infamous incident involving potential human health risks associated with chemical contamination of the environment was the Love Canal incident in New York State. According to Ross and Amter (2002), "the problem of soil and ground water polluted by toxic industrial wastes burst suddenly into the national consciousness in 1978 with the discovery of Love Canal," which was followed shortly after by the enactment of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, also known as Superfund), which mandates US EPA to assess the hazards associated with waste sites and to take remedial action, if necessary, to protect human health and the environment (US Congress, 1980; US EPA, 2017f). Also in 1980, the US Congress created ATSDR, an agency of the US Department of Health and Human Services, to implement the health-related sections of CERCLA. The agency was mandated to assess the presence and nature of health hazards at National Priorities List (NPL) and other waste sites, as well as help prevent or reduce further exposure and illnesses that result from exposure to any such hazards (ATSDR, 2005). In 1984, RCRA was amended to authorize ATSDR to conduct public health assessments at sites when it is requested to do so by US EPA, states, or individuals (ATSDR, 2005).

Quantitative site-specific risk assessment – the technique used to evaluate health risks associated with the chemicals found at contaminated sites today – has only been in existence since the early 1980s. Site-specific human health risk assessments were developed by US EPA in compliance with CERCLA (1980), as amended in 1986 by the Superfund Amendments and Reauthorization Act (SARA) (US EPA, 1989). In 1983, the NAS published *Risk Assessment in the Federal Government: Managing the Process*, which outlined the four steps to be used in conducting risk assessments (NRC, 1983): hazard identification, dose-response assessment, exposure assessment, and risk characterization. US EPA incorporated the four-step risk assessment process into its guidelines for site-specific risk assessment, which were first published in 1986 (in the "Superfund Public Health Evaluation Manual") and revised in 1989 as the Risk Assessment Guidance for Superfund (RAGS Volume I, Part A) (US EPA, 1986b, 1989). US EPA has also published a number of supplemental guidance documents to RAGS and other guidelines regarding risk assessment since the 1980s. Thus, modern site-specific risk assessment is a well-defined process that is the result of decades of development.

Many states have developed risk assessment guidelines that are conceptually similar to US EPA's RAGS, but which apply to inactive waste sites within the states. Minnesota, for example, enacted the Minnesota

Rules Part 7045.0131 in 1983, which complements CERCLA (MPCA, 2001). Other states that have promulgated their own risk assessment guidelines include New Jersey, Massachusetts, California, and Michigan (NJDEP, 2012; MADEP, 2001; CalOEHHHA, 2015; MDEQ, 2004).

Although municipalities, industries, and government agencies were aware of waste disposal practices and technologies before 1970, there was no uniform regulation of chemical waste disposal prior to the enactment of the National Environmental Protection Act in 1970 and no national hazardous waste legislation prior to the enactment of RCRA in 1976 (Colten and Skinner, 1996). In addition, it was reasonable that PFCs were not part of the initial group of compounds that were measured in groundwater (by 3M or by state and federal agencies), and that they could not, in fact, have been measured at this time because there were no analytical methods to detect them and because 3M had evidence indicating that populations that had been highly exposed to PFCs (*i.e.*, workers) did not have health effects as a result of that exposure.

12.3 Summary

Historically, scientists' and state officials' lack of understanding of human health risks from and exposure to industrial wastes influenced how they viewed disposal practices. When 3M began commercial production of PFCs in the early 1950s, and for decades thereafter, there was little understanding by public health professionals, chemical manufacturers, or the general public that, from a public health perspective, land disposal of industrial chemicals could reach and potentially impact groundwater. Moreover, even when such recognition began, it was in the context of specific organic chemicals (*e.g.*, VOCs, such as trichloroethylene and perchloroethylene), for which measurement methods existed and which were used in many different industries and applications throughout the US (USGS, 2006).

13 Comments on Plaintiff's Opinions

13.1 Comments on Dr. DeWitt's Opinions

Dr. DeWitt's overall conclusion is that PFCs pose a substantial present and potential hazard to human health, based on what she identified as particularly strong evidence for associations between PFCs and cancer, developmental toxicity, and immunotoxicity (DeWitt, 2017a). It is my opinion that, in developing this conclusion, Dr. DeWitt did not perform an appropriate weight-of-evidence analysis (see Section 3 of this report). Specifically, Dr. DeWitt overstated the evidence for PFCs' association with health effects, did not consider the inconsistencies between animal study and human study results, did not conduct an independent analysis of the studies that are the bases of the MDH HBVs and HRLs, and did not critically evaluate the conclusions of various health agencies that made determinations regarding PFCs. Moreover, she performed no reliable evaluation of exposures to residents of southern Washington County. For example, she did not compare the serum concentrations of residents to serum concentrations at which health effects occurred in animal studies. Thus, Dr. DeWitt's conclusion as to the present and potential hazard of PFCs to residents of southern Washington County is not supported by a comprehensive and balanced weight-of-evidence analysis of the potential health effects of PFCs and by consideration of potential exposures to residents of southern Washington County. The bases for these statements are provided in this section.

Much of the evidence that Dr. DeWitt cited in her report (DeWitt, 2017a) and in her deposition (DeWitt, 2017b) is also discussed in Section 6 of this report. As noted above, Dr. DeWitt overstated the evidence from many of the animal studies that she cited. My concerns about the interpretation of studies are discussed briefly in this section and in more depth in Section 6.

Throughout her report, Dr. DeWitt claimed that health effects that were reported for one PFC are also likely to induce similar toxicities in other PFCs "by extension" (DeWitt, 2017a). She did not provide any citations to support these broad statements. Not only are these statements inconsistent with the underlying literature (see Sections 6 and 7 of this report), but also, these statements contradict the recently published text book on PFCs that Dr. DeWitt edited:

Caution needs to be exercised in projecting the biological activities of any of the chemicals in this family based on results from others. For example, considering the three chemicals for which lifetime studies in rats are available, the [carcinogenicity] outcomes were different with no increase in tumors seen with perfluorohexanoic acid (PFHxA), liver adenomas seen with PFOS, and adenomas of the liver, testis, and pancreas seen with PFOA. (Kennedy and Symons, 2015)

Furthermore, Dr. DeWitt implied throughout her report that studies conducted by or sponsored by 3M were tainted by bias and therefore not credible. However, in her report and at her deposition (DeWitt, 2017b), Dr. DeWitt did not acknowledge that if 3M had not initiated those studies, such studies would not have been conducted at all. Dr. DeWitt also did not discuss how the early 3M studies were planned and conducted appropriately, in accordance with the knowledge that 3M had at the time, as discussed below and in Section 12.

13.1.1 Cancer

Dr. DeWitt's discussion of the carcinogenicity of PFCs overstated the available evidence. For example, she stated that the IARC (2016a) cancer assessment reflects "substantial evidence that PFOA is correlated with increased incidences of cancer" among exposed populations (DeWitt, 2017a, p. 4), when in fact, IARC has concluded that there is "limited evidence" for the carcinogenicity of PFOA in humans and classified PFOA as a Group 2B carcinogen (*i.e.*, possibly carcinogenic to humans) (IARC, 2016a).⁹⁷ Similarly, Dr. DeWitt stated that ATSDR (2015) determined that carcinogenicity was consistently associated with PFOA and PFOS serum levels in epidemiology studies (DeWitt, 2017a), when in fact ATSDR stated:

There was also equivocal evidence of carcinogenicity... Although several studies have found significant increases in cancer risk, the results should be interpreted cautiously since most studies did not control for potential confounding variables (particularly smoking), the number of cancer cases was low, and a causal relationship between perfluoroalkyls and cancer cannot be established from these studies. Additionally, the lack of consistency across facilities may be suggestive of a causative agent other than PFOA or PFOS. (ATSDR, 2015)

In addition, Dr. DeWitt's statements regarding the carcinogenicity of PFOA and PFOS are contradicted by the textbook on PFCs that she edited:

Epidemiologic studies have been reported for several levels of population exposure. Limited evidence of associations with kidney and testicular cancer has been reported in studies among community members exposed to drinking water contaminated by PFOA. Studies in workers exposed to higher levels of both PFOA and PFOS have not shown consistent evidence for an association with any specific cancer type. Studies in populations exposed to low levels of PFOA and PFOS have shown equivocal results for a variety of cancers with no consistent associations. Based on evidence reported to date, the prospect for developing a carcinogenic outcome following exposure to PFOA and PFOS is remote. For other perfluoroalkyl acids, there is not sufficient evidence regarding their potential carcinogenicity. (Kennedy and Symons, 2015)

In her expert report in this matter, Dr. DeWitt did not adequately discuss inconsistencies across carcinogenicity studies. For example, she cited several studies as providing evidence of the carcinogenicity of PFOA and PFOS (DeWitt, 2017a, pp. 24-25), but did not describe many other studies that did not show an association between these PFCs and carcinogenicity (see, for example, Grice *et al.*, 2007; Vassiliadou *et al.*, 2010; Steenland and Woskie, 2012). Dr. DeWitt also cited studies that she claimed showed increased risk of cancer from PFOA or PFOS, but these studies' results were not statistically significant (*e.g.*, Eriksen *et al.*, 2009; Raleigh *et al.*, 2014; Steenland *et al.*, 2015), and therefore are not considered by the general scientific community to be evidence of PFCs' carcinogenicity.

Dr. DeWitt's assessment that there is "strong" evidence that PFOA is carcinogenic (DeWitt, 2017a, p. 4) is not consistent with the underlying studies and with regulatory guidance. Her conclusion is based on two animal studies in which rats were fed 300 ppm PFOA in the diet (DeWitt, 2017a, pp. 25-28). In the study by Butenhoff *et al.* (2012b), male rats had an increased incidence of testicular Leydig cell adenomas, and in the study by Biegel *et al.* (2001), male rats had an increased incidence of hepatocellular

⁹⁷ It should be noted that a number of chemicals, agents, and environmental/mechanical phenomena are included in the Group 2B class, such as radiofrequency magnetic fields given off by cell phones (IARC, 2011).

adenomas and pancreatic acinar cell adenomas, plus one pancreatic carcinoma. Adenomas are benign tumors and carcinomas are malignant. Agency guidelines by US EPA (2005), NTP (2015), and IARC (2016b) all require findings of malignant tumors or a combination of benign and malignant tumors for evidence of carcinogenicity. The findings of benign adenomas only in the Butenhoff *et al.* (2012b) study and only one malignant carcinoma out of 76 total pancreatic tumors in the Biegel *et al.* (2001) study do not provide strong or sufficient evidence of PFOA's carcinogenicity according to US EPA, NTP, and IARC guidelines.

Dr. DeWitt's opinion that the carcinogenic potential of PFOA cannot be discounted as a species-specific response to peroxisome proliferation (DeWitt, 2017a, p. 22) is not reliably supported either by the studies that she cited or by other relevant evidence that she did not discuss in her report. In support of her opinion that PFOA could cause liver cancer in humans, Dr. DeWitt (2017a, p. 22) provided three lines of evidence: (1) that "hepatocellular hypertrophy and nonneoplastic liver lesions" were observed in mice lacking the PPAR α gene (*i.e.*, PPAR α -knockout mice) in a study by Filgo *et al.* (2015),⁹⁸ (2) that humans are responsive to agents such as the lipid-lowering fibrate class of drugs that act *via* PPAR α , and (3) that peroxisome proliferators may "pose a risk of carcinogenesis" in pigs, which are considered comparable to humans in terms of their response to peroxisome proliferators, as shown in a study by Luci *et al.* (2007). These three lines of evidence do not hold up to careful scrutiny.

- In the study by Filgo *et al.* (2015), there were no statistically significant neoplastic effects observed in the PPAR α -knockout mice. The only non-neoplastic liver effects in the PPAR α -knockout mice were a statistically significant trend for an increase in the size of hepatocytes (one type of liver cell) and a statistically significant decrease in the number of mice with enlarged Ito cells (another type of liver cell). Neither an increase in the size of hepatocytes nor a decrease in the number of mice with enlarged Ito cells translates to an increased risk of developing cancer, which involves an increase in the number of cells.
- Although humans are certainly responsive to lipid-lowering drugs that act *via* PPAR α , as discussed in Section 5.1, there are important differences between humans and rodents with respect to PPAR α -mediated effects in the liver, with humans being much less sensitive to such effects than rodents. In addition, there is a lack of evidence from epidemiology studies that the incidence of liver cancer is increased for individuals treated chronically with lipid-lowering drugs (discussed in Corton *et al.*, 2014). Experts from government, academia, and industry who reviewed differences in response to the lipid-lowering drugs between rodents and humans concluded that liver cancer associated with PPAR α activation was either "not relevant to humans" or "unlikely to be relevant to humans" (Corton *et al.*, 2014).
- On balance, the results from the study by Luci *et al.* (2007) do not indicate that peroxisome proliferators would pose a risk of carcinogenesis in pigs. As discussed by Luci *et al.* (2007), liver cancer in rodents exposed to peroxisome proliferators may be due to increased concentrations of hydrogen peroxide (a reactive molecule that can cause cell damage) and disruption in the balance between programmed cell death (an important physiological process that can prevent damaged cells from developing into tumors) and cell proliferation. In contrast to rodents, for which exposure to peroxisome proliferators is associated with increased concentrations of hydrogen peroxide and decreased programmed cell death, hydrogen peroxide was decreased and genes that promote programmed cell death were increased in pigs treated with clofibrate (a peroxisome proliferator). The only effect in the clofibrate-treated pigs that could potentially pose a risk of carcinogenesis was increased expression of two genes (*c-jun* and *c-myc*) that promote cell

⁹⁸ It should also be noted that, as discussed in Sections 6.1.1.1 and 6.1.1.2 of this report, liver hypertrophy associated with PFOA and PFOS exposure in rodents is reversible upon cessation of exposure.

proliferation. As discussed by Luci *et al.* (2007), the *c-myc* gene can also promote programmed cell death.

Dr. DeWitt also presented a 2-year rat study (Butenhoff *et al.*, 2012a) in which thyroid follicular cell tumors increased in rats treated with PFOS as evidence of the carcinogenicity of PFOS (DeWitt, 2017a, pp. 30-31). Dr. DeWitt overstated the results in this study by referring to an increase in combined thyroid follicular cell adenomas and carcinomas in male rats. There were no thyroid follicular cell carcinomas in these rats, only adenomas, which are benign. Dr. DeWitt also did not address the fact that the rats in this study that were treated with PFOS for 1 year and allowed to recover for 1 year showed an increase in the adenomas, while the rats that were treated with PFOS for 2 years did not. If PFOS had caused the thyroid follicular cell adenomas, the adenomas should also have appeared in the rats treated for 2 years. Dr. DeWitt did not discuss this inconsistency in her report. Thus, the increase in thyroid follicular cell tumors does not provide evidence of PFOS carcinogenicity. Moreover, the increase in "combined hepatocellular adenomas and carcinomas in female rats" that Dr. DeWitt referred to as providing additional support for the carcinogenicity of PFOS (Dr. DeWitt (2017a, pp. 30-31) included only one carcinoma in one rat.⁹⁹ As noted above, the finding of only one liver carcinoma in this study does not provide strong or sufficient evidence of PFOS's carcinogenicity according to US EPA, NTP (2015), and IARC (2016b) guidelines.

Dr. DeWitt discussed two studies in Sprague-Dawley rats, supported by 3M, regarding the PFC N-ethyl perfluorooctanesulfonamido ethanol (N-EtFOSE).¹⁰⁰ She concluded that these studies demonstrated that N-EtFOSE is carcinogenic. Dr. DeWitt's conclusion is overly broad and not supported by a more complete analysis of the studies and relevant supporting material.

Dr. DeWitt stated that the consideration of historical control data by Riker Laboratories/3M Co. (1987) in a chronic bioassay of N-EtFOSE in Sprague-Dawley rats was basically inappropriate. While it is correct that the incidence of hepatocellular carcinomas in female rats was somewhat outside control limits, Riker's use of historical control data, along with the lack of tumors in the female controls, was reasonable support for their conclusion that N-EtFOSE was not a liver carcinogen in the rat. For example, as discussed by Keenan *et al.* (2009): "HCD [historical control data] should be considered as one of many sources of information that add to the 'weight of evidence' approach when assessing the potential carcinogenic effect of a compound."

Dr. DeWitt also discussed a subsequent analysis of the Riker Laboratories/3M Co. (1987) study by Pathology Associates International (1998), which concluded that N-EtFOSE should be considered a liver carcinogen in rats. However, Dr. DeWitt did not discuss other relevant material in the Pathology Associates International (1998) report. Pathology Associates International (1998) stated that epigenetic mechanisms (*e.g.*, peroxisome proliferation) played a likely role in the development of liver proliferative lesions (which could lead to tumors) with N-EtFOSE and that agents that promote liver enlargement and tumors in rodents *via* peroxisome proliferation have not been reported to cause liver damage, including cancer in humans.

Dr. DeWitt discussed a subsequent study by Covance Laboratories, Inc. (2001) of N-EtFOSE in Sprague-Dawley rats and listed statistically significant increases in combined liver adenomas and carcinomas in female rats at the highest dose and statistically significant increases in combined thyroid follicular cell adenomas and carcinomas in male rats again at the highest administered dose. However, Dr. DeWitt did not discuss how there was a single liver carcinoma in the female rats, which was not statistically

⁹⁹ The rest of the tumor findings were adenomas, which are benign.

¹⁰⁰ I note that N-EtFOSE is not listed in the complaint (Minnesota, Attorney General, 2011). Dr. DeWitt does not provide a rationale for discussing N-EtFOSE.

significant (Covance Laboratories, Inc., 2001). Nor did Dr. DeWitt discuss how the study authors considered the significance of the thyroid follicular cell carcinoma in male rats questionable, given that it was not statistically significant by itself and did not show a clear dose-response relationship (Covance Laboratories, Inc., 2001).

Thus, the evidence for the carcinogenicity of N-EtFOSE is weak and inconsistent. Moreover, liver findings, such as liver enlargement and proliferative lesions, are likely mediated by epigenetic mechanisms (especially peroxisome proliferation), which would be of limited, if any, relevance to humans.

13.1.2 Developmental Toxicity

Dr. DeWitt overstated the evidence for an association between developmental toxicity effects and exposure to PFCs. She stated that ATSDR (2015) determined that decreases in birth weight were consistently associated with PFOA and PFOS serum levels in epidemiology studies (DeWitt, 2017a, p. 49). However, she did not note that ATSDR also stated, "[a]lthough significant associations were found, decreases in birth weight were small and may not be biologically relevant. No studies found an increased risk of low birth weight in infants (<2,500 g) in highly exposed residents" (ATSDR, 2015).

Dr. DeWitt relied on the US EPA (2016a,b) evaluations of PFOA and PFOS and on reviews of epidemiology studies of PFCs exposure and developmental effects (ATSDR, 2015; Rappazzo *et al.*, 2017) for her conclusions regarding the developmental toxicity of PFCs. She did not conduct an independent analysis of PFCs' developmental toxicity or of the epidemiology studies the reviews she relied upon discussed.

Dr. DeWitt did not address inconsistencies among developmental toxicity studies for PFCs or among agency evaluations of developmental toxicity evidence for PFCs. For example, she noted that ATSDR (2015) found that decreased birth weight was associated with PFOA serum levels in epidemiology studies, but did not address the fact that the C8 Science Panel (2011b) concluded there was no probable link between PFOA and lower birth weight or any birth defects¹⁰¹. In addition, the PFCs textbook that Dr. DeWitt edited does not conclude that there is consistent evidence of reproductive or developmental effects from PFC exposure in humans:

Human population studies have reported inconsistent associations between PFAS¹⁰² exposure and reproductive and developmental outcomes... None of these outcomes show consistent associations with any PFAS, and when positive associations have been reported, their magnitude has been small... One meta-analysis of nine studies reported a significant association of lower birth weight with more PFOA at low general population levels, but is contradicted two other studies with much greater exposure contrasts, and not supported by three studies showing no association of PFOA with [low birth weight]. (Khalil *et al.*, 2015)

In her discussion of studies of developmental toxicity and PFCs exposure, Dr. DeWitt did not take into account the presence of maternal toxicity and its potential effects on development. For example, Dr. DeWitt (2017a, p. 32) highlighted studies by Thibodeaux *et al.* (2003) and Lau *et al.* (2003). In both of these studies, developmental effects occurred at dose levels that were also associated with maternal

¹⁰¹ It should also be emphasized that the C8 Science Panel studies were done in the context of litigation and had a lower threshold for assessing possible causality than is typically used in epidemiology studies or accepted by the general scientific community.

¹⁰² PFAS is an abbreviation for per- and polyfluorinated alkyl substances, of which PFCs are a subgroup.

toxicity. When developmental effects occur at the same doses as maternal toxicity, the effects may be due to systemic toxicity in the dam rather than a direct result of chemical exposure to the fetus.

The Lau *et al.* (2006) study was used as the basis of both the US EPA (2016c) Lifetime HA and the MDH (2017b) HBV for PFOA. Dr. DeWitt claimed that this study provides evidence of PFOA causing developmental effects (DeWitt, 2017a, pp. 32-33), but she did not conduct a critical analysis of this study's results. As noted in Sections 6.1.1 and 8.1.1 of this report, neither of the endpoints chosen by US EPA (2016c) and MDH (2017b) as the critical effects (delayed skeletal ossification and accelerated male puberty) for PFOA exhibited a normal dose-response pattern. Dr. DeWitt did not address these irregularities, although she did state in general that some agents do not always generate a "standard" dose-response curve for health effects (DeWitt, 2017a, pp. 9, 13). The citation that she provided for this proposition, Vandenberg *et al.* (2012), is not broadly accepted in the scientific community, for a number of reasons (see, for example, Rhomberg and Goodman, 2012 and Lamb *et al.*, 2014), including the authors' use of anecdotal information, their assumption that statistically significant findings in and of themselves are indicative of causation, and their failure to consider other risk factors when interpreting epidemiological evidence as evidence of endocrine disruption. Dr. DeWitt has not provided a reliable basis to conclude that the standard dose-response pattern is not applicable to PFCs. The dose-response relationship represents one of the most fundamental concepts in the field of toxicology (Eaton and Gilbert, 2013), and the lack of a dose-response in the critical effects chosen by US EPA (2016c) and MDH (2017b) calls into question whether the effects were actually related to PFOA treatment.

13.1.3 Immunotoxicity

In her discussion of the immunotoxicity of PFCs, Dr. DeWitt did not address several issues regarding the studies she highlighted, including the lack of consistency across studies, the relevance of secondary vs. primary immune outcomes, the lack of dose-response patterns, and the presence of systemic toxicity.

Across the PFOA immunotoxicity studies conducted by Dr. DeWitt's group and cited in her report (DeWitt, 2017a, pp. 42-43), there is inconsistency in the reported NOELs and LOELs. For example, DeWitt *et al.* (2008) reported a NOEL for reduced antibody IgM production in C57BL/6N mice of 1.88 mg/kg-day PFOA and a LOEL of 3.75 mg/kg-day PFOA. A follow-up study using the same strain of mice (DeWitt *et al.*, 2009) reported a NOEL for antibody IgM production of 7.5 mg/kg-day PFOA and a LOEL of 15 mg/kg-day PFOA. In a third study with C57BL/6-Tac mice, DeWitt *et al.* (2016) again reported a NOEL for antibody IgM production of 7.5 mg/kg-day PFOA and a LOEL of 30 mg/kg-day PFOA. Dr. DeWitt and her coauthors did not address these differences in NOELs in their publications, and Dr. DeWitt did not address them in her report.

Dr. DeWitt also did not address the relevance of certain immunotoxicology outcomes. Antibody production is considered a secondary outcome (NTP, 2016), *i.e.*, it does not necessarily reflect a functional deficit. For example, in the DeWitt *et al.* (2008) study, immune function as measured by the SRBC assay was not affected at doses up to 30 mg/kg-day PFOA. This group also did not observe any developmental immune function effects of PFOA in C57BL/6 mice at doses of 0.5 or 1.0 mg/kg-day PFOA (Hu *et al.*, 2010).

In some of the immunotoxicology studies that Dr. DeWitt discussed in her report, the immune effects occurred at the same doses that systemic toxicity was observed and may have been secondary to systemic effects rather than directly caused by the chemical exposure. For example, in the study by DeWitt *et al.* (2009), the PFOA dose associated with reduced antibody production was also associated with a 10% reduction in body weight, indicating possible systemic toxicity. In the study by DeWitt *et al.* (2016),

body weights were reduced by 15-20% at the LOEL for reduced antibody production, again indicating systemic toxicity.

Dr. DeWitt also did not address the lack of a dose-response pattern in some studies, aside from her general statement that some agents did not always generate a "standard" dose-response curve, as noted above (DeWitt, 2017a, pp. 9, 13). In the DeWitt *et al.* (2016) study, the authors demonstrated a reduced TIAR (a primary effect) in mice treated with PFOA, but the reduction was roughly equivalent at all doses above the NOEL, indicating the lack of a dose-response. Contrary to Dr. DeWitt's general statement about dose-response curves, if PFOA caused the reduced TIAR, it would be expected that higher doses of PFOA would elicit greater effects.

Dr. DeWitt did not cite any specific studies of PFOS's effects on the immune system, but relied on the NTP (2016) review to conclude that PFOS, as well as PFOA, was immunotoxic. The NTP (2016) review has many of the same limitations as those that are present in Dr. DeWitt's report; specifically, most of the animal studies reviewed in the NTP evaluation that reported PFOA or PFOS dose-dependent immune effects also reported systemic toxicity at the same doses (in addition to the studies noted above, see, for example, Dong *et al.*, 2009, 2011; Zheng *et al.*, 2009). The only animal study that reported a PFOS dose-dependent reduction in immune response (Peden-Adams *et al.*, 2008) has not been replicated in publications by this or other research groups. Thus, while the NTP (2016) review is comprehensive in its coverage of the immunotoxicology of PFOA and PFOS, much of the animal evidence it relied on does not provide reliable support for its conclusions. These limitations are also discussed in Gradient's comments submitted to NTP in response to NTP's draft systematic review of PFOA and PFOS immunotoxicity studies (Gradient, 2016).

13.1.4 Thyroid Disease

The C8 Science Panel found a probable link between PFOA exposure and thyroid disease (C8 Science Panel, 2012c), as noted by Dr. DeWitt. However, Dr. DeWitt did not note that this finding was based on inconsistent and contradictory results, as described in Section 7.1.5.2 of this report. By the C8 Science Panel's own admission, "each finding in isolation was not compelling, plausibly a result of chance or other errors," and there was "a lack of coherence among them" (C8 Science Panel, 2012c).

Dr. DeWitt critiqued the work by Chang *et al.* (2007) that indicated that reports of reduced free thyroid hormone in the presence of PFOS were due to a negative bias in the analog methodology for hormone measurement,¹⁰³ and that the alternative method of direct equilibrium dialysis followed by radioimmunoassay (ED-RIA) yielded more-reliable results for measuring serum free T4 levels in the presence of PFOS. In discussing this study, Dr. DeWitt stated that "one study is considered insufficient to discount a generally accepted method" (DeWitt, 2017a, p. 46). However, a discrepancy between the analog method and the equilibrium dialysis method has been observed in a number of other studies (Seacat *et al.*, 2002; Luebker *et al.*, 2005b). The Chang *et al.* (2007) study adds to this literature by providing a methodological explanation for the discrepancy.

Dr. DeWitt gave weight to the likelihood of thyroid effects from PFOA and PFOS and, by inference, to other PFCs. I note, however, that, as discussed in Section 6 of this report, there is no indication that the changes in thyroid hormone levels and TSH observed in monkeys or rats exposed to PFOS are of

¹⁰³ Thus, PFOS-associated decreases in serum total T4 and free T4 concentrations in multiple studies without increases in TSH or thyroid gland pathology would be a result of the analytical methodology and not due to PFOS exposure.

sufficient magnitude to be biologically adverse (*i.e.*, to result in hypothyroidism).¹⁰⁴ Moreover, there is no evidence of hypothyroidism in studies where this endpoint was evaluated.

13.1.5 Consideration of Dose

Dr. DeWitt stated in her report that it is inappropriate to compare serum measurements in animals with humans because there are toxicokinetic differences between animals and humans in relation to PFCs, and "serum levels may underestimate exposures" (DeWitt, 2017a, p. 21). This is speculative; Dr. DeWitt did not provide any evidence that PFC exposure is underestimated when serum concentrations are reported. The vast majority of epidemiology studies that evaluated associations of health effects with PFCs are based on serum measurements, as are the US EPA Lifetime HAs for PFOA (US EPA, 2016a) and PFOS (US EPA, 2016b). Furthermore, Dr. DeWitt reported the serum PFOA concentrations of the mice in her own study and compared them to concentrations in human populations (DeWitt *et al.*, 2008).

Throughout her report, Dr. DeWitt did not compare the doses that elicited health effects observed in animal studies of PFCs to the possible PFC exposures of the residents of southern Washington County. Had she conducted this analysis, Dr. DeWitt would have found that the southern Washington County residents' PFOA and PFOS exposures, as reflected in the biomonitoring results for 2008, 2010, and 2014 (MDH, 2008-2014), did not put them at risk of the effects observed in animals (as discussed in Section 10). Dr. DeWitt also overstated the risk to humans from exposure to PFHxS, PFBA, and PFBS. As discussed in Sections 8 and 10, the LOELs for animal studies of these PFCs are higher than those observed in those of PFOA and PFOS, and the potential exposures of the residents of southern Washington County to these PFCs fall far below any that might put them at risk of health effects.

I also note that Dr. DeWitt stated that the PFOS maternal serum concentrations measured in the human study by Fei *et al.* (2008a) (described by Dr. DeWitt as 35 mg/L) were higher than the PFOS serum concentrations at the HED calculated by US EPA from the Butenhoff *et al.* (2009a) rat study (10.87 mg/L) (DeWitt, 2017a, p. 35). This is incorrect. The PFOS serum concentrations in the Fei *et al.* (2008a) study are presented in µg/L, rather than mg/L, and thus are more than 300-fold lower than the HED serum concentrations of PFOS calculated by US EPA.

Furthermore, in her deposition (DeWitt, 2017b, pp. 242-245), Dr. DeWitt overestimated the potency of PFOS and PFOA in the rat studies of neurodevelopmental toxicity and carcinogenicity, as reflected in her characterization of the HED. Specifically, she estimated that the HED for the Butenhoff *et al.* (2009a) neurotoxicity study of PFOS was 0.00003 mg/kg-day (DeWitt, 2017b, line 20, p. 244),¹⁰⁵ when in fact US EPA (2016b) calculated an HED for this study of 0.00084 mg/kg-day, a 28-fold difference. Dr. DeWitt then stated that, in the Butenhoff *et al.* (2012b) PFOA cancer study, exposure of rats to 300 ppm PFOA in feed yielded an HED of 0.03 mg/kg (DeWitt, 2017b, lines 9-10, p. 245). This is incorrect. While US EPA (2016a) did not use a carcinogenic endpoint in developing its RfD for PFOA, it did calculate an HED of 0.58 mg/kg-day for the Butenhoff *et al.* (2012b) study, a 20-fold difference from Dr. DeWitt's calculation.

¹⁰⁴ The NRC (2005) considers hypothyroidism to be the first biologically adverse effect associated with exposure to an agent that affects thyroid hormone levels.

¹⁰⁵ While Dr. DeWitt states PFOA on line 15, page 244, it is clear from the preceding discussion on page 243 that here she is discussing PFOS.

13.1.6 Consideration of Causation

Dr. DeWitt did not carefully consider the difference between correlation and causation, as exemplified in her deposition (DeWitt, 2017b, pp. 131-132) exchange regarding cottage cheese and obesity. Dr. DeWitt reached the conclusion in this example that the cottage cheese was causing obesity but did not consider the more plausible explanation that overweight people might introduce cottage cheese into their diet as an attempt to lose weight. Similarly, Dr. DeWitt did not consider in her expert report (DeWitt, 2017a) or in her deposition whether the health effects that were reported to be associated with PFCs in humans were caused by the PFC exposure.

13.1.7 Consideration of Risk Assessment

Dr. DeWitt's discussions of risk assessment were overstated, unclear, and occasionally factually incorrect. In her report (DeWitt, 2017a) and her deposition (DeWitt, 2017b), Dr. DeWitt mischaracterized and overstated the conclusions of IARC (2016a) and ATSDR (2015) regarding the strength of PFC associations with health effects, as noted in this section. In her deposition, Dr. DeWitt frequently referred to a "more likely than not" probability that PFC exposure leads to adverse health outcomes (*e.g.*, DeWitt, 2017b, pp. 134, 144). As noted in Section 7.1.5 of this report, the "more likely than not" designation in a legal context is not the same as a statistically significant association in a scientific context and would not be acceptable in the scientific literature as evidence of an association.

In her deposition, Dr. DeWitt's statement regarding slope factors is not consistent with the definition of a slope factor. She stated, "[i]f a cancer slope factor is greater than 1 in a million... then that would be a high probability of cancer being an adverse health outcome" (DeWitt, 2017b, p. 98). A CSF is the upper-bound proportion of a population that would be affected from lifetime exposure per each mg/kg-day of exposure (US EPA, 2011c), not an individual or population risk *per se*, although it can be used to calculate an individual or a population risk (such as 1 in 1 million). Furthermore, Dr. DeWitt's implication that a cancer risk greater than 1 in 1 million indicates a high probability of cancer is incorrect. A risk of 1 in 1 million is vanishingly small and is on the low end of US EPA's range of acceptable risk. The upper end of US EPA's acceptable risk range is 1 in 1,000 (US EPA, 2015).

13.1.8 Drinking Water Guidance

Dr. DeWitt described the methodology that US EPA (2016a,b) and MDH (2011a,b, 2017b,c) used to derive their PFC drinking water guidance values (DeWitt, 2017a, pp. 56-60), but she did not conduct a critical evaluation of the studies that provided the bases of those values. In particular, Dr. DeWitt did not evaluate the results of the Lau *et al.* (2006) and Luebker *et al.* (2005a) studies that provided the bases for the PFOA and PFOS RfDs used by both agencies in setting their guidance values for these PFCs. The lack of robustness in the developmental endpoints in the Lau *et al.* (2006) study that were chosen by US EPA and MDH as the basis for their PFOA guidance value was already discussed in this section as well as in Sections 6.1.1 and 8.1. For PFOS, both agencies chose the Luebker *et al.* (2005a) study as the basis of their guidance values. Both agencies chose reduced weight gain in the F2 pups as the critical effect. This endpoint also lacks robustness (*i.e.*, the effect was transient and may have been related to litter size or random effects of culling), as discussed in Sections 6.2.1 and 8.2.

It should also be noted that the draft versions of the US EPA Lifetime HA guidance for PFOA (US EPA, 2014e) and PFOS (US EPA, 2014f), released in 2014, were based on different critical effects for both chemicals. US EPA released the final 2016 guidance documents (US EPA, 2016a,b), based on new critical effects, without an additional comment period, so there was no opportunity for the public to

comment on the new choices of studies and critical effects on which the final Lifetime HAs were based. In her report (DeWitt, 2017a, pp. 32-35), Dr. DeWitt incorrectly referred to US EPA's 2014 draft PFOS RfD, and the study (Butenhoff *et al.*, 2009a) and critical effect (neurodevelopment) on which it was based, as the final US EPA PFOS guidance.

Dr. DeWitt also did not perform a rigorous analysis of MDH's methodology for the derivation of its RfDs and HBVs for the PFCs at issue in this matter. She did not evaluate each individual parameter that MDH included in its guidance value derivations. As I note elsewhere in this report (Section 8), the RfDs derived by MDH for the PFCs at issue in this matter could be higher and still be health-protective.

Dr. DeWitt's statement that "[c]oncentrations in drinking water above the HRLs were believed to pose human health risks" (DeWitt, 2017a, p. 57) is misleading. The definition of an HRL is "the concentration of a groundwater contaminant, or a mixture of contaminants, that can be consumed with little or no risk to health and which has been promulgated under rule" (MDH, 2017d). This does not mean that any concentration of a chemical above its corresponding HRL poses a risk. US EPA makes this statement regarding RfDs, which can be applied to guidance values in general: "It should be noted that exposures above an RfD... do not necessarily imply unacceptable risk or that adverse health effects are expected" (US EPA, 2000a). Moreover, Dr. DeWitt has conducted no reliable exposure analysis for residents of southern Washington County, and thus, her conclusions regarding potential health risks are not supported.

13.1.9 State of Knowledge

Dr. DeWitt (2017a, p. 19) presented "a brief chronology of a few of the numerous toxicological studies on PFCs performed by 3M or their subcontractors." Dr. DeWitt inferred that 3M was aware of a number of toxicological issues with PFCs at times when it continued to manufacture them. Using some of the studies DeWitt presented as examples, however, it appears that Dr. DeWitt took the findings of these studies out of context, and they do not support Dr. DeWitt's inferences regarding actions by 3M or its contractors over time.

1963 FC-95 LD₅₀. Dr. DeWitt (2017a, p. 20) described that in 1963, 3M had determined that FC-95 was as acutely toxic as phenobarbital and morphine (based on LD₅₀ values of approximately 100-1,000 mg/kg). This statement was presumably meant to show that PFOS toxicity was known to be on par with that of other foreboding-sounding chemicals. The LD₅₀, however, is a blunt measure of toxicity. For perspective, the LD₅₀ range presented by Dr. DeWitt is also consistent with those of the more benign-sounding chemicals caffeine and aspirin.

1978 Monkey Study. Dr. DeWitt (2017a, p. 20) stated that in 1978, 3M concluded that FC-95 was considerably more toxic to monkeys than anticipated because all of the dosed monkeys died in a 90-day subchronic toxicity study. In this study (Goldenthal *et al.*, 1979), monkeys were administered doses of 0, 10, 30, 100, or 300 mg/kg-day FC-95 by gavage. This method of administration is *via* a tube inserted into the esophagus, which results in the animals receiving all of a daily dose of a chemical at once, rather than the dose being spread out throughout the day, as would occur with occupational and environmental exposures. Clearly, the doses selected for this study were too high for the determination of dose-response data for milder toxicity. Any substance can cause toxicity if administered at sufficiently high doses (particularly with gavage administration). 3M initiated a second monkey study the same year (Goldenthal *et al.*, 1978b) with more appropriate doses (0, 0.5, 1.5, or 4.5 mg/kg-day) for the evaluation of the dose-response for sub-lethal effects of FC-95. The findings from these and other contemporaneous animal studies served to alert 3M to the need to measure and examine parameters that would inform whether similar effects were occurring in their workers. Notably, indications of adverse effects were not observed, indicating to 3M that the adverse effects observed in the animal studies were excessive relative to the

exposures experienced by 3M employees and/or because there were important differences between the responses of animals and humans to PFOS exposure.

1980-1983 PFC Teratology. Dr. DeWitt (2017a, p. 20) stated that 3M conducted several teratology studies of PFCs in rats between 1980-1983 and that these studies suggested that PFCs induced lens abnormalities and other birth defects in rat fetuses. She also concluded here and later in her report (DeWitt, 2017a, pp. 36-38) that the lens abnormalities, which 3M suggested may have been caused by a sectioning artifact, should not have been dismissed. With regard to PFOA and PFOS, these studies were unremarkable when statistical significance and maternal effects are taken into consideration (*i.e.*, structural abnormalities or other developmental effects were found in the highest dose groups, in which significant reduction of weight and food consumption were also observed in the pregnant dams). Considering maternal effects is important, because at doses that are maternally toxic, one cannot tell whether the chemical is acting specifically on the fetus or affecting maternal health to such an extent that fetal health is indirectly compromised. Thus, teratogenic agents (as a form of developmental toxicity) are typically considered to be those that exert a selective or greater toxicity to the developing organism relative to the adult (Lewandowski, 2015). The relevant studies involving PFOA and PFOS (or in the form of a product) in the timeframe of 1980-1983, or just after, are described below.

Regarding PFOA, Riker Laboratories, Inc. [Gortner] (1981) dosed rats with 0, 0.05, 1.5, 5, or 150 mg/kg-day PFOA. Maternal toxicity was seen in the high dose group in the form of significant reductions in body weight, as well as ataxia and death in some dams. There were no dose-related fetal effects; PFOA was not teratogenic in this study. A fetal lens finding was observed to occur in individual fetuses of all dose groups, including the control group. It was interpreted histopathologically as either a freehand sectioning artifact or a normal area of primary lens fiber degeneration. Riker Laboratories, Inc. [Gortner] (1982) dosed pregnant rabbits with 0, 1.5, 5, or 50 mg/kg-day PFOA in drinking water. Dams in the 50 mg/kg-day dose group lost significantly more weight than the control group. There were some small effects on rib development in the 5 mg/kg-day dose group, but these findings were not considered to be malformations. PFOA was not teratogenic in this study. Finally, Staples *et al.* (1984) of DuPont conducted a developmental toxicity study of PFOA (in the form of APFO) with both inhalation and oral exposure routes. Rats were exposed *via* inhalation to 0, 0.1, 1, 10, and 25 mg/m³ APFO for 6 hours per day. In the oral portion of the study, rats were administered 0 and 100 mg/kg-day APFO by gavage. A teratogenic response from APFO was not demonstrated in either portion of the study, despite maternal toxicity. The types of lens changes previously reported were detected in several fetuses, but were determined not to be related to APFO administration because they occurred at similar incidences among all groups, including the control group. Lens clefts were determined to be postmortem artifacts that were caused by cutting through the center of the eyes. US EPA (2003b) also dismissed the lens abnormalities in this study as an artifact of the tissue-preparation technique.

Regarding PFOS, Gortner (1980) administered FC-95 orally to pregnant rats at 0, 1, 5, and 10 mg/kg-day. The proportion of fetuses with teratogenic changes in the lens of the eye was significantly higher than in the control group at the 10 mg/kg-day dose level, at which maternal toxicity (decrease in body weight) also occurred. With regard to the lens abnormalities, Gortner (1980) explicitly stated that the effect occurred at all dose levels, except the controls, but was only statistically significant at the high dose, at which there was maternal toxicity. In a later study that was similar in design to the Gortner (1980) study, Wetzel *et al.* (1983) administered T-3351 (identified elsewhere as PFOS) to mated female rats by oral intubation at 0, 1, 5, or 10 mg/kg-day. The doses of 5 and 10 mg/kg-day resulted in maternal toxicity, including death in 2 of 25 dams dosed with 10 mg/kg-day T-3351, and significant body weight and food consumption reductions. Fetotoxicity and an increased incidence of external visceral anomalies and skeletal variants occurred in fetuses from the 10 mg/kg-day dose group dams, but no apparent teratogenicity occurred at dose levels ≤ 5 mg/kg-day. Thus, despite being of similar design to the Gortner

(1980) study, OECD (2002) noted that this study did not find lens abnormalities even in the presence of maternal toxicity.

In my opinion, the authors' interpretations of the lens abnormalities in each of the above studies is consistent with standard practice for interpreting developmental studies. The authors considered the presence of the effect in the control group and statistical significance in comparing dose groups with the control group, as well as whether maternal toxicity was present when statistically significant findings were found. These considerations would be appropriate even without the explanation of a sectioning artifact. Furthermore, in my review of later developmental studies of PFOA (see Section 6.1.1.5) and PFOS (see Section 6.2.1.5) exposure, I am not aware of any significant findings of lens abnormalities or any other teratological effects from exposure to PFOA and PFOS (*e.g.*, Thibodeaux *et al.*, 2003; Butenhoff *et al.*, 2004b). Although Dr. DeWitt may have disputed the sectioning artifact as an interpretation of the lens abnormality finding, the results of the above studies stand alone in supporting that lens abnormalities are not a direct effect of exposure to PFOA and PFOS.

Dr. DeWitt (2017a, p. 55) concluded that published studies of the legacy PFCs PFOA and PFOS did not dramatically increase in number until the 2000s, sometimes based on studies performed in the 1980s and 1990s. She stated, "[t]herefore, the 3M Company had data about the toxicity of legacy compounds that may have been shared with regulators, but was not part of the publicly available peer-reviewed literature that is vitally important for the advancement of scientific knowledge and for regulators and policy makers at the local and state level" (DeWitt, 2017a, p. 55). Of relevance here is that the increase in publications on PFCs in the 2000s coincided with the discovery that PFOA and PFOS were present and widely distributed in the blood of the general population and wildlife (*e.g.*, Giesy and Kannan, 2001). Whereas PFOA and PFOS exposure had largely been considered a worker issue, of primary interest to 3M internally, the discovery of a greatly expanded exposed population led to increased interest from both 3M and the greater scientific community in understanding the toxicity of these compounds. This may also explain why studies of PFCs performed in earlier years, which evolved to carry broader relevance, but not published, were prepared for publication years later.

13.1.10 Appendix B: Estimating "Safe" PFOS Doses

Dr. DeWitt (2017a) presented a rough calculation made by Dr. John Butenhoff (3M, c. 1998) regarding a "safe" dose of PFOS and compared it to the US EPA (2016d) Lifetime HA for PFOS and the MDH (2017c) HBV for PFOS. Dr. Butenhoff's approach was developed *circa* 1998, prior to the availability of more relevant information, and is superseded by subsequent analyses using updated methodology. Therefore Dr. DeWitt's comparison of Dr. Butenhoff's calculation of a "safe" dose of PFOS to current PFOS guidance values is irrelevant.

13.2 Comments on Dr. Grandjean's Opinions

Dr. Grandjean's overall conclusion, as reflected in his expert report (Grandjean, 2017a) and reiterated at his deposition (Grandjean, 2017b), is that PFCs pose a substantial present and potential hazard to human health, stating that there are "convincing associations" between PFOA and PFOS and multiple health outcomes and that, although less studied, human and animal evidence of PFBA suggests it is also a substantial present and potential hazard to human health (Grandjean, 2017a, p. 4). Note, however, that Dr. Grandjean did not discuss any epidemiological studies of PFBA. Dr. Grandjean also indicated that more protective drinking water limits for PFCs than those currently in place are justified.

There are several overarching issues that reduce confidence in Dr. Grandjean's final conclusions. First, and perhaps most importantly, Dr. Grandjean often overstated the consistency and strength of the evidence for PFC toxicity. He selectively chose positive results in some cases and, in general, downplayed null associations. Second, Dr. Grandjean often did not discuss the clinical relevance of findings from the studies he reviewed. In certain cases, when positive effects have been observed for PFCs, the clinical relevance of those effects is not established, and thus, such findings do not provide a reliable basis for conclusions regarding the hazard presented by PFCs.

Further, in several cases, Dr. Grandjean relied heavily on other agency and panel reviews of the toxicity of PFCs, without performing a critical analysis of the materials. For example, with regard to the findings of studies by the C8 Science Panel, as noted in Section 7.1.5 of this report, the Panel was asked to draw conclusions about whether or not there were likely associations between PFC exposures and adverse health effects in the context of a specific legal case, and the Panel's definition of a "probable link" between a PFC exposure and an adverse effect was that there was a >50% chance that the association was not random (*versus* the typical 95% probability of a non-random association used in scientific literature) (C8 Science Panel, 2017).

Finally, Dr. Grandjean consistently drew very broad conclusions about toxicity across numerous PFCs (mainly, PFOA and PFOS, and to a lesser degree, PFHxS and PFBA), even within sections of his report in which he only discusses the evidence available for a single compound.

In the following sections, I discuss the health endpoints on which Dr. Grandjean focused most heavily, specifically: immunotoxicity, reproductive and developmental toxicity, and cancer. Within each endpoint, as appropriate, I provide examples, integrating epidemiological and toxicological evidence, to support my opinion that Dr. Grandjean's overall conclusion that PFCs pose a substantial present and potential future hazard to humans is not consistent with a balanced analysis of the available studies of these compounds.

13.2.1 Immunotoxicity

Epidemiological Evidence

Dr. Grandjean devoted a large part of his report to the potential for immunotoxicity from exposure to PFCs. He indicated that the immune system is a sensitive target for PFC toxicity, or "perhaps the most sensitive" (Grandjean, 2017a, p. 39). Further, he noted that exposure levels similar to or below those reported in the East Metro Minnesota area (referred to in this report as southern Washington County) are associated with immunotoxicity.

Dr. Grandjean focused heavily on the studies of childhood vaccine response, specifically, noting, "[s]uch effects are linked to an increased occurrence of infectious diseases" (Grandjean, 2017a, p. 40). As discussed below, however, Dr. Grandjean overstated the strength and consistency of the available evidence and thus did not sufficiently support his conclusions that there is "convincing" evidence of an association between PFCs and immune effects (Grandjean, 2017a, p. 4).

With regard to Dr. Grandjean's studies of PFCs and serum vaccine antibodies in the Faroese population, as noted in Sections 7.1.4 and 7.2.4 of this report, Grandjean *et al.* (2012) reported that maternal and child serum PFOA concentrations (at age 7) were statistically significantly associated with decreases in childhood anti-diphtheria antibody concentrations, and a subanalysis remained significant for an association with decreases below the clinically protective level (according to the authors) of diphtheria and tetanus antibodies (*i.e.*, 0.1 IU/mL). Note, however, that the serum diphtheria antibody titer levels

were within the range of what the CDC would consider an indication of at least some immunity (*i.e.*, >0.01 IU/mL) (Tiwari, 2011). No significant decreases in either antibody concentration were found after PFOS exposure.

In a follow-up analysis of the Grandjean *et al.* (2012) cohort, Grandjean *et al.* (2017) investigated the associations observed in the original study subjects through age 13. In the overall analyses, anti-diphtheria antibodies at age 13 decreased slightly with increasing PFOA and PFOS exposure at age 7 or 13, but the associations were not statistically significant. The results of the subgroup analyses, based on whether the child had received a diphtheria vaccine booster, whether the child had had an emergency room visit, and anti-diphtheria antibody status, were inconsistent. For example, the analysis of children that received no booster or had an emergency room visit showed a significant association between a doubling of maternal PFOA serum concentration and lowered anti-diphtheria antibody concentrations (measured in both analyses at 13 years of age), but in the same analysis of 13-year old children who received no booster, had no emergency room visit, and had no antibody increase, there was no significant association between maternal PFOA serum concentration and anti-diphtheria antibody concentration. While anti-tetanus antibodies *increased* with increasing PFOA exposure in the same study, these results were not statistically significant.

Dr. Grandjean focused heavily on the issue of clinical significance, and suggested that the children in these studies had no long-term immunity to the diseases they had been vaccinated for (Grandjean, 2017a). However, he did not discuss that, while some of the results of his group's studies were clinically relevant, the children in these studies still retained some immunity against these diseases. Further, he did not discuss how, in his follow-up analysis of the children after they had reached 13 years of age, the results were attenuated and generally not statistically significant. Further, the results of the subgroup analyses Dr. Grandjean and his coauthors performed were inconsistent and indicated that children between ages 7 and 13 were likely to have a sufficient antibody response to the diseases they had been vaccinated for, regardless of PFOA exposure. The results for PFOS in these two studies were similar, particularly the lack of statistical significance in the follow-up study and the lack of consistency across subgroup analyses by booster and emergency room visit status.

Dr. Grandjean also referenced Granum *et al.* (2013), which measured vaccine antibody levels and incidence of common infectious disease in a sub-cohort of 99 mother-child pairs in the Norwegian Mother and Child Cohort Study. Maternal serum PFOS concentration was statistically significantly negatively associated with rubella anti-vaccine antibody levels ($p = 0.007$), but not measles, *Haemophilus influenzae* type b, or tetanus antibody levels. Maternal PFOS exposure was also not associated with incidence of common cold or gastroenteritis episodes.

The Grandjean *et al.* (2012, 2017) and Granum *et al.* (2013) studies did not follow children to determine whether the lowered antibody levels led to a higher risk of the associated disease later in life. Perhaps most importantly, there was no consistently decreased response to vaccines observed within or across these studies.

With regard to other immune endpoints, particularly atopic and infectious disease, the evidence is inconsistent, but predominantly negative. Overall, Dr. Grandjean overstated the evidence for an association between PFC exposure and the endpoints and, in some cases, did not discuss the full set of findings in the study. For example, when discussing Goudarzi *et al.* (2017), a study from the Hokkaido Study on Environment and Children's Health investigating doctor-diagnosed childhood infectious disease, Dr. Grandjean stated that these authors "reported higher incidence rates at elevated prenatal exposures to PFOS and PFHxS" (Grandjean, 2017a, p. 43). While there were statistically significant, slight increases in all four of the infectious diseases studied in this population, this was only apparent in the second and fourth quartiles of PFC exposure (and not in the third). For PFHxS, the results for all children were not

statistically significant, and there was an apparent inverse exposure-response relationship. The results were only statistically significant in the female-only analysis, and even then, there was no exposure-response, with the lowest odds of disease in the highest quartile of exposure. The lack of an exposure-response relationship weakens the confidence in the likelihood of there being an association between PFHxS exposure and increased incidences of infectious diseases.

When discussing the study by Looker *et al.* (2014), Dr. Grandjean stated: "A study carried out in connection with the C8 studies encompassed adults, whose serum samples were analyzed before and about three weeks after flu (A/H3N2) vaccination [125]. Thus, the elevated serum-PFOA concentrations were associated with a weakened vaccine antibody response also in adults" (Grandjean, 2017a, p. 42). In fact, Looker *et al.* (2014) indicated that while there were slight PFOA-associated decreases in influenza type A/H1N1 post-vaccination titers (but not in influenza type A/H3N2 or influenza type B post-vaccination titers, and there were no associations between any of these and PFOS exposure), the changes were not associated with increased self-reported cold or flu, for either PFOA or PFOS.

Dr. Grandjean devoted a large section of discussion in his expert report to the results of an investigation of Chemolite employees conducted as part of Dr. Frank Gilliland's dissertation. While several publications came out of these investigations, the results for the immune endpoints (serum lymphocyte counts) were not published. Dr. Grandjean contended that these results are important and consistent with other findings about immune endpoints and PFC exposure and presumably should have been published. This inference is not consistent with a full reading of the study. For example, Drs. Gilliland and Mandel stated in the results section that, despite the significant correlation between total serum fluorine and peripheral blood lymphocytes, "the relationship was complex, with significant interactions between total serum fluorine and BMI, cigarette use, and alcohol use" (Gilliland and Mandel, 1993b). Drs. Gilliland and Mandel also noted that the magnitude of associations was not clinically significant from an infectious disease perspective (Gilliland and Mandel, 1993b).

Overall, Dr. Grandjean overstated the strength and consistency of the available evidence for an association between PFCs and immune effects, and did not sufficiently support his claims that there is "convincing" evidence of an association between PFCs and immune effects (Grandjean, 2017a, p. 4). A full weight-of-evidence analysis, as presented in Section 7 of this report, indicates there is no reliable association between PFOA and PFOS exposure and immune effects. In particular, there is little biological plausibility for these PFCs causing decreases in antibody titers to some vaccines, but not others (or for these PFCs causing increases in some antibody titers). Further, studies of PFC exposure and physician-diagnosed infectious diseases generally found weak or no association between PFOS and PFOA exposure and risk of the infectious diseases studied.

Toxicological Evidence

Dr. Grandjean overstated the toxicological evidence of PFCs' effects on the immune system. He also did not address the inconsistencies across studies. He relied on the NTP (2016) review of immunotoxicity of PFOA and PFOS to support his conclusions. The NTP (2016) review has multiple limitations. Specifically, most of the animal studies reviewed in the NTP evaluation that reported PFOA or PFOS dose-dependent immune effects also reported systemic toxicity at the same doses (see, for example, DeWitt *et al.*, 2009, 2016; Dong *et al.*, 2009, 2011; Zheng *et al.*, 2009). The only animal study that reported a PFOS dose-dependent reduction in immune response (Peden-Adams *et al.*, 2008) has not, to my knowledge, been replicated in publications by this or other research groups. Thus, while the NTP (2016) review is comprehensive in its coverage of the immunotoxicology of PFOA and PFOS, much of the animal evidence upon which it relied does not constitute reliable evidence of the immunotoxic effects of PFOA and PFOS. These limitations are also discussed in Gradient's comments submitted to NTP in response to NTP's draft systematic review of PFOA and PFOS immunotoxicity studies (Gradient, 2016).

13.2.2 Reproductive and Developmental Outcomes

Epidemiological Evidence

Dr. Grandjean concluded that, based on the epidemiological evidence and supporting toxicity evidence, PFCs pose a risk of reproductive and developmental effects. In contrast, many of the specific conclusions contained in his report are not consistent with such a strong overall conclusion about the reproductive and developmental toxicity of PFCs. As noted below, Dr. Grandjean stated in his expert report that these outcomes "may not be highly sensitive targets of PFC toxicity," and that PFCs may induce only "minor effects" at concentrations associated with those at issue in this case (Grandjean, 2017a, p. 54). In addition, while Dr. Grandjean grouped PFCs when making his overall causal conclusions, at times, he presented only studies of a single chemical for a specific endpoint. More generally, he reported predominantly positive results, and did not acknowledge inconsistencies within and across studies.

Dr. Grandjean stated, "only a small number of published articles relate to developmental exposure and vulnerable subgroups such as pregnant women and children" (Grandjean, 2017a, p. 26). This is not correct. There are several large pregnancy and birth cohort studies of PFC exposure (e.g., the Danish National Birth Cohort), as well as smaller cohorts, that have assessed an array of outcomes, including infertility, miscarriage, birth weight, and post-natal growth and development (see Sections 7.1.4 and 7.2.4.1 of this report). As discussed below, in general, these studies show weak or null associations between PFCs and the investigated outcomes.

Reproductive Effects. In his discussion of female fertility and PFC exposure, Dr. Grandjean cites Fei *et al.* (2009), a study of a Danish population, that reported significant associations between PFOS exposure and TTP. He did not, however, discuss the re-analysis of the cohort of women studied in Fei *et al.* (2009) conducted by Bach *et al.* (2015), which found no association between PFOS exposure and decreased fecundability. Dr. Grandjean also cited Velez *et al.* (2015), which found some associations between PFOA and PFHxS exposure and ability to conceive. He did not, however, report that the results for PFOS from this study were null.

Dr. Grandjean left out at least one available study whose results were null (Jorgensen *et al.*, 2014), which reported no significant increase in the odds of infertility and no significant decrease in fecundability as a result of PFC exposure in the INUENDO Cohort. In a subset analysis of male partners of the women studied (n = 401), there was also no association between male partner serum PFOS concentration and prolonged TTP. Overall, as noted in Section 7.2.4 of this report and supported by a systematic review by Bach *et al.* (2016b), there is little evidence that PFCs have an effect on either male or female fertility, and the few associations reported in parous women are likely not causal.

With regard to male reproductive effects, Dr. Grandjean cited several studies that reported associations between increasing serum PFOA and PFOS concentrations and lowered sperm concentrations and counts. He also noted that despite inconsistencies, "subtle associations between higher PFOS and lower testosterone or abnormal sperm morphology have been found in some of the studies and cannot be ignored" (Grandjean, 2017a, p. 52). As noted in Section 7.2.4 of this report, however, there is little evidence of an association between PFOS exposure and markers of male fertility. Most studies measuring male hormones found no changes outside of the range of normal, indicating the declines may not be associated with clinically significant effects. Further, as noted by Dr. Grandjean, findings regarding sperm morphology have been inconsistent, with some finding *inverse* associations between PFOS and adverse morphological effects.

In his discussion of early onset puberty and menopause (Grandjean, 2017a, p. 51), Dr. Grandjean did not discuss the potential for reverse causation on account of the pharmacokinetics of PFOS and PFOA (*i.e.*, the possibility that the observed effects may have been responsible for the differences in serum PFC concentrations). For example, because menstrual blood is one route of removal of PFOA and PFOS from the body, girls with an earlier first menarche would have lower serum PFOA and PFOS concentrations than those with later first menarche (Wong *et al.*, 2014; Wu *et al.*, 2015). Similarly, women who reach menopause at a later age would be expected to have lower serum concentrations of PFOA and PFOS relative to those reaching menopause at an earlier age. Further, Dr. Grandjean did not discuss at least one important recent study of this endpoint, which showed no significant associations between either PFOA or PFOS and earlier age at menopause in women enrolled in the C8 Cohort (Dhingra *et al.*, 2016).

When evaluating the evidence of birth and growth alterations, Dr. Grandjean discussed several of the many inconsistent studies (also discussed in Section 7 of this report), and while he highlighted some of these studies' positive results, he also noted, that the association between PFC exposure and decreased birth weight "can be affected by distribution factors, rather than toxicity, and dietary intakes of, e.g., n-3 fatty acids play an important role and is difficult to control for [184] in observational population studies" (Grandjean, 2017a, p. 56).

Dr. Grandjean's final conclusion was that fertility and pregnancy outcomes may not be highly sensitive targets of PFC toxicity, and that the evidence suggests that only "minor" adverse effects can occur at "elevated levels of background exposure" to PFCs (Grandjean, 2017a, p. 54). However, in the following paragraph, Dr. Grandjean stated that the evidence shows that "associations between PFC exposure and adverse effects on human reproductive functions, in particular risks of adverse effects on fetal growth" (Grandjean, 2017a, p. 55). This statement is inconsistent with the statements made in the preceding paragraphs of his report.

Developmental Toxicity and Birth Defects. In his discussion of the possibility of developmental abnormalities as a result of PFCs exposure, Dr. Grandjean cited several DuPont internal documents that reported cases of eye and tear duct birth defects in three children of employees at the DuPont Washington Works Plant and the Shimizu Corporation in Japan (Grandjean, 2017a, p. 55). He provided no discussion of epidemiological studies investigating potential birth defects from exposure to PFCs. As discussed in Section 7 of this report, however, there is very little evidence of an association between PFCs exposure and birth defects. Case reports are generally used for hypothesis generation, and it is unusual to place heavy weight on such reports, particularly when the cases observed are present in small numbers and in the presence of more reliable, formal observational studies of a chemical exposure (Hennekens and Buring, 1987).

While I did not explicitly discuss the association between birth defects and PFOA exposure in this report, I evaluated birth defects in relation to PFOS exposure. I identified three studies of this association, none of which found increases in developmental abnormalities in children of exposed mothers, including those enrolled in the C8 Cohort. I reviewed these studies again, and the two that also included PFOA exposure also found no significant associations between PFOA and the studied birth outcomes (Stein *et al.*, 2009; Vesterholm Jensen *et al.*, 2014). I also identified (albeit without a formal literature search) at least two other studies showing a lack of association between PFOA and congenital anomalies, including in the C8 Cohort (Nolan *et al.*, 2010; Savitz *et al.*, 2012a).

Dr. Grandjean discusses neurobehavioral effects and PFCs exposure in Section VII.F of his expert report (see Grandjean, 2017a, p. 68). There is very little evidence of an association between PFCs and neurobehavioral effects. As noted in Section 7.2.4 of this report, at least six studies showed no associations between PFOS exposure and neurobehavioral effects, including motor skills and ADHD (Fei *et al.*, 2008b; Ode *et al.*, 2014; Strom *et al.*, 2014; Hoyer *et al.*, 2015; Liew *et al.*, 2015; Quaak *et al.*,

2016). These studies were predominantly prospective cohort studies, whereas some of the studies with positive findings cited by Dr. Grandjean (*e.g.*, Hoffman *et al.*, 2010; Gump *et al.*, 2011) were cross-sectional in design, and thus, cannot inform the likelihood of a causal relationship between PFCs exposure and neurobehavioral effects.

Endocrine Effects. Dr. Grandjean indicated that PFCs, at exposure levels similar to or below those reported for the East Metro Minnesota area, pose a substantial "present and potential hazard to human endocrine functions" (Grandjean, 2017a, p. 57). Dr. Grandjean stated that endocrine-disrupting effects are typically defined as those causing *adverse* effects that have an endocrine MoA (*i.e.*, affects the function of the endocrine system). It is critical to note that this definition contains the word "adverse" – *i.e.*, harmful – indicating that endocrine disruption requires not only a change in the endocrine system, but one that is associated with an apical, functional outcome, such as impaired reproductive function (NAS, 2017). However, in this section of his expert report, Dr. Grandjean discussed studies that reported small changes in reproductive hormones that are of an unknown clinical significance. These changes were often in the range of normal (*i.e.*, subclinical) (for example, those reported by Tsai *et al.*, 2015).

With regard to duration of breastfeeding, the weight of evidence does not establish an association between PFCs and this effect. There are very few studies available, and the mechanism whereby PFCs could affect breastfeeding duration is unknown and may not necessarily be hormonally mediated (Romano *et al.*, 2016).

Toxicological Evidence

In Section VII.B.2 of his expert report, Dr. Grandjean (2017a, pp. 56-57) discussed an experimental study conducted by 3M in 1981 (presumably, Riker Laboratories, Inc. [Gortner], 1981) that showed birth defects in the eye lens of rats exposed to PFOA. He stated that eye lens changes were observed in two other studies (specific studies not stated, but perhaps Gortner, 1980 and Staples *et al.*, 1984) and that these effects have been interpreted by the authors and others to be a sectioning artifact. Dr. Grandjean states that this explanation is not satisfactory to him. However, when interpreting these studies, the authors considered the presence of the effect in the control group and statistical significance in comparing dose groups with the control group, as well as whether maternal toxicity was present when the findings were statistically significant. These considerations, which suggest that PFOA and PFOS did not exert a direct teratological effect, are consistent with standard practice for interpreting developmental studies and would be appropriate even without the interpretation of a sectioning artifact.¹⁰⁶ US EPA (2003b) also dismissed the lens abnormalities in this study as an artifact of the tissue-preparation technique. Furthermore, in my review of later developmental studies of PFOA (see Section 6.1.1.5), I am not aware of any significant findings of lens abnormalities or any other teratological effects from exposure to PFOA (*e.g.*, Butenhoff *et al.*, 2004b).

13.2.3 Cancer

Epidemiological Evidence

Dr. Grandjean concluded that, "[b]ased on the available evidence, exposure to PFCs has a substantial potential to cause cancer, most clearly in regard to cancers of the kidneys and the testicles, and highly likely also in regard to prostate cancer and bladder cancer. A possible risk of breast cancer is also of concern" (Grandjean, 2017a, p. 78). He focused on kidney, testicular, and prostate cancers, and while he presents evidence of other cancers, noted that the epidemiological evidence is limited for these other sites

¹⁰⁶ More details on this point are provided in my comments on the expert report of Dr. Dewitt in Section 13.1.7.

(Grandjean, 2017a). In his analysis, Dr. Grandjean also leaned heavily on cancer classifications made by IARC and the C8 Science Panel. As discussed in this report (*e.g.*, see Sections 6.1.2, 7.1.2, and 7.1.5), however, these classifications serve very specific purposes and, in some cases, are not supported by the balanced analysis of the available science. At his deposition (Grandjean, 2017b, pp. 83-84), Dr. Grandjean also referred to work by Dr. Sunding as support for his conclusion regarding the carcinogenic effects of PFCs on individuals residing in Minnesota, in the area in question. In Section 13.3 of this report, I discuss the limitations of Dr. Sunding's analysis, such as the lack of individual-specific exposure information. Because of these limitations, Dr. Sunding's analysis cannot be used as a reliable basis to draw causal inferences (as Dr. Grandjean has used it).

Dr. Grandjean did not conduct an appropriate full, independent weight-of-evidence analysis to reach conclusions regarding the causal associations between PFCs and cancer. Below, I discuss the three cancer types (prostate, kidney, and testicular) upon which Dr. Grandjean places the most weight.

Prostate Cancer. Dr. Grandjean discussed the prostate cancer epidemiology in great detail in several sections of his expert report. He referenced the limited evidence of positive findings found in some early studies (relying heavily on the dissertation of Dr. Frank Gilliland) and suggested that PFC exposure may contribute to the etiology of this cancer type. Although Gilliland and Mandel (1993a) found a statistically significant increase in prostate cancer mortality for the group exposed for 10 years, they found only six prostate cancer cases and urged caution in interpreting these results, due to the low numbers of cases. Note also that when not restricted by years of employment, there was no statistically significant association between PFOA and prostate cancer in all workers or the subset of Chemolite Chemical Division workers in this analysis. This finding agrees with the Gilliland dissertation (Gilliland, 1992), which stated:

Ten years of employment in the CD [Chemical Division] was associated with a significant three fold increase in prostate cancer mortality. There was no association between prostate cancer mortality and employment (ever/never) in the Chemical Division. Given the small number of deaths from prostate cancer in this study and the natural history of the disease, the association between employment in the CD and prostate cancer must be viewed as hypothesis-generating and should not be overinterpreted.

Dr. Grandjean also discussed problems with the control group in studies of cancer mortality in the Decatur plant, stating that:

Film plant workers were used as the control group for these occupational studies, although it was known that they had at least some exposure and therefore constituted a very imperfect control group (see Section V.A.1). According to Alan De Waard, nearly all (95% plus) new employees on the Decatur site started in the chemical plant, and many started in the fluorochemicals drying jobs. (Grandjean, 2017a, p. 29)

However, the evidence suggests that PFC exposures differed substantially between the film plant worker controls and the highly exposed chemical plant workers. Alexander *et al.* (2003) reported that the geometric mean serum PFOS concentration for chemical plant employees was 0.9 ppm, and for film plant employees, it was 0.1 ppm. The authors indicated that the majority of film plant jobs had no direct workplace exposure to fluorochemicals and that their serum concentrations measured in film plant employees were thought to be due, to a large extent, to environmental exposure in proximity to the chemical plant. Further, even though these were not entirely unexposed controls, their exposure was still well below that of the average chemical plant worker, and as such, comparing these two groups still provides valuable information regarding the risk of cancer in more highly exposed workers. Note that

there were insufficient prostate cancer cases for Alexander *et al.* (2003) to calculate risk of this cancer type.

A subsequent analysis of PFOS exposure and prostate cancer risk by Raleigh *et al.* (2014) found no increased risk of either prostate cancer incidence or mortality. In Dr. Raleigh's dissertation (the basis for the published paper), she indicated that while continuous analyses of the entire exposed population showed no statistically significant increase in risk of prostate cancer, there was a non-significant, but apparent, dose-response relationship in prostate cancer mortality in analyses of six time-dependent exposure groups (Raleigh, 2013). She noted, however, that the results showing these apparent increases in prostate cancer were imprecise, based on only a few cases, and "were not beyond chance" (Raleigh, 2013). Lastly, it is critical to note that Raleigh *et al.* (2014) found no associations between PFOA and prostate cancer using a referent population of workers from another 3M plant (tapes and adhesives) that had no occupational PFC exposure. This population would eliminate the issue of the healthy worker effect.

Overall, while there were some isolated findings of increased prostate cancer risk in early occupational mortality studies, more recent analyses with strong methodologies (*e.g.*, Raleigh *et al.*, 2014, which utilized incidence data as well as a worker comparison population) have shown no associations between PFCs and this type of cancer.

Kidney Cancer. Dr. Grandjean's discussion of kidney cancer is very brief, and his analysis relied heavily on the C8 Science Panel and IARC analyses. Dr. Grandjean noted the studies and findings relied upon by the C8 Science Panel, but focused his discussion on two studies that reported exposure-response relationships between PFOA exposure and kidney cancer (Steenland and Woskie, 2012; Vieira *et al.*, 2013).

In fact, however, neither Steenland and Woskie (2012) nor Vieira *et al.* (2013) reported such exposure-response relationships. As noted in Section 7.1.2, Steenland and Woskie (2012) found that kidney cancer incidence was statistically significantly increased in all exposure groups relative to unexposed workers from other plants; however, the CIs included 1.0 in all exposed groups except the fourth quartile ($\geq 2,700$ ppm-years; SMR = 2.66, 95% CI: 1.15-5.24). Further, there were no kidney cancers observed in the third quartile of exposure. The combined SMR for all exposure groups was 1.28 (95% CI: 0.66-2.24). In Vieira *et al.* (2013), a study of cancer outcomes in a community exposed to PFOA from a manufacturing plant, the combined analyses showed no increased risk of kidney cancer across all districts or in any individual district. Only in the analysis assuming 10-year residency and latency was any level of PFOA exposure associated with kidney cancer. In this case, a statistically significant increase in risk (adjusted odds ratio [AOR] = 2.0, 95% CI: 1.3-3.2) was reported only in the "high" exposure group, but the magnitude of risk (AOR = 2.0) was the same and not statistically significant in the "very high" exposure group (Vieira *et al.*, 2013).

Dr. Grandjean did not discuss the results of Raleigh *et al.* (2014), which found no increased risk of developing or dying of kidney cancer in PFOA-exposed workers. Finally, Dr. Grandjean did not include discussion of other non-occupational studies of PFOA exposure and kidney cancer, including Vassiliadou *et al.* (2010), who studied the general population in Greece, and Barry *et al.* (2013), who studied the C8 Cohort and found no statistically significant exposure-response relationships.

Finally, while Dr. Grandjean applied his conclusions regarding cancer risk to both PFOA and PFOS, he did not discuss the literature on PFOS exposure and kidney cancer. While not well studied in non-occupational populations, there are a few occupational cohort studies of mortality (Mandel and Johnson, 1995; Alexander *et al.*, 2003) that showed no increased risk of death from kidney cancer among workers exposed to PFOS.

Overall, Dr. Grandjean did not sufficiently support his assertions that PFOA or any of the other PFCs are associated with an increased risk of kidney cancer. Rather, as discussed in Sections 7.1 and 7.2 of this report, there is little evidence of an increased risk of kidney cancers in workers, communities near point sources, or the general population as a result of exposure to PFCs.

Testicular Cancer. Dr. Grandjean did not discuss details regarding the results of individual studies to support his conclusions regarding PFCs exposure and testicular cancer. He noted that mortality studies are unlikely to identify all cases of testicular cancer, and while this is true, these data are still informative. Instead, Dr. Grandjean relied on two studies of cancer incidence in PFC-exposed communities (Vieira *et al.*, 2013 and Barry *et al.*, 2013).

Dr. Grandjean did not accurately describe the results of Vieira *et al.* (2013). The authors reported no statistically significant risk in testicular cancer incidence in residents living near a DuPont manufacturing facility in analyses across all exposure levels and communities. The only statistically significant result was for the Little Hocking Water District (AOR = 5.1, 95% CI: 1.6-15.6); the CI was wide, however, indicating a lack of precision. When the authors then analyzed PFOA serum concentration exposure categories assuming a 10-year residency and latency, there were no clear exposure-response patterns; in fact, the association was inverse in the lower exposure groups. The authors also noted that they were unable to adjust for other important risk factors for testicular cancer, including prenatal exposure to estrogens, which may have confounded results.

Barry *et al.* (2013) also suggested a possible exposure-response relationship between PFOA exposure and testicular cancer. However, as noted in Section 7.1.4 of this report, the CIs were wide and all overlapped "1." An important limitation of both of these studies is the potential for exposure misclassification (*i.e.*, error in the exposure estimates) based on modeled, rather than measured, serum PFOA concentrations.

With regard to occupational studies, several studies have shown no associations between PFOA exposure and testicular cancer (Gilliland and Mandel, 1993a; Steenland and Woskie, 2012) or between PFOS and testicular cancer (Mandel and Johnson, 1995). While Dr. Grandjean critiqued the results of these mortality studies, he nonetheless relied on mortality studies when discussing other often survivable cancers, including kidney and prostate cancer.

Overall, there are limited studies of PFCs exposure and testicular cancer, and the weight of evidence does not establish a causal link between PFOA or other PFCs and testicular cancer.

Toxicological Evidence

Dr. Grandjean discussed evidence that PFOA is carcinogenic based on two animal studies in which rats were fed 300 ppm PFOA in the diet. In the study by Butenhoff *et al.* (2012b), male rats had an increased incidence of testicular Leydig cell adenomas, and in the study by Biegel *et al.* (2001), male rats had an increased incidence of hepatocellular adenomas and pancreatic acinar cell adenomas, plus one pancreatic carcinoma. Adenomas are benign tumors and carcinomas are malignant. Agency guidelines by US EPA (2005), NTP (2015), and IARC (2016b) all require findings of malignant tumors or a combination of benign and malignant tumors for evidence of carcinogenicity. The findings of benign adenomas only in the Butenhoff *et al.* (2012b) study and only one carcinoma in the Biegel *et al.* (2001) study do not provide strong or sufficient evidence of PFOA's carcinogenicity according to US EPA, NTP, and IARC guidelines.

13.2.4 Other Endpoints

In my report, I selected endpoints that have been the focus of recent agency reviews by US EPA and NTP. Dr. Grandjean overstated the strength of the evidence of several endpoints that have not been the focus of agency reviews, such as diabetes, obesity, and liver disease. He often referred to findings from the C8 Science Panel of "probable links" as support for his conclusions. However, he rarely discussed the C8 Science Panels' conclusions of no "probable link" in some instances.¹⁰⁷

The C8 Science Panel concluded that there is no probable link between PFOA and type 2 diabetes (C8 Science Panel, 2017). US EPA echoed the C8 Science Panel's conclusions in the Agency's 2016 assessment of PFOA (US EPA, 2016), stating that studies from occupational cohorts, exposed communities, and the general population, "show a lack of association of PFOA with diabetes, metabolic syndrome, and related endpoints." With regard to PFOS, while the evidence is more limited relative to PFOA, US EPA (2016) indicated that the body of literature does not show associations between PFOS and metabolic syndrome, insulin resistance, or insulin levels in adults or adolescents.

Dr. Grandjean overstated the available evidence for PFC exposure and diabetes and obesity and did not discuss all the available studies, particularly those published after the C8 Science Panel reports. For example, Karnes *et al.* (2014) conducted retrospective and prospective analyses of type 2 diabetes in the C8 Cohort and found that cumulative exposure was not associated with risk of type 2 diabetes for either analysis; similarly, there was no association when authors assessed the association when exposure was lagged 10 years prior to diabetes diagnosis (a 10-year lag).

With regard to the association between PFCs and liver toxicity, Dr. Grandjean did not discuss the findings of the C8 Science Panel, which found no probable link between PFOA and liver disease (C8 Science Panel, 2017). More generally, Dr. Grandjean overstated the weight of the evidence and selectively reported positive findings, without considering adversity. For example, Dr. Grandjean did not discuss the findings of Melzer *et al.* (2010) (although he cited this paper elsewhere), which found no associations between PFOA or PFOS and current liver disease in the NHANES survey. Further, while he cited the analysis by Darrow *et al.* (2016) as providing evidence of an association between PFOA and liver effects, he did not discuss the clinical relevance of the changes in liver enzymes, nor the fact that the authors stated there was no increased risk of frank liver disease.

13.2.5 State of Knowledge

General Comments

By not providing context, Dr. Grandjean in his expert report (Grandjean, 2017a) and at his deposition (Grandjean, 2017b) mischaracterized early studies or actions by 3M. In several instances, Dr. Grandjean attributed motives to 3M without evidence. For example, in his expert report, Dr. Grandjean stated:

- "One additional action taken by 3M was to search for blood samples that were free of organofluorine compounds. 3M also initiated efforts beginning in 1993 to show that organic fluorine in blood could be from entirely natural sources. Both efforts were unsuccessful, but

¹⁰⁷ I note that, as discussed in Section 7.1.5 of this report, the C8 Science Panel analysis was not done according to standard epidemiological criteria, but used a less-rigorous criterion for making its "probable link" determinations in the context of a legal matter. Therefore, in my analysis, I do not rely on the Panel's findings regarding the evidence for associations between PFOA and health effects.

revealed a strategy to pursue studies that might benefit company interests" (Grandjean, 2017a, p. 25 [emphasis added]).

- "3M either closed its eyes to the evidence, or chose purposefully not to find it, or being generous to 3M, it seems possible that 3M may have mistakenly relied on the absence of evidence, despite the old dictum that 'the absence of evidence is not evidence of absence,' which later became famous in U.S. politics" (Grandjean, 2017a, p. 27).
- "This apparent shift in focus is concerning, as it suggests that 3M was seeking to bias the results in order to undercut the earlier study" (Grandjean, 2017a, p. 29).

In each of these statements, Dr. Grandjean implied improper actions on the part of 3M or its contractors (*e.g.*, covering up findings, succumbing to industry influence). Such implications are inconsistent with a full reading of the record, as presented in full in Section 11 of this report. I provide a brief summary of the record here.

Dr. Grandjean criticized 3M for not publicly disclosing its knowledge or undertaking in-depth studies:

Given that 3M was an innovator in PFC production and the primary if not sole manufacturer in the U.S. for many years, it is not surprising that much of the early examination of PFC properties was undertaken or sponsored by 3M. What does appear to be remarkable is how little and how late 3M's knowledge on environmental dissemination and toxicity was publicly disclosed and how little this information inspired the company to conduct in-depth studies to reveal and understand any PFC-associated risks. (Grandjean, 2017a, p. 25)

While 3M has studied PFCs for decades and published much of its early work, it appropriately increased its efforts and publications on PFCs in the 2000s. These efforts coincided with the discovery that PFOS and PFOA were present and widely distributed in the blood of the general population and wildlife (*e.g.*, Giesy and Kannan, 2001). Whereas PFOA and PFOS had largely been considered a worker exposure issue, of primary interest to 3M internally, the discovery of a greatly expanded exposed population led to increased interest from both 3M and the greater scientific community in understanding the toxicity of these compounds.

In a discussion of early epidemiology studies of PFC exposure, Dr. Grandjean (2017a, p. 30) suggested that 3M should have conducted more epidemiology studies prior to the 1990s: "Systematic evidence from 3M's occupational medicine records was analyzed only from the 1990s and then reached the public at a delay, and only in parts, and without detailed reference to the caveats. Such studies were apparently not a matter of priority." At various points in his deposition, Dr. Grandjean made similar statements regarding what he concluded was a lack of follow-up by 3M in regard to study findings (*e.g.*, Grandjean, 2017b, p. 162).

In fact, several occupational health studies of 3M workers were conducted by 3M prior to the 1990s (*i.e.*, Schuman and Mandel, 1980; Ubel *et al.*, 1980; Roach, 1982a; Mandel and Schuman, 1989). Moreover, it is important to note that reliable analytical methodologies for measuring specific PFCs (such as PFOS and PFOA) in blood were not existent or limited prior to the 1990s. While early analytical methods for measuring total organic fluorine in the blood of individual (*i.e.*, not pooled) occupationally exposed workers began to be used in the 1970s, they were laborious, non-specific, and had high levels of detection (in the ppm range) (Hansen *et al.*, 2001; Lindstrom *et al.*, 2011). Further advancements in the 1980s to early 1990s allowed for the direct analysis of individual PFCs, such as PFOS and PFOA, with lower sensitivity (in the high ppb level), but these analyses were difficult to perform and not reliably

quantitative. It was not until the 1990s that significant advances in analytical chemistry made it possible to reliably measure individual PFCs (such as PFOS and PFOA) in humans. Thus, epidemiology studies based on occupational data collected prior to the 1990s could not have been based on blood concentrations of specifically PFOS and PFOA (or not reliably so).

Specific Examples

Dr. Grandjean (2017a, p. 25) stated: "At about the same time, a further report from Dr. Taves and his colleagues [Guy *et al.*, 1976] showed that there was widespread contamination of human tissues with organofluorine compounds which likely derived from commercial sources such as PFOA (or PFOS, as it seemed later on) [47]." For context, it should be noted that Guy *et al.* (1976) also stated that "other chemicals are usually not toxic in blood concentrations similar to those found here for organic fluorine" and that "there may have been a decrease in the concentration of organic fluorine in human plasma since the late 1950s." Thus, Dr. Grandjean did not provide the necessary context for the findings he cited.

Dr. Grandjean (2017a, p. 26) stated: "After the institution of regular blood testing at 3M's production facility in 1976, the 3M medical service team noticed, at least by September of 1984, an increasing trend in worker organic fluorine concentrations in blood." The evidence I have seen does not support this statement. Roach and Sorenson (1984) compared the results of blood monitoring for organic fluorine between 1982 and 1983 at the 3M Decatur plant. The results indicated a continuing downward trend in workers in nearly all production areas, which the authors attributed to improved exposure controls. The authors concluded, "[w]hile all studies to date fail to demonstrate human disease or patterns of adverse effect associated with organic fluorine concentrations in the blood, it is imperative that we continue every effort to reduce exposure and [organic fluorine] levels to the lowest degree possible" (Roach and Sorenson, 1984).

Dr. Grandjean (2017a, p. 26) also stated that:

While a published scientific article in 1980 reported on the presence of organic fluorine compounds in serum from exposed workers [84], and drinking water contamination in Ohio and West Virginia by PFOA was discovered and later publicly disclosed [71], PFC industries, such as 3M and DuPont, conducted some evaluations of potential health effects over time, but the results of these investigations were generally in the form of internal reports that were not published in the peer reviewed literature [71].

As explained in Section 11 of this report, the discovery that PFOS and PFOA were present and widely distributed in the blood of the general population and wildlife beginning in the 1990s led to increased interest from both 3M and the greater scientific community in understanding the toxicity of these compounds. This led to more publications, including of earlier studies that had previously existed only as internal 3M reports.

Regarding the bioaccumulation of PFCs, Dr. Grandjean (2017a, p. 28) stated: "Likewise, 'no widespread potential for bioaccumulation' is counter to predictions from physicochemical properties, and it is not clear how 3M justified the conclusion (which was later shown to be erroneous)." Similarly, at his deposition (Grandjean, 2017b, pp. 171-172), Dr. Grandjean concluded that, based on the physical-chemical properties of PFCs that he reviewed in an edition of the "Rubber Bible,"¹⁰⁸ dating from the 1950s, 3M must have known about the potential for dissemination of PFCs in the environment. Based on information discussed in Section 11 of this report and presented below, it is my opinion that Dr.

¹⁰⁸ The "Rubber Bible" is a handbook of physical-chemical properties of many chemicals (*e.g.*, Weast *et al.*, 1987-1988).

Grandjean's speculation about what 3M "must have known" is inconsistent with other relevant information.

While it has long been understood that there are relationships between physical-chemical properties and the potential for bioaccumulation in organisms (Arnot and Gobas, 2006), most bioaccumulative chemicals concentrate in fatty tissues because they are lipophilic, or "fat-loving." Lipophilic compounds move from water into octanol (a solvent that is similar to fat). Thus, for many persistent organic chemicals, one can use the partitioning of a compound between octanol and water (*i.e.*, the octanol-water partition coefficient, or log K_{ow}) to predict its bioaccumulation potential. Generally, the more lipophilic the compound, the higher the tendency for the compound to accumulate in biota (Conder *et al.*, 2008). Using K_{ow} values to predict the bioaccumulation of chemicals like PFOS and PFOA is not appropriate, however, because these chemicals accumulate in proteins rather than fats (3M Co. *et al.*, 2003; Kennedy *et al.*, 2004; Conder *et al.*, 2008). Thus, before they could be measured in biota, PFOA and PFOS would not have been recognized to be bioaccumulative. For example, 3M carried out testing on PFOS according to contemporaneously available scientific knowledge and, in the late 1970s, reasonably came to the initial conclusion that this compound would not appreciably bioaccumulate in biota (Welter, 1979). Dr. Grandjean did not discuss that PFOS and PFOA are atypical bioaccumulative chemicals in that they do not possess the classic properties for predicting bioaccumulation.

Regarding a study by Roach (1982b), Dr. Grandjean (2017a, p. 28) stated:

3M had an excellent opportunity to gather evidence emanating from health surveillance of exposed workers, such as those employed at the 3M Chemolite (Cottage Grove, Minnesota) manufacturing plant. A cross-sectional study of worker health was summarized by the 3M medical officer in 1982. There was a high prevalence of high blood pressure, and elevation of cholesterol, changes that the authors believed to be more likely due to lifestyle, not occupational exposures. It is not clear how this conclusion was reached, as the fact that other factors may have contributed to the outcomes does not mean that the occupational exposure had no impact. In particular, elevated cholesterol – as an indication of liver toxicity – was soon found to be a major effect of PFC exposure and should not have been ignored in 1982.

Dr. Roach described his analysis of the findings as follows: "These differences, however, have a high degree of variability and may be due to a number of factors such as incomplete fasting, diet, rotating shifts, etc." (Roach, 1982b). In a similar study of workers at the 3M Decatur plant, there were no significant differences in the distribution of liver enzymes, hematology parameters, and cholesterol between film plant and chemical plant employees (Roach, 1982a). There was also no significant correlation between these test results and blood levels of total organic fluorine. 3M would continue to conduct biomonitoring and clinical chemistry testing periodically at its Cottage Grove, Decatur, and Antwerp plants (Mandel and Burris, 1995; Gilliland and Mandel, 1996; Olsen *et al.*, 1998, 1999, 2000, 2003a). Because of the liver effects seen in animals, much of the testing in humans focused on tests aimed at evaluating hepatic function. Overall, these studies did not demonstrate a consistent relationship between PFOS/PFOA concentrations and blood parameters, liver function abnormalities, or hormone levels. Moreover, there were no substantial changes in hematological, lipid, hepatic, thyroid, or urinary parameters, consistent with the known toxicological effects of PFOS or PFOA. Most studies found no statistically significant differences in the mean percentage of test results that were above or below the reference ranges (*i.e.*, normal values) of the clinical chemistry and thyroid parameters.

In his expert report, Dr. Grandjean (2017a, p. 28) stated:

The most extensive early epidemiology study was conducted in connection with the thesis project carried out by Frank Gilliland, MD from the University of Minnesota School of Public Health. This study provided surveillance data on employees who voluntarily participated in the program at the Chemolite plant. Although Gilliland identified several associations that suggested adverse health effects, 3M later undermined the findings from the 1990 medical examination data stating that they could not be repeated in subsequent 1993 and 1995 medical surveillance examinations. This was also the conclusion of an article with 3M's Dr. Geary W. Olsen as the first author and published in the Journal of Occupational & Environmental Medicine in 1998; Dr. Gilliland was a co-author on that paper. One potential reason for the difference between the conclusions by Dr. Gilliland and Dr. Olsen is that Dr. Olsen in the later article relied on measured PFOA concentrations, while Dr. Gilliland had access to measured total organic fluorine only, thus perhaps including other PFCs that may have contributed to the changes.

Dr. Grandjean was referring to Gilliland (1992), a thesis paper, and Olsen *et al.* (1998), a subsequent publication. In his thesis, Gilliland (1992) conducted a cross-sectional analysis of the association between total serum fluorine concentration and reproductive hormones. He reported an association between total serum fluorine concentration and decreases in free testosterone and increases in estradiol; however, the magnitude of change was not reported (*i.e.*, the absolute or percentage change in hormone levels were not provided). As noted above, total organic fluorine blood measures are less reliable than specific PFOA measures. Further, in this analysis, the authors were limited by single blood draws from a commercial clinical laboratory (Gilliland, 1996).

Because of the possible positive findings, 3M sponsored additional study on reproductive hormones with more robust methods. Findings were reported in a publication authored by Drs. Gilliland, Olsen, Mandel and others (Olsen *et al.*, 1998). Serum PFOA concentrations from male 3M Cottage Grove plant production workers in 1993 and 1995 were compared to levels of several reproductive hormones. In this analysis, serum PFOA concentration was not statistically significantly associated with estradiol or testosterone levels and was not consistently associated with other measured hormone levels. Although a 10% increase in mean estradiol levels was observed among employees who had the highest serum PFOA concentrations, this association was confounded by BMI. The authors concluded that their results provided "reasonable assurance that, in this production setting, there were no significant hormonal changes associated with PFOA at the serum levels measured" (Olsen *et al.*, 1998).

The authors exchanged several letters regarding the findings and the content of the final publication of the Olsen *et al.* (1998) paper (Olsen, 1996; Gilliland, 1996, 1998). In one of these exchanges, Dr. Olsen presented the results of a statistical comparison of the total serum fluorine concentrations measured in 1990 by Dr. Gilliland and the serum PFOA concentrations measured for the 1998 publication. Dr. Olsen found that these two measures were highly correlated (*i.e.*, reported concentrations were similar for both measures), indicating total serum fluorine was an adequate surrogate for serum PFOA concentration in this case, and thus, differing measures were unlikely to be the reason for any conflicting findings between the two analyses. Thus, it is not appropriate to consider that Olsen *et al.* (1998) "undermined" the findings of Gilliland (1992). The authors simply could not replicate the earlier findings. Further, email communications just prior to publication indicate that Dr. Gilliland was satisfied with the content of the manuscript for publication (Gilliland, 1998).

Lastly, Dr. Grandjean (2017a, p. 31) stated:

In a review of 3M-sponsored subacute toxicity studies by 1979, a conclusion on PFOS was that 'FC-95 was the most toxic of the three compounds studied and certainly more toxic than anticipated. [...] Unless there are adequate data through human epidemiological evaluations that can reasonably assure relative safety of these compounds following long term exposure, lifetime rodent studies should be undertaken as soon as possible.'

Indeed, the absence of adverse effects in 3M production plant workers, a population that was reasonably assumed to have the highest exposures to PFOS, provided those assurances to 3M that the adverse effects observed in animals were not occurring in workers.

13.2.6 Serum Analyses from Minnesota Residents

In his discussion of the serum concentrations of PFCs in the residents of southern Washington County, Dr. Grandjean (2017, p. 18) exaggerated the difference between the residents' serum concentrations and those of the general US population by comparing the maximum concentrations of the residents to the average concentrations of the general population. As noted in Section 10.5 of this report, the average serum PFOA and PFOS concentrations in southern Washington County residents were approximately 3-4 times higher than those in the general US population in 2008, 2010, and 2014 (MDH, 2008-2014), not 10-100 times higher, as Dr. Grandjean implied. Furthermore, the residents' serum PFC concentrations have been declining over time in parallel with the US population values (CDC, 2015, 2017a).

13.2.7 Alternative Drinking Water Limits

Dr. Grandjean stated that the US EPA and MDH drinking water guidance values for PFOA and PFOS are too high, possibly by a factor of 100-1,000 (Grandjean, 2017a), although he presented no detailed calculation of an alternative RfD for these PFCs, nor did he state what drinking water guidance values for these PFCs he would consider to be protective. As discussed in Section 8 of this report, such a conclusion is inconsistent with the underlying science and with standard risk assessment methodology, which indicate that the US EPA and MDH PFOA/PFOS drinking water guidance values are very health-protective.

Dr. Grandjean referenced a "safe" serum PFOS concentration that was calculated by Dr. John Butenhoff *circa* 1998 (3M, c. 1998). However, Dr. Butenhoff's approach to calculating this "safe" PFOS concentration was developed prior to the availability of more-relevant information and is superseded by subsequent analyses using updated methodology. Dr. Grandjean's discussion of this "safe" concentration is not relevant to the development of drinking water guidance values for PFOS in 2016-2017.

In support of his opinion that the US EPA drinking water guidance values for PFOA and PFOS should be even lower than those of MDH, Dr. Grandjean cited a study of mammary gland development in mice dosed with PFOA (White *et al.*, 2011b). US EPA (2016a) did not consider studies of mammary gland development, including this one, in its derivation of an RfD for PFOA. US EPA (2016a) noted that it was not possible to assess the quality of the mammary gland studies, because the authors did not describe the methodology used to score the tissue for histological criteria (US EPA, 2016a) and also noted that there is uncertainty regarding the functional impact (*i.e.*, adversity) of this endpoint (US EPA, 2016a).

Dr. Grandjean's opinion is that the current US EPA and MDH drinking water exposure limits for PFOA and PFOS are too high, based in particular on his own study of diphtheria and tetanus antibody levels

(Grandjean *et al.*, 2012), summarized above and discussed in earlier in both this section and in Section 7.1. While this study found decreased diphtheria and tetanus antibody levels, below a clinical level of 0.1 IU/mL, with increasing exposure to PFOA (results for PFOS were not statistically significant), these results are not consistent with the follow-up study. The weight of the evidence indicates that the endpoint of decreased antibody levels after vaccination is not suitable to use as a POD for PFCs, because it is unlikely to be associated with a clinically relevant, adverse health effect.

Dr. Grandjean proposed a serum-based benchmark response of 5% (*i.e.*, a 5% reduction in antibodies) as a basis for a drinking water exposure limit, suggesting that the use of a 5% benchmark response is standard for epidemiology studies. However, US EPA (2012) guidance notes that a 10% benchmark response is customarily used, with lower values appropriate for frank effects (disease, for example), and a benchmark response greater than 10% is used for precursor effects (reduced antibody numbers is a precursor effect, rather than a frank effect, *i.e.*, it is not a disease state).

13.3 Comments on Dr. Sunding's Opinions

In his expert report, Dr. David Sunding evaluated associations between PFC exposure and adverse birth outcomes, population fertility rates, and cancer incidences in affected Minnesota communities (as defined by Dr. Sunding) (Sunding, 2017). Dr. Sunding's analyses, with the exception of those on adverse birth outcomes (based on birth certificates), do not contain information on disease outcome at the individual level. Furthermore, none of Dr. Sunding's analyses contain information regarding exposure to PFCs at the individual level. These studies are of ecological or cross-sectional design and, while useful for hypothesis generation, cannot be used to reliably draw causal inferences regarding health effects from PFC exposure (Olsen *et al.*, 2014). In addition, Dr. Sunding's analyses do not adequately consider some key confounders (*e.g.*, smoking). Dr. Sunding's claims regarding population disease burden are not appropriate, because he has not established a causal link between PFCs and disease outcomes, and thus, his analysis does not constitute an appropriate basis for calculating damages resulting from health impacts.

Responses to specific claims by Dr. Sunding are discussed below.

Adverse Birth Outcomes

Regarding adverse birth outcomes (Sunding, 2017, pp. 23-27), Dr. Sunding concluded that mothers who lived in the affected community of Oakdale, Minnesota,¹⁰⁹ before 2006 were more likely to have a low-birth-weight baby or preterm birth, compared to mothers residing in unaffected communities.¹¹⁰ Dr. Sunding further concluded that, after 2006, mothers who resided in Oakdale were somewhat more likely to have a low-birth-weight baby, compared to those residing in unaffected communities.

Dr. Sunding did not adjust his association analysis for important potential confounders, such as maternal smoking, drinking, or drug use, as well as access to prenatal healthcare and gestational age. These factors can be associated with adverse birth outcomes and thus can affect observed associations between chemical exposures and adverse birth outcomes (CDC, 2017c,d). In addition, Dr. Sunding used residential ZIP Code/county to approximate maternal PFC exposure in the affected and unaffected groups; this crude exposure metric is a very imperfect measure, for a number of reasons. For example,

¹⁰⁹ Where elevated concentrations of certain PFCs have been measured in some drinking water wells.

¹¹⁰ Dr. Sunding evaluated birth outcomes at the individual level and exposures on a geographic level in a cross-sectional study.

individuals in the same ZIP Code may not consume water from the same source and may have different water consumption patterns.¹¹¹

Fertility

In his analysis of general fertility rates in Oakdale before or after 2006, Dr. Sunding reported that these rates were lower in Oakdale compared to unaffected communities (Sunding, 2017, pp. 27-30). Dr. Sunding did not account for other community-level factors that may affect general fertility rates, such as marriage age¹¹² and available family planning resources (CDC, 2012).

Cancer Incidence

Dr. Sunding conducted an analysis to determine whether cancer incidences were elevated in Washington County as compared to other counties in Minnesota and found that non-Hodgkin's lymphoma, kidney cancer, bladder cancer, and chronic lymphocytic leukemia are statistically significantly more common in Washington County than in other Minnesota counties, when controlling for certain demographic characteristics (Sunding, 2017, pp. 30-42).

Dr. Sunding relied on MDH's online cancer database and two MDH cancer reports for cancer incidence data for his analysis. While Dr. Sunding did include county-level factors in his analysis (*i.e.*, race, age, and income), he did not account for or discuss the potential influence of smoking prevalence, which will impact the incidences of smoking-related cancers, such as bladder cancer (CDC, 2017e).

In his analyses, Dr. Sunding also reported that the proportions of adults and children with cancer or dying of cancer in Oakdale, but not other affected communities, were statistically significantly higher than those in the control area. Proportional mortality ratio studies are not appropriate in this context, because increases in one outcome could be a consequence of decreases in another. For example, the increased proportion of mortality from cancer may be reflective of a decreased proportion of mortality from diseases other than cancer.

¹¹¹ Similar uncertainties in exposure measurements are applicable to Dr. Sunding's other analyses.

¹¹² This metric is a reflection of maternal age at the birth of first child (CDC, 2012).

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