Molecular and Integrative Toxicology

Jamie C. DeWitt *Editor*

Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances

Humana Press

ED_002330_00119699-00001

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ED_002330_00119699-00002

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ED_002330_00119699-00003

Jamie C. DeWitt
Editor

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ED_002330_00119699-00004

Editor
Jamie C. DeWitt
Department of Pharmacology
and Toxicology
Brody School of Medicine,
East Carolina University
Greenville, NC, USA

ISSN 2168-4219 ISSN 2168-4235 (electronic)
Molecular and Integrative Toxicology
ISBN 978-3-319-15517-3 ISBN 978-3-319-15518-0 (eBook)
DOI 10.1007/978-3-319-15518-0

Library of Congress Control Number: 2015934825

Springer Cham Heidelberg New York Dordrecht London
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Preface

This book will be of interest to toxicologists, environmental chemists, risk assessors, and others with an interest in the class of compounds known as perfluoroalkyl and polyfluoroalkyl substances (PFASs). Most of the chapters are written for those with a background in toxicology or chemistry, but background information and references to review articles are included to provide a starting point for those seeking additional information.

PFASs are commonly used in myriad industrial and commercial applications. PFASs have provided industries and the general public with products of convenience and safety, including oil- and water-repellent textiles and papers, coatings, and fire-retardants. However, the unintended presence of PFAS in environmental media, including biota, may have adverse effects on the health of exposed organisms. Reports of cancers of the liver, pancreas, and testes in rodents exposed to PFASs began appearing in the early 1990s; in the ensuing decades, the number of publications related to PFAS toxicity has increased dramatically, revealing that many of these substances are associated with toxicities in nearly every system studied. Although the potential health risks of these compounds have been assessed by governmental agencies and organizations in many countries and health advisories and recommendations have been established, many questions about PFAS toxicity remain. Therefore, one goal of this book is to highlight what is known about the toxicity of PFASs in experimental animal models and in exposed humans.

The chapters are organized by themes: human and wildlife exposure/body burdens, reviews of metabolism and toxicological effects by organ system/developmental stage, and aspects of PFAS toxicity that are driving PFAS research and regulatory oversight.

Human and wildlife exposure/body burdens: Chap. 1 provides an excellent and thorough summary of the major scientific advances related to PFASs research over the past several decades, including an overview of the major toxicological findings and considerations for evaluation of newly synthesized PFASs. Chapter 2 focuses on the current methods for the extraction and analysis of PFASs in biological matrixes as well as their analytical challenges and new developments.

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Chapters 3 and 4 detail PFAS concentrations in human populations, mainly in the USA; Chap. 3 focuses on the general human population whereas Chap. 4 highlights highly exposed human populations. Finally, the section on PFAS exposure considerations concludes with concentrations in wildlife populations as well as some of the challenges associated with measuring such compounds in wildlife samples.

Reviews of metabolism and toxicological effects by organ system/developmental stage: While not every single toxicological effect published will be captured by these chapters, they cover the toxicological effects most commonly reported in human and experimental animal studies. This section starts with two chapters related to metabolism: Chap. 6 addresses the metabolism and pharmacokinetics of PFASs in biological organisms and Chap. 7 focuses on the effects of PFAS exposure on the metabolic processes themselves and how other effects may arise from toxicological changes in these metabolic processes. Chapter 8 captures the major outcomes that have been observed in studies of developmental exposure and Chaps. 9, 10, and 11 address toxicities to the three main controlling systems, neurotoxicity, immunotoxicity, and endocrine toxicity, respectively. The last two chapters of this section focus on broader toxicological issues. Chapter 12 details evidence of carcinogenicity in exposed organisms and Chap. 13 summarizes major epidemiological findings in human populations. Together, these eight chapters capture the major toxicological research associated with PFAS exposures.

Aspects of PFAS toxicity that are driving PFAS research and regulatory oversight: Chap. 14 synthesizes the data that were used to generate appropriate pharmacokinetic models to estimate human serum concentrations. Chapter 15 is actually a case study of the use of two PFAS compounds in literature-based evaluations of environmental contaminants and reflects an effort by the National Toxicology Program's Office of Health Assessment and Translation to increases its transparency in systematic review procedures of environmental contaminants. Chapter 16 is a thorough compendium of the human health risk assessment associated with these compounds and brings to light the need for effective communication among the many groups affected by such assessments. Chapter 17 focuses on the available toxicological data available for alternative fluorinated technologies that are being explored to replace longer chain PFASs that have been associated with toxicological effects. The final chapter summarizes the major points associated with PFAS toxicology and includes recommendations for future research.

I thank and gratefully acknowledge the chapter authors. In spite of enormous demands on his or her time, each author's expertise, dedication and willingness to participate in this project made this volume possible.

Greenville, NC, USA Jamie C. DeWitt

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Contributors

Barbara D. Abbott Toxicity Assessment Division (B105-04), National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA

Abee L. Boyles Office of Health Assessment and Translation, Division of the National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), Research Triangle Park, NC, USA

Robert C. Buck The Chemours Company FC, LLC, E. I. duPont de Nemours and Company, Inc., Wilmington, DE, USA

John L. Butenhoff Ph.D., DABT SaluTox, L.L.C., Lake Elmo, MN, USA (Formerly, Medical Department, 3M Company, St. Paul, MN, USA)

Antonia M. Calafat Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Sonia Dagnino Department of Chemical and Environmental Engineering, University of Arizona, Tucson, AZ, USA

Jamie C. DeWitt Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC, USA

Suzanne E. Fenton Ph.D. National Toxicology Program (NTP) Laboratory, Division of the NTP, National Institute of Environmental Health Sciences NIEHS/NIH, DHHS, Research Triangle Park, NC, USA

Hui Gao Department of Pharmacology, Qingdao University Medical College, Qingdao, Shandong, China

Qixiao Jiang Department of Pharmacology, Qingdao University Medical College, Qingdao, Shandong, China

ED_002330_00119699-00010

Kayoko Kato Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Deborah E. Keil Ph.D., M.T. (ASCP), D.A.B.T. Department of Microbiology and Immunology, Montana State University, Bozeman, MT, USA

Gerald L. Kennedy Consultant to DuPont, Wilmington, DE, USA

Wilmington, DE, USA

Naila Khalil M.B.B.S., M.P.H., Ph.D. Center for Global Health, Department of Community Health, Boonshoft School of Medicine, Wright State University, Dayton, OH, USA

Naomi Kudo School of Pharmaceutical Sciences, Josai University, Sakado, Saitama, Japan

Christopher Lau Ph.D. Developmental Toxicology Branch, Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, USA

Miryoung Lee Ph.D. Division of Epidemiology, Lifespan Health Research Center, Department of Community, Boonshoft School of Medicine, Wright State University, Dayton, OH, USA

Espen Mariussen Protection and Societal Security Division, Norwegian Defence Research Establishment (FFI), Kjeller, Norway

Geary W. Olsen D.V.M., Ph.D. Medical Department, 3M Company, St. Paul, MN, USA

Benjamin J. Place Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD, USA

Casey E. Reed National Toxicology Program (NTP) Laboratory, Division of the NTP, National Institute of Environmental Health Sciences NIEHS/NIH, DHHS, Research Triangle Park, NC, USA

Jessica L. Reiner Chemical Sciences Division, Hollings Marine Laboratory, National Institute of Standards and Technology, Charleston, SC, USA

Joseph V. Rodricks Ph.D., DABT ENVIRON International, Arlington, VA, USA

Andrew A. Rooney Office of Health Assessment and Translation, Division of the National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), Research Triangle Park, NC, USA

NIEHS, Research Triangle Park, NC, USA

Kyle Steenland Ph.D. Department of Environmental and Occupational Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA

ED_002330_00119699-00011

J. Morel Symons DuPont Epidemiology Program, Newark, DE, USA

Henrik Viberg Department of Organismal Biology, Division of Environmental Toxicology, Uppsala University, Uppsala, Sweden

Vickie R. Walker Office of Health Assessment and Translation, Division of the National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), Research Triangle Park, NC, USA

John Wambaugh Ph.D. National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

Xiaoyun Ye Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Lei Zhang Department of Pharmacology, Qingdao University Medical College, Qingdao, Shandong, China

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Editor Biography

Dr. Jamie C. DeWitt is an Assistant Professor in the Department of Pharmacology and Toxicology at the Brody School of Medicine at East Carolina University. She received BS degrees in Biology and Environmental Science from Michigan State University and PhD degrees in Environmental Science and Neural Science from Indiana University-Bloomington. Dr. DeWitt also completed postdoctoral training in Developmental Cardiotoxicity from Indiana University-Bloomington and in Immunotoxicology at the U.S. Environmental Protection Agency through a cooperative training agreement with the University of North Carolina at Chapel Hill. Dr. DeWitt is the immediate past-president of the North Carolina chapter of the Society of Toxicology and the Senior Councilor for the Immunotoxicology Specialty Section of the Society of Toxicology. She also was awarded the Outstanding Young Investigator Award from the Immunotoxicology Specialty Section in 2013. Dr. DeWitt's main research focus is on how toxicants found in the environment can lead to neurodevelopmental and neurodegenerative disorders via disruption of the developing immune system. She is especially interested in microglia, which are immune cells that reside in the central nervous system (CNS). These cells are often viewed as the interface between the CNS and the rest of the body and therefore are thought to be critical for proper neuronal development and homeostasis. Additionally, Dr. DeWitt and members of her lab work to understand how environmental toxicants impact both the adult and developing immune and nervous systems. Ultimately, she hopes that her work will not only improve our understanding of how exogenous chemicals alter these systems but also improve regulatory oversight and testing to reduce the number and amounts of potentially hazardous chemicals into the environment.

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Chapter 1
Perfluorinated Compounds: An Overview

Christopher Lau

Abstract This chapter provides an overarching introduction to the subject matter of this volume: toxicology and health effects of perfluorinated compounds (PFCs). It highlights briefly the unique chemical and physical properties of these chemicals, their production history and commercial uses, and their environmental and health concerns. The distribution profiles of these persistent contaminants in environmental media, wildlife and humans, as well as potential pathways of human exposure are characterized. General toxicological features of these chemicals include major species and sex differences in pharmacokinetic disposition among congeners with different carbon-chain lengths and functional groups, and differential potencies based on their primary mechanism of action, i.e., activation of the nuclear receptor PPARα. Current understanding of adverse effects associated with PFC exposure based on laboratory animal models include hepatotoxicity, tumor induction, developmental toxicity, immunotoxicity, neurotoxicity and endocrine disruption. Associations and probable links between exposure to some PFCs and adverse health outcomes in humans have been suggested by recent epidemiological reports. Ecological and human health risk assessments of these chemicals are still in their infancy. Each of these sub-topics will be expanded into full discussion in subsequent chapters. Importantly, a new generation of fluorinated chemicals has already emerged in commerce, with little information regarding their environmental fate and distribution as well as potential health effects. Materials presented in this volume should provide a scientific basis to inform the design of safe replacement

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Disclaimer This chapter has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does the mention of trade names of commercial products constitute endorsement or recommendation for use.

C. Lau, Ph.D. (✉)
Developmental Toxicology Branch, Toxicity Assessment Division,
National Health and Environmental Effects Research Laboratory,
Office of Research and Development, US Environmental Protection Agency,
Mail Drop B105-04, 109 T.W. Alexander Dr, Research Triangle Park, NC 27711, USA
e-mail: lau.christopher@epa.gov

© Springer International Publishing Switzerland 2015

J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_1

ED_002330_00119699-00015

products for the current crop of perfluorinated chemicals that promises to be less persistent and prevalent in the environment.

Keywords Perfluoroalkyl acids • Toxicology • Health effects • Epidemiology • Overview

The topic of "Perfluorinated Chemicals" (PFCs) has appeared with increasing frequency in popular news articles as well as research publications, particularly in the fields of environmental sciences and toxicology. Indeed, before the turn of the century, there were fewer than 300 research papers devoted to this subject; since then, well over 3,000 papers have been published on these chemicals, describing their worldwide detection in various environmental media, human populations and wildlife animals, their toxicological characteristics and mechanisms of toxicity, as well as their human and ecological health risks. In fact, several reviews have appeared periodically during the past decade describing interim progress in specific areas of research (e.g., Kennedy et al. 2004; Beach et al. 2006; Lau et al. 2004, 2007; Houde et al. 2006, 2011; Kovarova and Svobodova 2008; Suji et al. 2009; Olsen et al. 2009; Giesy et al. 2010; D'Hollander et al. 2010; Young and Mabury 2010; Lindstrom et al. 2011; Lau 2012; DeWitt et al. 2012; Post et al. 2012; Butt et al. 2014; Ahrens and Bundschuh 2014; Chang et al. 2014). This volume summarizes our current understanding of the adverse health effects of this interesting group of chemicals. Many aspects of PFCs are described in detail, ranging from chemical detection to exposure assessment, from pharmacokinetics to toxicity characterization and associated modes of action, from epidemiological surveys to health risk assessment, and from description of emerging replacement compounds to discussion of research needs in the future. The goal of this introductory chapter is to provide a brief overview of these aspects, highlighting the salient features of historical discoveries about these chemicals and contemporary progress that has enhanced our knowledge base. It is strongly recommended that readers consult individual chapters for in-depth discussions of specific topics of interest covered in this volume.

So, what are these perfluorinated chemicals and why have they drawn such immense interest for investigation? Buck et al. (2011) have published an excellent review on the terminology, classification and origins of these chemicals, and only a brief description is provided here. By definition, perfluorinated chemicals are organic compounds where every hydrogen atom bonded with a carbon atom on the alkane backbone is replaced by a fluorine atom. They may include perfluoroalkanes, perfluoroalkyl acids (PFAAs) and their precursors, and a number of surfactants and fluoropolymers. Perfluoroalkanes are a unique group of chemicals used primarily for clinical purposes in oxygenation and respiratory ventilation, but are not a subject for discussion in this volume. Perfluoroalkyl acids found in the environment are compounds with a perfluoroalkyl backbone (typically with carbon-chain lengths ranging from 4 to 14) attached to a functional group. These chemicals are largely man-made, as naturally occurring perfluorinated organic chemicals are rare (Key et al. 1997). To date, three groups of PFAAs have been identified: perfluoroalkyl

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sulfonates (PFSAs), perfluorocarboxylates (PFCAs) and perfluoroalkyl phosphonates or phosphinates (PFPAs). While the first two groups have been detected ubiquitously since the early 2000s, the presence of PFPAs was first reported by D'eon et al. (2009) in Canadian surface waters and waste water treatment plants, and subsequently by Busch et al. (2010) in landfill leachates in Germany, Esparza et al. (2011) in water and sludge in the Netherlands, and Liu et al. (2013) in sewage sludge in China, suggesting their wide distribution, similar to that seen with PFSAs and PFCAs. Little is known about the toxicity of PFPAs except their pharmacokinetic profiles in the rat (D'eon and Mabury 2010) and a preliminary report on their developmental toxicity in mice (Tatum-Gibbs et al. 2010). Thus, an in-depth description of their potential adverse effects awaits additional investigation. Fluoropolymers and PFAA derivatives (such as alcohols, amides and esters for PFSAs, and telomer alcohols (TAs) and polyfluoroalkyl phosphates (PAPs) for PFCAs) are known to be degraded or metabolized to PFAAs as terminal products; hence, for intent and purpose, the focus of discussion regarding adverse health effects of perfluorinated chemicals in this volume is limited to PFSAs and PFCAs. However, although they have received less attention, several PFAA precursors such as N-ethyl-N-(2-hydroxyethyl)-perfluorooctanesulfonamide (N-EtFOSE; Berthiaume and Wallace 2002; Lau et al. 2003; Xie et al. 2009), 4:2, 6:2, 8:2 and 10:2 telomer alcohols (Kudo et al. 2005; Mylchreest et al. 2005; Phillips et al. 2007; Andersen et al. 2008; Fasano et al. 2009; Anand et al. 2012; Serex et al. 2014) and polyfluoroalkyl phosphate esters (D'eon and Mabury 2011a, b; Rosenmai et al. 2013; Rand and Mabury 2014) have been included in toxicological evaluations.

The physical and chemical properties of PFAAs, their industrial applications and production history, and their fate and transport have been well described by Kissa (2001), Prevedouros et al. (2006), and Wang et al. (2014a, b). In brief, these chemicals are highly stable even at high temperature, nonflammable, not readily degraded by strong acids, alkalis or oxidizing agents, and not subject to photolysis. The unique stability of these chemicals thus renders them practically nonbiodegradable and very persistent in the environment. Unlike other persistent organic pollutants (such as dioxin and PCB) that are lipophilic, PFAAs are both hydrophobic and lipophobic, which lends them most useful as surfactants and polymers. By attaching a charged moiety such as carboxylate, sulfonate, or phosphonate/phosphinate to the perfluoroalkane chain, the molecule becomes more water soluble. While all amphoteric PFAAs of various chain lengths share some surfactant properties, the eight-carbon chemicals perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have been most effective in commercial uses. Indeed, over 200 industrial and consumer applications are known for these chemicals, ranging from water-, soil-, and stain-resistant coatings for textiles, leather, and carpets, to grease-proof paper products for food contact, floor polishes, insecticide formulations, ski wax, electroplating, and electronic etching bath surfactants, photographic emulsifier, aviation hydraulic fluids, and fire-fighting foams (Renner 2001).

Perfluoroalkyl acids are fairly contemporary chemicals, being in large-scale production only since the 1950s. Two methods of production have dominated the industrial manufacturing process: electrochemical fluorination (ECF) and telomer-

ization. The ECF process is based on the reaction between organic feedstocks such as 1-octanesulfonyl fluoride ($C_8H_{17}SO_2F$) and anhydrous hydrogen fluoride, which is fueled by an electrical current, causing the hydrogen atoms on the carbon backbone to be replaced by fluorine atoms, with resultant products such as perfluorooctane sulfonyl fluoride (POSF, $C_8F_{17}SO_2F$). During this process, fragmentation and rearrangement of the alkane backbone can occur, producing fluorinated organic molecules of various carbon-chain lengths and a mixture of linear, branched and cyclic isomers of the chemical. Typically 70–85 % of the mixture is linear and 15–30 % is a mixture of branched isomers. The telomerization process involves a telogen such as pentafluoroethyl iodide ($F(CF_2CF_2)I$) that polymerizes with tetrafluoroethylene (CF_2CF_2) to form telomer intermediates of desired carbon chain length; the resultant telomer iodides are then reacted with ethylene via free radical addition. The telomerization process thus yields straight-chain alcohols ($F(CF_2CF_2)$ nCH_2CH_2OH) that can be converted into final products for commercial application. Both linear and branched isomers of PFAAs have been detected in the environment, in humans and wildlife (De Silva and Mabury 2009; De Silva et al. 2009; Benskin et al. 2010a, b; Beesoon et al. 2011; Zhang et al. 2013a). Their comparative toxicities have not been investigated systematically. The branched isomers are generally eliminated at a faster rate than the linear isomers (Benskin et al. 2009a, b), but the linear precursor perfluorooctane sulfonamide (PFOSA) has been shown to be eliminated faster than the branched precursor (Zhang et al. 2013b).

Production of PFAAs has been scaled up steadily since the 1950s; for instance, an estimate of 3,500 tonnes of PFSA was produced in 2000, and an annual average of PFCA production was estimated between 150 and 350 tonnes in the past 60 years (Lau et al. 2004; Wang et al. 2014a). Production of PFOS and POSF-related chemistry was phased out by 3M in 2002, and DuPont ceased production of PFOA by the end of 2013. In the interim, shorter chain PFAAs such as perfluorobutane sulfonate (PFBS) and perfluorohexanoic acid (PFHxA) have largely taken the place of the eight-carbon chemicals in U.S. commerce. Since 2002, production of some of the PFAAs has gradually been shifted from North America and Europe to other developing countries; China in particular has scaled up production of PFOS considerably from an estimate of 30 tonnes per year in 2001 to an average of 250–300 tonnes per year since 2006 (Lim et al. 2011; Wang et al. 2014a, b). A substantial amount of PFAAs is emitted to the environment, either directly as manufacturing by-products or indirectly through degradation of precursor and intermediates materials (e.g. POSF-based intermediates, TAs, PAPs, and fluoropolymers). These chemicals are distributed globally, even to remote locales such as the Arctic and the Antarctic, by atmospheric and/or oceanic transfers (Yamashita et al. 2008; Armitage et al. 2009; Dreyer et al. 2009; Bengtson Nash et al. 2010; Young and Mabury 2010). PFAAs are found in all environmental media: air, water, soil, sediment, sludge from waste water treatment plants, biosolids for agricultural application, and house dust. Because of the persistent nature of PFAAs, these emitted contaminants settled on soils are transported to groundwater and surface water for consumption, and their removal at waste water treatment plants is expensive and inefficient. Recent studies have also demonstrated that PFAAs can be taken up into edible plants grown in

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soils amended by biosolids derived from waste water treatment plants (Lechner and Knapp 2011; Blaine et al. 2013, 2014). To a certain extent, once these anthropogenic chemicals are produced, they are essentially accumulated and re-circulated through different environmental media. In general, the levels of PFAAs detected are higher in urban and industrial areas in developed countries than in rural and sparsely populated locales (Kunacheva et al. 2012).

Giesy and Kannan (2001) were first to report the presence of PFAAs in wildlife animal samples. Since then, a plethora of studies has documented the prevalence of these chemicals in a wide variety of wildlife species including mammals, amphibians, reptiles, birds, fish, and a number of invertebrates in a wide range of locales in North America, Europe and Asia. Several worldwide surveys of PFAAs in wildlife have been conducted, and findings have been summarized periodically (Houde et al. 2006, 2011; Lau et al. 2007; Butt et al. 2010; Murakami et al. 2011; Gebbink et al. 2011; Rigét et al. 2013). Generally, the levels of PFAAs detected in wildlife are highest around industrialized areas, mirroring the environmental distribution of these chemicals. However, it is notable that extensive contamination of wildlife has occurred in remote locales including the Arctic (Butt et al. 2010; Rotander et al. 2012; Braune and Letcher 2013; Rigét et al. 2013). There were some uncertainties regarding the potential for bioaccumulation and biomagnification of PFAAs through food webs (Conder et al. 2008; Xu et al. 2014), but recent studies have suggested that PFCAs and PFSAs with perfluoroalkyl chain lengths shorter than C7 and C6 are not likely to bioaccumulate (Suja et al. 2009; Loi et al. 2011; Martin et al. 2013; Naile et al. 2013; Hong et al. 2014).

The presence of organic fluoride in humans was first reported by Taves and coworkers (Taves 1968; Shen and Taves 1974). These compounds were later identified as PFOS or PFOA (Taves et al. 1976; Ubel et al. 1980; Belisle 1981). The extent of human exposure to these chemicals was not confirmed and elaborated upon until the turn of the twenty-first century when significant advances in analytical chemistry enabled routine detection of individual PFAAs in various biological and environmental matrices in the sub-ppb range by high performance liquid or gas chromatography followed by electrospray ionization tandem mass spectrometry (HPLC-ES/MS/MS) (Hansen et al. 2001; Sottani and Minoia 2002; Martin et al. 2002; Hebert et al. 2002). Improvements of these analytical methods have continued in the past decade (reviews: Martin et al. 2004; Trojanowicz and Koc 2013; Valsecchi et al. 2013). Equipped with such sensitive detection methods, a number of biomonitoring surveys of PFAA production workers and individuals from specific populations (Olsen et al. 2003a, b, c, 2004) revealed wide-spread presence of PFOS, PFOA and related chemicals. These initial discoveries led to systematic surveys of perfluorinated compounds in the general populations of industrialized countries in North America, Europe and Asia (Lau et al. 2007). The National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention in the U.S. provides perhaps the most comprehensive description of serum PFAAs in the adult population across the country from 1999 to the present. The changing trends of notable PFAAs over the past decade in the U.S. have been described by Kato et al. (2011). In brief, PFOS, PFOA, perfluorohexane

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sulfonate (PFHxS) and perfluorononanoic acid (PFNA) have been routinely detected since 1999, with PFOS levels being by far the highest. Since, 3M ceased production of POSF-related chemicals in 2002, a significant drop of PFOS levels (by about two-thirds) was noted; in contrast, the levels of PFNA, though lowest among the four PFAAs, have been on a steady rise, tripling during the past 10 years. Survey results from other populations from Europe, Asia and Australia are in general agreement with the NHANES findings (Kärrman et al. 2007; Fromme et al. 2009; Zhang et al. 2010; Glynn et al. 2012; Okada et al. 2013; Yeung et al. 2013a, b; Toms et al. 2014). However, several "hot spots" have been identified where the areas were known to be contaminated and levels of PFAAs in the local populations were significantly elevated above those reported in the general population. These include, for instance, Arnsburg, Germany in 2006, and in the U.S., mid-Ohio and West Virginia areas in 2007, and Minnesota in 2008.

Pathways for human exposure to PFAAs have been explored actively and summarized in several recent reviews (Fromme et al. 2009; D'Hollander et al. 2010; Haug et al. 2011; Cornelis et al. 2012). Dietary intake (Tittlemier et al. 2007; Picó et al. 2011; Domingo 2012; Klenow et al. 2013), migration from food package materials (Begley et al. 2005; Trier et al. 2011; Xu et al. 2013), drinking water (Rumsby et al. 2009; Domingo et al. 2012; Post et al. 2013); indoor air and house dust (Shoeib et al. 2005, 2011; Strynar and Lindstrom 2008; Liu et al. 2014), and consumer products (Vestergren et al. 2008; Trudel et al. 2008) are likely important routes of human exposure. Among these, dietary intake has generally been considered the major source of exposure, particularly through consumption of PFAA-contaminated fish and seafood (Haug et al. 2010; Domingo et al. 2012), while exposure through consumption of vegetables and beverages (such as cow milk and beer) is comparatively low (Herzke et al. 2013; Barbarossa et al. 2014; Stahl et al. 2014).

Toxicological properties of PFAAs have been described in several reviews (Lau et al. 2004, 2007; White et al. 2011; Mariussen 2012; Lau 2012; Dewitt et al. 2012; Corsini et al. 2014). This chapter only provides an overarching description of these characteristics, detailed accounts with specific relevant citations are furnished in the ensuing chapters. In general, PFAAs are well absorbed after oral ingestion, are not metabolized, and are excreted primarily in urine and to a lesser extent in feces. These chemicals have a high affinity for protein binding (e.g. serum albumin, fatty acid binding proteins). PFAAs are distributed broadly among tissues (Kennedy et al. 2004; Harris and Barton 2008), but with the exception of the short chain chemicals (C6 or less), the chemicals are taken up and stored preferentially in the liver. In fact, liver, kidney and blood compartments can account for greater than half of the body burden of PFAAs. During pregnancy, these chemicals can cross the placental barrier readily in both laboratory animals and humans, although the maternal levels of PFAAs tend to be higher than those in the fetus (Thibodeaux et al. 2003; Lau et al. 2003; Midasch et al. 2007; Glynn et al. 2012). After birth, lactational transfer of PFAAs has been well documented (Kärrman et al. 2007; Fenton et al. 2009; Liu et al. 2011).

In laboratory studies, the adverse effects of PFAAs vary widely based on their perfluoroalkyl chain lengths and functional groups, as well as species and sex

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differences of the animal models (Kudo and Kawashima 2003; Lau et al. 2007; Lau 2012). Two prominent features must be considered to account for these variations: differential pharmacokinetic disposition and potency among the homologues of these chemicals. As illustrated in Table 1.1, the serum elimination half-lives of PFAAs can vary greatly, from hours to years. Typically, chemicals with long perfluoroalkyl chain lengths (>C4 for PFSAs, >C6 for PFCAs) are much more persistent in the body; half-lives tend to increase from rodents (hours-days) to monkey (days-months) and to humans (months-years), and those in males are slightly longer than in females (with the exaggerated exceptions of PFOA and PFNA in rat where tremendous differences in $t_{½}$ between males and females were seen). Differential renal reabsorption involving organic anion transporters likely contributes to these varying pharmacokinetic profiles of PFAAs (Andersen et al. 2008; Weaver et al. 2010; Han et al. 2012). The response potency of individual PFAAs can also vary significantly among chain lengths, between functional groups and target species (Ishibashi et al. 2011; Wolf et al. 2012; Rosen et al. 2013; Buhrke et al. 2013) (Table 1.2). Based on the experimental model of peroxisome proliferator-activated receptor-alpha (PPARα) activation in transfected COS-1 cells, Wolf et al. (2008, 2012) surmised that (a) PFAAs of increasing chain lengths induced increasing activity of the mouse and human PPARα, (b) PFCAs were stronger activators than PFSAs, and (c) the mouse PPARα appeared to be more sensitive to PFAAs than the human PPARα. Hence, only nominal adverse effects were seen with PFBA in rodents (Das et al. 2008; Butenhoff et al. 2012) compared to PFOA (Kennedy et al. 2004; Lau et al. 2006), in part because of the faster clearance rate of the former homologue (hours vs. days) and the weaker potency in its effects.

Because multiple PFAAs are typically found in the environment, humans and wildlife, their cumulative risks and potential interactions must be considered. Several *in vitro* studies have addressed the combined effects of selected PFAAs (Wei et al. 2009; Ding et al. 2013; Carr et al. 2013; Wolf et al. 2014). In general, binary combinations of PFAAs behaved additively at low and moderate concentrations, but this response might change into antagonism at very high concentrations. Further investigation with a diverse set of PFAAs (different chain lengths and functional groups) and confirmation of *in vitro* findings with *in vivo* studies are needed to clarify this key issue.

The specific characteristics of individual toxicities associated with PFAA exposure from various experimental models will be elaborated in great details in the following chapters. Only the salient features of each notable adverse effect are mentioned here. PFAAs are not known to be genotoxic or mutagenic (Eriksen et al. 2010; Florentin et al. 2011; Jacquet et al. 2012; Butenhoff et al. 2014). Hepatic effects are a sensitive hallmark response of PFAA exposure. Liver hypertrophy associated with vacuole formation and peroxisome proliferation typically have been observed when a significant body burden of PFAAs was achieved, particularly for the more persistent and potent long-chain homologues. Correspondingly, transcriptional activation of nuclear receptor (prominently PPARα)-related genes in the liver was routinely detected. Hepatosteatosis was also a common feature of chronic exposure to PFAAs. However, these effects were largely reversible upon cessation

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Table 1.1 Serum/plasma elimination half-lives of various PFAAs

	PFBS		PFHxS		PFOS		PFBA		PFHxA		PFOA		PFNA	
Species	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Rat	4.0 h	4.5 h	N/A	29.1 days	62–71 days	38–41 days	1.0–1.8 h	6–9 h	0.4–0.6 h	1.0–1.6 h	2–4 h	4–6 days	1.4 days	30.6 days
Mouse			25–27 days	28–30 days	31–38 days	36–43 days	3 h	12 h	~1.2 h	~1.6 h	17 days	19 days	26–68 days	34–69 days
Rabbit											7 h	5.5 h		
Dog											8–13 days	20–30 days		
Cattle					56 days							19.2 h		
Pig	43 days		2 years		1.7 years				4.1 days		236 days			
Chicken					15–17 days							3.9 days		
Monkey	3.5 days	4.0 days	87 days	141 days	110 days	132 days	1.7 days		0.1–0.8 days	0.2–1.5 days	30 days	21 days		
Human	28 days		8.5 years		5.4 years		3 days		32 days		2.3–3.8 years			

Modified with kind permission of Springer Science + Business Media (From Lau 2012)
New data: PFHxS, Sundström et al. 2012; humans (PFHxA), Russell et al. 2013; pig, Numata et al. 2014; cattle (PFOS), http://europepmc.org/abstract/MED/23790943

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Table 1.2 Relative response potency of various PFAAs

A. Animal models		
COS-1 cells (mouse)	Hepatocytes (mouse)	CV-1 cells (seal)
PFNA (C9)	PFOA (C8)	PFOA (C8)
PFOA (C8)	PFHxA (C6)	PFHpA (C7)
PFUnDA (C11)	PFDA (C10)	PFNA (C9)
PFHpA (C7)	PFNA (C9)	PFPeA (C5)
PFDA (C10)	PFHpA (C7)	PFHxS (C6)
PFDoDA (C12)	PFPeA (C5)	PFHxA (C6)
PFHxA (C6)	PFHxS (C6)	PFDA (C10)
PFPeA (C5)	PFBA (C4)	PFBA (C4)
PFBA (C4)	PFOS (C8)	PFOS (C8)
PFHxS (C6)	PFBS (C4)	PFUnDA (C11)
PFOS (C8)	PFUnDA (C11)	PFDoDA (C12)
PFBS (C4)	PFDoDA (C12)	PFBS (C4)
B. Human models		
COS-1 cells	Hepatocytes	HepG2 cells
PFOA (C8)	PFNA (C9)	PFDoDA (C12)
PFNA (C9)	PFHpA (C7)	PFDA (C10)
PFHpA (C7)	PFHxS (C6)	PFNA (C9)
PFHxA (C6)	PFHxA (C6)	PFOA (C8)
PFPeA (C5)	PFOA (C8)	PFHpA (C7)
PFBA (C4)	PFDA (C10)	PFHxA (C6)
PFHxS (C6)	PFOS (C8)	PFBA (C4)
PFUnDA (C11)	PFBS (C4)	
PFBS (C4)	PFBA (C4)	
PFOS (C8)	PFPeA (C5)	
PFDA (C10)	PFDoDA (C12)	
PFDoDA (C12)	PFUnDA (C11)	

Adapted from the following sources: COS-1 cells, Wolf et al. 2012; Hepatocytes, Rosen et al. 2013; CV-1 cells, Ishibashi et al. 2011; HepG2 cells, Buhrke et al. 2013)
The ranking is in descending order, with the most potent chemical on top of the list

of chemical treatment. Profound developmental toxicity has been described with gestational and lactational exposure to PFOS, PFOA and PFNA. Neonatal morbidity and mortality were seen with exposure to high doses of these chemicals in rodents, while growth deficits and developmental delays were noted in offspring exposed to lower doses. Immunotoxicity of PFOS, PFOA and PFNA has been demonstrated; these chemicals appear to suppress both acquired and innate immunity in mice and compromise their immune responses mediated by T cells and B cells. Both PFOA and PFOS have been shown to induce tumors in rodents and fish. In particular, liver adenomas, pancreatic acinar cell tumors and testicular Leydig cell adenomas have been detected in rats treated with PFOA chronically. This

"tumor triad" profile is typically associated with the PPARα-mediated molecular signaling pathway. Interestingly, liver tumors involving this mode of action have been considered not to be relevant to humans (Corton et al. 2014).

Some endocrine disrupting effects have been attributed to PFAAs, most notably induction of hypothyroxinemia and reduction of serum testosterone in rats, the latter effect produced by PFOA was likely related to an elevation of hepatic aromatase activity. The neurotoxic potential of PFAAs has been addressed only scantily. Altered proliferation and differentiation of PC12 cells, altered synaptogenesis and synaptic transmission in cultured hippocampal cells and induction of apoptosis in cultured cerebellar cells by PFOS have been reported. Neonatal exposure to PFAAs in mice leading to deranged behaviors at adult ages have also been described. In contrast, in guideline tests for developmental neurotoxicity, neither PFOS nor PFHxA exhibited any significant adverse effects. Recent preliminary studies have suggested that developmental exposure to PFOA (particularly at low doses) might lead to obesity in adult ages in mice and humans (Hines et al. 2009; Halldorsson et al. 2012), but conflicting findings have also been reported (Ngo et al. 2014; Barry et al. 2014). Clearly, the endocrine disrupting, neurotoxic, and obesogenic potentials of PFAAs will require substantial additional elucidation.

To date, activation of PPARα is the only established mechanism of action for PFAAs (Rosen et al. 2008; Ren et al. 2009). Other putative mechanisms for PFAAs include gap junctional inhibition to disrupt cell-cell communication, mitochondrial dysfunction, interference of protein binding, partitioning into lipid bilayers, oxidative stress, altered calcium homeostasis, inappropriate activation of molecular signals such as MAPK, ERK1/2, and NF-κB. However, these alternative candidates lack direct or strong evidence to support a pathophysiological role in the multi-faceted effects of PFAAs. Characterization of the modes of action for PFAA toxicities remains an intriguing area of future investigation.

Prompted primarily by toxicological findings in laboratory animals, a flurry of epidemiological reports have appeared in recent literature. These studies can be grouped into four categories: biomonitoring of production workers, surveys of highly exposed populations, nationwide cross-sectional studies, and individual case studies. The levels of PFAAs found in production workers typically are about 100 times higher than those detected in the general population (e.g., NHANES). The health impacts of PFOS on workers have been carefully investigated by 3M, those of PFOA have been tracked by 3M and DuPont, and a single study to date reported the health status of PFNA production workers (Mundt et al. 2007). Significant positive associations were noted between PFOS/PFOA levels and elevated serum cholesterol, low density lipoproteins (LDL) and uric acid, and reduction of bilirubin in these workers. The C8 Study evaluated the health status of 69,000 people (including occupationally exposed workers) living near a fluoropolymer production plant in West Virginia, whose drinking water was contaminated with PFOA. The median level of PFOA in this cohort is about five times higher than that of the general population (NHANES). Probable links were found between exposure to PFOA and hypercholesterolemia, thyroid disease, pregnancy-induced hypertension, testicular and kidney cancers, and ulcerative colitis. Data mining studies were conducted with a number of surveys, including NHANES, Danish National Birth Cohort, Danish

Cancer Registry, Norwegian Mother and Child Cohort, Avon Longitudinal Study of Parents and Children, Taiwan Birth Panel and Birth Cohort in the Faroe Islands. Serum levels of PFAAs were generally in the range of those reported by NHANES. These cross-sectional studies essentially reflect a broad "snap-shot" analysis of representative populations. By and large, the positive associations between PFAA exposure and the adverse health outcomes were marginal and somewhat inconsistent. These included increased serum cholesterol, LDL and uric acid, thyroid, cardiovascular and kidney diseases, altered liver enzyme activities, lengthened time-to-pregnancy, early onset of menopause, low birth weight and small size of newborns, attention deficit hyperactivity disorder, reduced immune responses in children and delays in age of menarche. Individual case studies were based on regional hospitals and clinics in U.S., European and Asian countries, and findings are generally in line with those reported in the cross-sectional studies with larger cohorts. From these epidemiological investigations, the strongest and most consistent associations between PFAA exposure and adverse health effects in humans are elevated serum cholesterol, LDL and uric acid, suggesting metabolic disorders. This finding is not surprising as PFAAs were once referred to as "synthetic fatty acids", and their potential competition with the endogenous long-chain fatty acids for interactions with metabolic sensors such as hepatic nuclear receptors is entirely plausible. Paradoxically, reductions of serum cholesterol and triglycerides are the prevailing findings from PFAA studies with rodents, which are opposite in direction from the human scenarios. A better understanding of the fundamental differences in intermediary metabolism between rodents and humans is needed to resolve these apparently conflicting observations.

Only a few concerted efforts have been undertaken to date toward human health risk assessments of PFAAs as a group (So et al. 2006; Zhao et al. 2011; Borg et al. 2013). Toward that end, Scialli et al. (2007) and Peters and Gonzalez (2011) have expressed caution regarding the use of the toxic equivalency factor (TEF) approach for the combined risk assessment of PFAAs. On the other hand, risk assessments of PFOA in the general population (Butenhoff et al. 2004; Post et al. 2012) as well as in specific population at highly contaminated areas (Wilhelm et al. 2008) have been published. A similar assessment on the health impacts of PFOS was provided by Saikat et al. (2013). Yang et al. (2014) recently described the development of criteria for protection of aquatic life from PFOS and PFOA. In addition, regulatory agencies in the U.S. and Europe have issued health advisories on tolerable daily intake (TDI) of PFOS and PFOA in food (by European Food Safety Authority, UK Committee on Toxicity in Food, Consumer Products and the Environment, and German Federal Institute for Risk Assessment) and in drinking water (by U.S. Environmental Protection Agency, Minnesota Department of Health, and New Jersey Department of Environmental Protection); while PFOS was listed in Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009. Still, many challenges remain for human and ecological health risk assessment of PFCs. (a) There is a slew of poly- and perfluorinated chemicals in the environment- PFAAs and their precursors- and various related fluorinated chemicals that are only beginning to be identified; their aggregate risks must be considered. Limited information thus far on the additive effects of PFAA mixtures at low levels (i.e. environmental ranges) is

encouraging for modeling their complex interactions. (b) The pharmacokinetic profiles of PFCs (where information is available) vary greatly between species, with humans apparently at the extreme end of the spectrum; these different parameters may lead to variable and unpredicted body burdens of the chemicals. (c) Modes of action for apical toxicity endpoints may vary among PFAAs and between species (e.g. rodents vs. humans). Extrapolation of toxicity data from animal studies to humans (or other ecological species) must take into account the variables associated with toxicokinetics and toxicodynamics. A dosimetric anchoring approach recently advocated by Wambaugh et al. (2013) may be instrumental in addressing some of these issues. (d) Supporting epidemiological findings thus far indicate mostly associations between PFAA exposure and human health risks. An improved understanding of the biological and physiological underpinnings that drive these associations are needed to support the conclusions drawn from these findings.

Concerted research efforts by environmental chemists, ecologists, toxicologists and epidemiologists have provided a wealth of information on perfluorinated chemicals in the past 15 years. Our understanding of exposure sources, fate and transport, exposure profiles and pathways, adverse effects in laboratory animals and untoward health outcomes in humans has been greatly improved. However, in common with many scientific pursuits, novel discoveries often lead to additional queries that require further investigation. This axiom certainly applies to PFC research. There are still many lingering issues regarding the health effects of these chemicals, foremost of which are (a) the biological basis that accounts for the extraordinary persistence of PFAAs in humans (with half-life estimates in months to years), (b) the extrapolation of toxicity findings from animal studies to human health risks, and (c) the potential hazards of PFAA replacement chemicals. Improved insights into the mechanisms of PFAA toxicity (beyond PPARα activation) will reduce the uncertainties of cross species extrapolation (for example, induction of liver tumors by PFOA via PPARα activation in rodents that is unlikely in humans, *vide supra*). After production of PFOS, and more recently, PFOA by the major manufacturers ceased, a host of replacement compounds have emerged, which are typically polyfluorinated rather than perfluorinated, or have a carbon backbone with ether linkages (Wang et al. 2013). With the exception of one compound, ADONA (Gordon 2011), very little is known about the health safety of these new replacement chemicals. Indeed, Scheringer and colleagues (2014) have recently issued a "Helsingør Statement" on poly- and perfluorinated alkyl substances, in which these scientists outlined their concerns about continued development of these chemicals. Perhaps the manufacturers, environmental scientists, risk assessors and managers, as well as the general public should well heed their sentiment.

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Chapter 2
Analysis of PFASs in Biological Tissues and Fluids

Sonia Dagnino

Abstract In order to determine the toxicology and health effects on humans and wildlife of per- and polyfluorinated substances (PFASs), it is critical to develop rapid, accurate and sensitive methods for their analysis in biological tissues and fluids. In the past few years, extraction and analytical methods have been developed to allow their detection in very complex matrices and at very low levels. Biological tissues and fluids include: whole blood, serum, urine, feces, sweat, nails as well as organs: liver, kidneys and more. Although liquid chromatography coupled with mass spectrometry has been the most prevalent method over the past decade, other techniques can be applied, particularly for the detection and discovery of newly developed fluorinated materials. This chapter will review current methods for the extraction and analysis of PFASs in biological matrixes as well as their analytical challenges and new developments.

Keywords Perfluorinated • Biological fluids • Tissues • Extraction • Analysis

Abbreviations

ESI	Electrospray ionization
FTCA	Fluorotelomer carboxylate
FTOH	Fluorotelomer alcohols
FTUCA	Fluorotelomer unsaturated carboxylate
GC-NCI-MS	Gas chromatography-negative chemical ionization mass spectrometry
HPLC	High pressure liquid chromatography
IPE	Ion-pair extraction
IS	Internal standard

S. Dagnino (✉)
Department of Chemical and Environmental Engineering, University of Arizona, 1133 James E. Rogers Way, Tucson, AZ 85721, USA
e-mail: sonia.dagnino@gmail.com

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_2

ED_002330_00119699-00036

LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
MTBE	Methyltert-butyl ether
N-ethyl FOSA	N-Ethylperfluorooctanesulfonamide
N-ethyl FOSE	N-Ethylperfluorooctanesulfonamidoethanol
N-ethyl PFOSA	N-Ethylperfluorooctanesulfonamide
N-methyl FOSA	N-Methylperfluorooctanesulfonamide
N-methyl FOSE	N-Methylperfluorooctanesulfonamidoethanol
N,N-diethyl PFOSA	N,N-Diethylperfluorooctanesulfonamide
PAP	Polyfluoroalkyl phosphate esthers
PFAS	Per and Polyfluoroalkyl substances
PAP	Polyfluoroalkyl phosphate esthers
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonate
PFCA	Perfluorocarboxylic acids
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFDS	Perfluorodecanesulfonate
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxDA	Perfluorohexadecanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFOSA	Perfluorooctanesulfonamide
PFPA	Perfluorinated phosphonic acids
PFPeA	Perfluoropentanoic acid
PFSA	Perfluorosulfonic acids
PFTeDA	Perfluorotetradecanoic acid
PFUnDA	Perfluoroundecanoic acid
QqQ	Triple quadrupole
QTOF	Quadrupole time-of-flight
SAX	Strong ion exchange
SPE	Solid phase extraction
TBA	Tetrabutylammonium hydrogen sulfate
TFC	Turbolent flow chromatography
THPFOS	tetrahydroperfluorooctanesulfonate
TOF	Time-of-flight
UPLC	Ultra performance liquid chromatography
WAX	Weak anion exchange

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2.1 Introduction

In 1968, Taves was the first to report organic fluorine in human serum using nuclear magnetic resonance (Taves 1968). These pioneer studies, led to an increasing concern over the need to identify and measure perfluorinated materials in biological tissues and fluids. The advances in analytical instrumentation, particularly the introduction of Liquid Chromatography-Mass Spectrometry (LC-MS), has allowed the development of methods for the detection of perfluorinated compounds in complex matrices such as biological tissues and fluids. The extraction and analysis of PFAS in these tissues is essential for the understanding of their toxicity and modes of actions. Studies on human biomonitoring have often focused on the analysis of whole blood, serum or milk. The development of sensitive methods for the analysis of these fluids has allowed to correlate PFAS levels to health effects, such as birth defects, immunotoxicity, cancer and others (Fei et al. 2007; Granum et al. 2013; Klaunig et al. 2012; Nelson et al. 2010). Recently, other methods have been developed to allow the biomonitoring with non-invasive sampling by analyzing PFASs in nails, hair and urine. These matrices are interesting as they are easier to obtain, especially for sensitive population such as infants and children. Moreover, the analysis has often been focusing on historical PFASs, such as perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfonic acid (PFOS). New methods are being developed for the analysis of other classes of PFASs such as: fluorotelomer alcohols (FTOHs), polyfluoralkyls esthers (PAPs) and their metabolites.

2.2 Challenges for the Analysis of Per- and Polyfluorinated Compounds

Fluorine is an element of the periodic table that has special properties which contribute to the characterization of perfluoroalkyl and polyfluoroalkyl substances (PFASs). The relative atomic mass of fluorine is 18.9984 u. It is less than the unit which means that fluorine has a negative mass defect which is defined as the difference between the nominal mass of an atom and its accurate mass based on ^{12}C as 12.0000 (Sparkman 2002), as shown in Fig. 2.1. In the case of Fluorine, this difference is negative whereas in the case of hydrogen, the mass is larger than the unit (1.008 Da). Highly fluorinated compounds will therefore have lower monoisotopic masses than their respective nominal mass, in respect to compounds with only C–H bonds. This means, for instance, that PFOA, which has a nominal mass of 413 Da, has in fact an exact mass of 412.9664 (anion). These properties can be very useful for the identification of PFASs with high resolution instruments capable of measuring monoisotopic mass.

On the contrary to other organohalogens, Fluorine is a monoisotopic element. Thus, fluoro-organic compounds do not often offer characteristic isotopic patterns in mass spectrometry, which is one of the disadvantages of Fluorine. Properties of Fluorine related to its ionic interaction result in the bond between carbon atoms and

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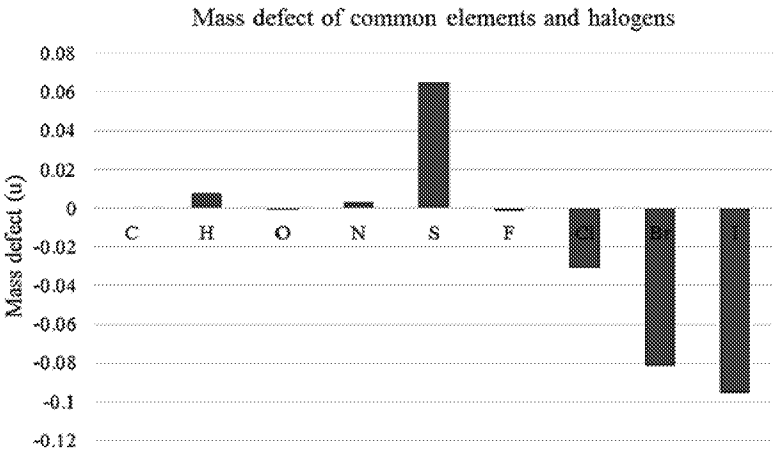

Fig. 2.1 Mass defect of common elements and halogens, the mass defect is calculated relative to the mass of carbon 12 equals 12.000

Table 2.1 Experimental aqueous solubility of selected per and polyfluorinated compounds with different carbon chain length and functional groups

Name	Number of fluorinated carbons	Functional group	Log AqS (mg/L) at 25 °C[a]
PFOA	8	-COOH	−2.02
PFDA	10	-COOH	−2.29
4:2 FTOH	4	-OH	2.99
6:2 FTOH	6	-OH	1.27
8:2 FTOH	8	-OH	−0.83
10:2 FTOH	10	-OH	−1.96
MeFOSE	8	$-SO_2N-C_2H_4OH$	−0.09
EtFOSE	8	$-SO_2N-C_2H_4OH$	−0.05

[a]LogAqS are reported from Bhhatarai and Gramatica (2011)

fluorine being considered the strongest single bond in organic compound chemistry (Blanksby and Ellison 2003). This peculiarity is reflected in the great stability and persistence of PFASs in their chemical applications but also in humans and the environment. The unicity of fluorine also contributes to the partitioning characteristics of perfluorinated compounds, which can be both hydro and lipophilic and have very different aqueous solubility depending on molecule length, and which functional group is attached to the molecule as illustrated in Table 2.1.

Challenges with the reliability and quality of PFASs analysis have been debated since the early 2000s (Martin et al. 2004). PFASs are man-made chemicals that can be produced by two methods: electrochemical fluorination and telomerization. Electrochemical fluorination results in the production of various carbon chain length PFASs and a mixture of linear, branched and cyclic isomers. Telomerization produces straight – chain telomer alcohols that can be converted into final products

(Lehmler 2005). These two methods produce PFASs mixtures that are composed of a family of target compounds as well as by-products of various chain lengths and isomers, which have the possibility to introduce a bias in the analysis. Although the impurity of the standards can be overcome by proper analytical methods and error adjustments, bias induced by presence of different isomeric composition cannot easily be avoided or estimated. This bias is introduced by the possibility of different ionization capacities of each isomer as well as their potential different fragmentation pattern. Uncertainty due to isomers can be overcome by isomeric profiling of standards and samples, which has been proven in recent work to be useful for a more precise quantification of PFOA and PFOS, and interesting regarding their toxicity evaluation (Zhang et al. 2014).

When dealing with analysis in biological tissues and fluids, one must consider the uncertainties due to the effects of the matrix on the detection with mass spectrometry techniques. An example is the well-known interference between PFOS and taurodeoxycholic acid (TDCA) in biological samples. TDCA is a bile salt that has been reported in the liver, bile tissues, and also serum and milk (Hansen et al. 2001; Keller et al. 2010; Reiner et al. 2012). TDCA and PFOS have the same unit mass of 499, and both contain a sulfonate group, that delivers the same transition 499–80 in classic LC-MS methods. This transition is often used as quantifier fragment for PFOS. Therefore, co-elution, especially in the presence of high concentrations of TDCA, can lead to overestimation at the m/z 499–80 transition. This interference has been overcome by using transition 499–99 which is unique to PFOS, but also by introducing purification techniques that eliminate TDCA from the sample. This interference can also be avoided by the use of accurate mass instrumentation. The Monoisotopic masses of PFOS and TDCA are 499.9375 and 499.2968 respectively, differing by 1,281.64 ppm, which makes it impossible to mistake one from the other with high resolution detection.

The interesting industrial properties of PFASs justify their presence in multiple applications. Unfortunately, this ubiquitous presence can affect their analysis as they can be present in laboratory material. Fluoropolymers such as polytetrafluoroethylene, Teflon® or other fluoropolymers can be used for vial caps, LC instrument tubing and internal instrument parts. In general, contact with fluoropolymers from laboratory materials should always be avoided to prevent contamination (Martin et al. 2004). This can be done by the use of alternative materials such as polypropylene. Contamination due to fluoropolymer parts in the instrument can be overcome by the replacement of the parts, when possible, or the installation of a guard column upstream of the LC-column to retain PFASs contamination (Martin et al. 2004).

One of the main challenges regarding monitoring of PFASs is the variety and rapid progression of the fluorinated materials currently on the market. Hundreds to thousands per and poly-fluorinated compounds are currently in use and new formulations are brought to market continuously (Gordon 2011; D'Eon and Mabury 2011a) for the replacement of the compounds that are considered toxic. Analytical standards cannot be synthesized fast enough for their confirmation and quantification. Therefore, the use of accurate mass equipment is necessary for the assessment of the exposure to these newly developed compounds. All challenges related to analytical equipment will be discussed further in the analytical section.

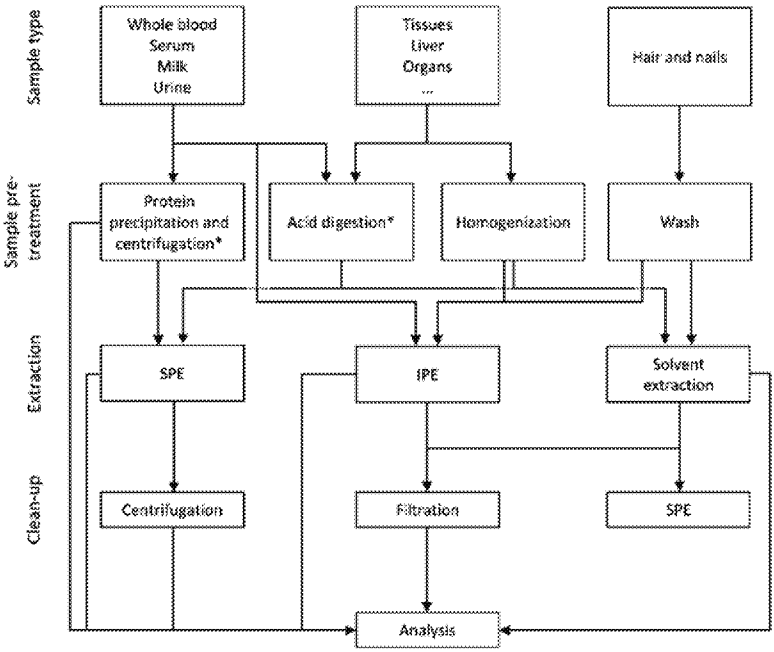

Fig. 2.2 Extraction and clean-up methods for the analysis of PFASs in biological tissues and fluids (Reprinted from van Leeuwen and de Boer 2007 with permission from Elsevier)

2.3 Sample Preparation

Several studies have reported measurements of PFASs in biological tissues and fluids. Most frequently methods are developed for perfluorocarboxylic acids (PFCAs) and perfluorosulfonates (PFSAs) with the addition of perfluorooctanesulfonamide (PFOSA). Similar methods are reported for the extraction in biological samples. The outline of the procedures used for different sample types is illustrated in Fig. 2.2. For the extraction of perfluorinated compounds in biological samples, ion-paired-extraction, solvent extraction and solid-phase extraction are among the most commonly used techniques, a selection of the methods applied and their description can be found in Table 2.3.

2.4 Sample Conservation and Pre-treatment

Although immediate analysis is considered as the best option, it is often not feasible to extract and analyze the sample immediately after collection. Therefore, proper sample conservation to avoid losses and contamination is necessary. Sample conservation is usually done by freezing. Polypropylene vials are considered as a good

option to conserve samples. One study has measured traces of PFOA in polypropylene bottles (Yamashita et al. 2004), thus, it is advised to rinse the sampling vials with semi-polar solvents when the analysis is targeting very low levels (<ng/L). Glass containers have been debated, as studies have reported the ability of some PFASs to stick to glass (Martin et al. 2004; van Leeuwen and de Boer 2007). For biological tissues and fluids sample, matrix components can shield the active sites at the glass surface. Karrman et al. did not find any loss for perfluorohexanesulfonic acid (PFHxS), PFOS, PFOA and perfluorononanoic acid (PFNA) in whole blood samples stored in glass and frozen over a period of 4 months (Karrman et al. 2006). Overall freezing in polypropylene vials seems to be a good solution for storage of samples prior to PFCAs and PFSAs analysis. Berger et al. evaluated losses of PFCAs and PFSAs in water stored in polypropylene tubes for 90 days. All compounds showed recoveries >70 % except for perfluoroundecanoic acid (PFUnDA) and perfluorododecanoic (PFDoDA) (<50 % recovery) (Berger et al. 2011). The use of high-density polyethylene or glass container is advised by Berger et al. (2011), although their effectiveness is not measured. Specific care must be taken for PFASs analysis where storage conditions in biological samples have not yet been described, such as FTOHs, di-PAPs and others. Additional studies are needed to assess the effects of sampling and storage conditions for these PFASs.

2.5 Ion Pairing Extraction

The Ion-Pairing Extraction (IPE) was first developed by Hansen et al. (2001) for a classic suite of PFASs (PFOA, PFOS, PFHxS, PFOSA, tetrahydroperfluorooctanesulfonate (THPFOS)). IPE is a sub-type of the liquid-liquid extraction. It involves the addition of a salt such as a tetrabutylammonium (TBA) solution (at a certain pH) for the ion-pairing of the target compounds, followed by an extraction with methyl-*ter*-butyl ether (MTBE). This method has initially been developed for the extraction of PFAS from serum and liver, and has been used since for a wide variety of extractions in human serum, nails or tissues (Kannan et al. 2004; Kim et al. 2011; Lee and Mabury 2011; Loi et al. 2013; Olsen et al. 2003), as well as for biota (Furdui et al. 2008; Llorca et al. 2009; Vestergren et al. 2012). The advantages of this method are its simplicity and the limited number of steps, as it does not require any sample pretreatment (i.e., protein precipitation). However, this method has also been proven to have some disadvantages, such as co-extraction of matrix components. This disadvantage could be improved by the introduction of a clean-up step, which is usually not performed (Kim et al. 2011; Lee and Mabury 2011; Loi et al. 2013) or limited to a 0.2 μm filtration (Kannan et al. 2004). Another disadvantage for serum and plasma extraction is the volume of the samples, 0.5–3 mL generally, which can be problematic for human biomonitoring where small volume sampling is preferred. This method has recently been proven effective for the extraction of PFASs other than PFCAs and PFSAs, such as fluorotelomer sulfonates (FTS), polyfluoroalkylphosphate esthers (mono and di-PAPs), perfluorophosphates (PFPA),

perfluorophosphinates (PFPiA) and others (Lee and Mabury 2011; Loi et al. 2013). It can be therefore considered useful for the monitoring of these rarely monitored PFASs.

2.6 Other Solvent Extractions

Other solvent mixtures have been explored for the extraction of PFASs in liquid and solid biological matrices. Addition of acetonitrile followed by sonication has been used for serum and whole blood (Glynn et al. 2012; Li et al. 2012). This method has been used for PFCAs (C6–C14) and PFSA (C4–C10) as well as PFOSA, with good recoveries (>70 %) and comparable detection limits in respect to classic IPE. Extraction with acetonitrile has also proven its efficiency for solid matrices such as hair and nails (Li et al. 2012; Perez et al. 2012). Hair and nails have to undergo a cleaning step before extraction to avoid contamination from the surface. PFSA and PFCAs have shown good recoveries and detection limits down to 0.03 ng/g for hair and nails with solvent extraction (Li et al. 2012). Acetonitrile extraction without sonication has also been demonstrated as a quick method for the extraction of a multitude of human tissues (liver, kidney, adipose tissue, brain, thyroid, muscle) (Maestri et al. 2006). The samples were homogenized and diluted in water, extracted with acetonitrile and subsequently enriched by SPE on a C18 column, followed by purification on a strong anion exchange column (SAX). This method allowed sufficient sample clean-up for detection limits as low as 0.1 ng/g by LC-MS. Solvent extraction other than IPE is usually followed by a clean-up step with solid phase extraction. Different phases have been used for the purification: Oasis-Wax (weak anion exchange) for nails and hair (Li et al. 2012), ENVI-Carb for serum and whole blood (Glynn et al. 2012) and C-18 followed by SAX for human tissues (Maestri et al. 2006). Solvents with MTBE have also been used for the extraction of more volatile compounds such as FTOHs from rat plasma. This technique produced a clean extract with good recoveries (86–113 %) and did not need further clean-up for an limit of detection (LOD) of 5 ng/mL (Szostek and Prickett 2004).

2.7 Solid Phase Extraction

Solid phase extraction has been widely used for the extraction of PFASs in environmental samples, especially in water (D'Eon et al. 2009; Ding et al. 2012) and biota samples (Delinsky et al. 2009; Ye et al. 2008). Solid-phase extraction (SPE) is a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture, according to their physical and chemical properties. SPE involves the filtration of the liquid sample trough a cartridge filled with a stationary phase whose composition and properties can vary. SPE can be used as a concentration step as well as a clean-up step. For the extraction of complex matrices usually a pre-treatment is required, such as: grinding, protein

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precipitation, or filtration to prevent the cartridges from clogging. This method has been generally preferred for liquid matrices, serum, milk and urine (Kuklenyik et al. 2004; Reiner et al. 2011; Zhang et al. 2014). Protein precipitation is usually performed in liquid matrices before extraction by the addition of solvent (methanol or acetonitrile) and centrifugation, but has also been used for solid matrices such as feces (Genuis et al. 2010) and various human tissues (Maestri et al. 2006). Currently, the most commonly used cartridge phases for PFASs extraction are Oasis-HLB and Oasis-WAX, although other sorbents have been used. Kaarman et al. (2006) developed a method using C-18 sorbent for PFCAs and PFSAs extraction in whole blood. The method showed good recoveries (>70 %) for PFCAs C6–C10, PFHxS and PFOS, but was not very efficient for higher chain PFCAs (C11–C13 < 70 %), PFOSA and PFBS (26 %). A rapid method was developed by Kuklenyik et al. (2004) for the analysis of human serum and milk with HLB automated SPE, followed by HPLC-TurboIonSpray-MS/MS. This method has the advantage of being extremely rapid, and it is currently used by the center for disease control and prevention (CDC) in the National Health and Nutrition Examination Survey (NHANES) for the measure of: C5–C12 PFCA, PFHxS, PFOS and PFOSA in human serum across the US population. Recently, a modified version of this method was applied to serum analysis for: C7–C11 PFCAs, PFHxS, PFOS and PFOSA that resulted in low detection limits (8–100 pg/mL) and good recoveries (>80 %, except for PFHxS >60 %) (Kato et al. 2011). Although Oasis-HLB seems like a good sorbent, its lower efficiency for smaller chains (<C5) and higher chains (>C10) PFASs, is a disadvantage.

In environmental samples applications, Oasis-WAX sorbents are very successful, which is leading recent publication to use this technique for biological fluids. SPE extraction with Oasis-WAX has been used as a purification method for whole blood of ski waxers workers after formic acid digestion and sonication (Nilsson et al. 2013), for purification of hair and nail extracts (Li et al. 2012) and urine samples (Zhang et al. 2014). This method is one of the first that has been proven effective not only for PFCAs and PFSAs, but also for the extraction of fluorotelomer alcohol metabolites in human blood (ski waxers), with recoveries ranging from 50 to 90 %. This method is interesting for further studies involving the monitoring of PFASs metabolites in humans.

Oasis-WAX sorbent has also been described as efficient for urine analysis of PFCAs (C7–C11), PFHxS, PFOS and PFOSA with recoveries >90 % and low detection limits (14–173 pg/mL). Only a handful of studies are available on urine analysis of PFASs (Genuis et al. 2013; Perez et al. 2012; Zhang et al. 2013), this method could be promising to allow further biomonitoring in this matrix, which is much easier to collect in respect to serum, blood and milk.

2.8 Alternative Methods Requiring No-Extraction

Recently, a few studies have reported the use of new online extraction methods for the determination of PFASs. Wang et al. (2011) developed a method using online-solid phase extraction for the determination of 18 PFCAs in human serum. Online

SPE involves the loading of the sample onto an online SPE cartridge by using the liquid chromatography device. The sample is then pushed across the sorbent where compounds are retained. This step is followed by a reversal of flow across the sorbent to elute the target analytes directly onto the analytical LC column. This method has the advantage of requiring less sample preparation, using only 100 μL of sample, while achieving detection limits close or lower than current extraction techniques (<0.09 ng/mL) (Wang et al. 2011). A similar method was reported by Schecter et al. also for human serum (Schecter et al. 2012) but with slightly higher detection limits (0.1–0.2 ng/mL). Kato et al. (2009) applied online-SPE to the analysis of blood-spots that are collected from newborns infants. This method is very interesting as it allows the use of only 75 μL of serum, while achieving good recoveries for PFOA and PFOS (100 %) and detection limits of 0.1–0.4 ng/mL. Analytical methods that require no extraction procedures are interesting as they avoid all bias and errors related to sample preparation. It also allows the development of high-throughput analysis, with the need of a very small volume of sample, and by decreasing preparation time, allowing the analysis of a greater number of samples.

2.9 Clean-Up

Clean-up steps can be added to the extraction method to minimize matrix interference and improve analysis. Further clean-up is usually necessary for complex sample types such as liver, adipose tissue and feces. Additional steps can be added to remove lipids, small solids in suspension or other matrix components. A summary of clean-up procedures reported in the literature is described in Table 2.2.

A common clean-up step is the filtration of the sample prior to analysis. Filtration is often applied after IPE and solvent extractions. A 0.2 μm syringe nylon filter is commonly used for this procedure (D'Eon and Mabury 2011b; Kannan et al. 2004) but care should be taken in the choice of filters as some brands have been reported to contain amounts of PFOS and PFOA (Yamashita et al. 2005). Solid-phase extraction does not usually require a clean-up step, but it can be used as a clean-up step after protein precipitation, acid digestion and solvent extraction particularly for the extraction of human tissues (Genuis et al. 2010; Glynn et al. 2012; Maestri et al. 2006). Recently, Turbulent-flow chromatography online technique has been used for the clean-up of urine, and hair and tissue extracts, for the analysis of PFASs (Perez et al. 2012, 2013). Turbulent flow liquid chromatography combines size exclusion and traditional stationary phase column to separate matrix components, such as proteins and target analytes in biological fluids. The process is very rapid, and can be used as an on-line extraction or purification method. Very low limits of detections have been achieved with this technique for PFCAs, PFSAs and PFASs metabolites (Table 2.3) (Perez et al. 2012, 2013).

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Table 2.2 Extraction, clean-up and analytical techniques for the analysis of PFASs in biological tissues and fluid

Matrix	Analytes			Sample volume	Extraction procedure		Clean-up		Analytical method	Ref.
	PFCA	PFSA	Other		Technique	Solvent	Technique	Final solvent		
Human Serum or whole blood	C_8	C_6, C_8	PFOSA	0.5–1 mL	IPE	MTBE	0.2 um nylon Mesh filter	Methanol	HPLC-ES-MS/MS	Kannan et al. (2004)
Human serum	C_8–C_{14}	C_6–C_{10}	Me-, Et-PFOSAA,	200 µL	IPE	MTBE	n.a.	Acetonitrile	HPLC-ESI-MS/MS	Kim et al. (2011)
Human serum	C_4–C_{11}	C_4, C_6, C_8, C_{10}	x:2 FTS (x = 4, 6, 8) C_6, C_8, C_{10} PFPA FOSA Me-, Et- PFOSAA, Masurf©FS-780a	2–3 mL	IPE	MTBE	n.a.	Methanol	HPLC-MS/MS	Lee and Mabury (2011)
Human whole blood	C_6–C_{14}	C_4, C_6, C_8	6:2 and 8:2 diPAP	3 g	IPE	MTBE	n.a.	Methanol	UPLC-MS/MS	Loi et al. (2013)
Human liver	C_8	C_6, C_8	PFOSA	nd	IPE	MTBE (Hansen)	n.a.		HPLC-ES-MS/MS	Olsen et al. (2003)
Human whole blood	C_8–C_{13}			4 mL	IPE		Derivatisation Silica gel	Toluene	GC-NCI-MS	De Silva and Mabury (2006)
Human nails	C_8	C_8		nd	Cleaning and IPE	MTBE	n.a.	Acetonitrile	HPLC-MS/MS	Xu et al. (2010)
Human serum	C_4–C_{12}			200 µL	Protein precipitation	Acetonitrile	n.a.	Acetonitrile	HPLC-ES-MS/MS TurboIonSpray	Olsen et al. (2011)

(continued)

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Table 2.2 (continued)

Matrix	Analytes			Sample volume	Extraction procedure		Clean-up		Analytical method	Ref.
	PFCA	PFSA	Other		Technique	Solvent	Technique	Final solvent		
Rat plasma	C_4–C_9	none	8:2 FTOH, FTCA, FTUCA, 7:2 sFTOH, 87:3 Acid, 7:3 UA,	nd	Protein precipitation	Acetonitrile	n.a.		LC-ES-MS/MS	Himmelstein et al. (2012)
Mouse serum	C_8			25 µL	Acid digestion with Formic acid		Protein precipitation	Acetonitrile	LC-ES-MS/MS	Jiang et al. (2012)
Rat blood, urine and feces	C_4–C_{11}	none	x:2 diPAP,, mono-PAP, FTCA, FTUCA (x,=4,6,8), 7:2 FTCA 8:2 FTOH-sulfate, -glucoronide	40–150 mg	Solvent extraction + sonication	Acetonitrile	Centrifugation 0.2 um nylon mesh filter	Acetonitrile scetonitrile: water (80:20) for feces	LC-MS/MS	D'Eon and Mabury (2011b)
Human serum and whole blood	C_6–C_{14}	C_4–C_8, C_{10}	FOSA	0.5 g	Solvent extraction + sonication	Acetonitrile	SPE-ENVI-Carb and centrifugation	Acetonitrile	HPLC-ESI-MS/MS HPLC-ESI-MS-qTOF	Glynn et al. (2012)
Human hair or human nail	C_6–C_{12}	C_6, C_8		0.1 g	Solvent extraction + sonication	Acetonitrile	SPE-WAX	Methanol: water	HPLC-ESI-MS/MS	Li et al. (2012)

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Human urine and hair	C_4–C_{13}, C_{16}, C_{18}	C_4, C_6, C_8, C10	6:2, 8:2, 10:2 FTCA PFOSA (only in hair)	0.25 g	Hair: solvent extraction + sonication urine: protein precipitation	Acetonitrile	Turbulent flow chromatograph	n.a.	TFC-LC-MS/MS	Perez et al. (2012)
Human serum, urine and sweat and feces	C_8–C_{11}, C_{13}	C_6, C_8		1 mL	Acid digestion (formic acid) Solvent extraction (feces)	Methanol (feces)	SPE (not specified) None (feces)	n.d.	LC-MS/MS	Genuis et al. (2010)
Human liver, kidney, brain, lung, and bone	C_4–C_{13}, C_{16}, C_{18}	C_4, C_6, C_8, C_{10}	6:2, 8:2, 10:2 FTCA, PFOSA	1 g	Solvent extraction	20 mM NaOH in methanol	Turbulent flow chromatograph	n.a.	TFC-LC-MS/MS	Pérez et al. (2013)
Human liver, kidney, adipose tissue, brain, basal ganglia, hypophysis, thyroid, gonads, pancreas, lung, skeletal muscle	C_8	C_8		n.d.	Solvent extraction	Acetonitrile	SPE-C18 and SAX	Methanol	LC-MS	Maestri et al. (2006)

(continued)

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Table 2.2 (continued)

Matrix	Analytes			Sample volume	Extraction procedure		Clean-up		Analytical method	Ref.
	PFCA	PFSA	Other		Technique	Solvent	Technique	Final solvent		
Rat plasma,			8:2 FTOH	250 µL	Solvent extraction	MTBE	n.a.	n.a.	GC-MS	Szostek and Prickett (2004)
Rat liver, kidney, adipose tissue			8:2 FTOH	0.5 g	Acid digestion (Perchloric acid) Solvent extraction	Hexane or heptane	SPE with silica sodium sulfate and silica	Isopropanol	GC-MS	Szostek and Prickett (2004)
Human serum	C_7–C_{12}	C_4, C_6, C_8	PFOSA Me-, Et- PFOSAA	1 mL	Acid digestion (formic acid) + sonication	Acetonitrile +1 % NH4OH	SPE-HLB	Acetonitrile	HPLC-ESI-MS/MS	Kato et al. (2011)
Human serum	C_5–C_{12}	C_6–C_8	PFOSA Me-, Et- PFOSAA	1 mL	Acid digestion (formic acid) + sonication	Acetonitrile +1 % NH4OH	SPE-HLB	Acetonitrile	HPLC-ES-MS/MS TurboIonSpray	
Human milk	C_5–C_{12}	C_6–C_8	PFOSA EtPFOSAA	1 mL	SPE-HLB	Acetonitrile +1 % NH4OH	n.a.	Acetonitrile	HPLC-ES-MS/MS TurboIonSpray	Kuklenyik et al. (2004)
Human serum	C_7–C_{12}	C_4–C_8	PFOSA Me-, Et- PFOSAA	100 µL	Online-SPE-HPLC, C18	Methanol	n.a.	n.a.	HPLC-ESI-MS/MS	Wang et al. (2011)

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Whole blood ski waxers	C_4–C_{14}, C_{18}	C_4, C_6, C_8, C_{10}	Metabolites: 3:3, 5:5, 7:3 FTCA 6:2, 8:2,10:2 FTUCA	0.5 mL	Acid digestion (formic acid) + sonication	n.a.	SPE-WAX	Methanol	UPLC-MS/MS	Nilsson et al. (2013)
Breast milk	C_8–C_{12}	C_6–C_9	MePFOSAA EtPFOSAA	1 mL	Protein precipitation	Methanol	SPE on HLB	Methanol	HPLC-ESI-MS/MS	Kim et al. (2011)
Blood spots from babies	C_8, C_9	C_6, C_8		75 µL	Acid digestion (formic acid) Solvent extraction + sonication	Methanol	n.a.	n.a.	Online SPE-HPLC-MS/MS	Kato et al. (2009)
Human serum and urine	C_7–C_{11} (isomers)	C_6, C_8 (isomers)	PFOSA	0.5 mL	Serum: SPE HLB	Methanol	Centrifugation	Methanol: water	HPLC-MS/MS	Zhang et al. (2013)
				50 mL	Urine: SPE WAX					
Human whole blood	C_6–C_{13}	C_4, C_6, C_8, C_{10}	PFOSA THPFOS	0.75 mL	Acid digestion and sonication	n.a.	SPE (C18) and filtration 0.2 um	Methanol	HPLC-ES-MS	Karrman et al. (2005)
Rat plasma			8:2 FTOH	50 µL	Heating at 50 °C for 10 min	n.a.	Headspace solid-phase microextraction	n.a.	GC-MS	Fasano et al. (2006)

(continued)

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Table 2.2 (continued)

Matrix	Analytes			Sample volume	Extraction procedure		Clean-up		Analytical method	Ref.
	PFCA	PFSA	Other		Technique	Solvent	Technique	Final solvent		
Human serum	C_8–C_{10}	C_6, C_8	PFOSA Et-PFOSA-AcOH Me-PFOSA-AcOH	0.1 mL	Online-SPE	n.a.	n.a.	n.a.	Online-SPE-HPLC-ID-MS/MS	Schecter et al. (2012)

n.a. non applicable, *n.d.* not determined

[a]Composition of Masurf©FS-780 is reported here as a mix of 4:2, 6:2, 6:2–8:2, 8:2, 8:2–10:2, 10:2 diPAP, 6:2 FTMAP, EtPFOSAA-phosphate, C6–C8 PFPA C6, C6–C8, C8, C6–C10, C8–C10, C6–C12 PFPiA

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Table 2.3 Recoveries of extraction methods and performance of analytical methods used to quantify PFSAs in biological tissues and fluids

Matrix	Extraction mode	Matched matrix	Calibration	LOD or LOQ			Recovery	Ref.
				PFCA	PFSA	Other		
Human serum,	IPE	Yes	$^{13}C_4$-PFOS $^{13}C_4$-PFOA	0.03–0.06 ng/mL	0.05–0.07 ng/mL	0.07–0.12 ng/mL	PFCA >90 % PFSA >80 % Other >99 %	Kim et al. (2011)
Human breast milk	SPE-HLB	No	$^{13}C_4$-PFOS $^{13}C_4$-PFOA	0.02–0.06 ng/mL	0.06 ng/mL	0.09–0.16 ng/mL	PFCA >90 % PFSA >80 % Other >95 %	Kim et al. (2011)
Human Serum	IPE	No	THPFOS, PFBS	3–20 ng/mL	1–1.3 ng/mL	1.3–6 ng/mL	PFBS = 75 %	Kannan et al. (2004)
Human serum	Online-SPE	No	$^{13}C_2$-PFOA and $^{13}C_4$-PFOS,	0.04–0.22 ng/mL	0.01–0.08 ng/mL	0.01 ng/mL	n.d.	Wang et al. (2011)
Human serum	SPE-HLB	Yes	$^{13}C_2$-PFOA, THPFOS	0.1–3.2 ng/mL	0.3–0.4 ng/mL	0.2–0.6 ng/mL	PFCA 22–90 % PFSA >80 % Other 40–90 %	Kuklenyik et al. (2004)
Human milk	SPE-HLB	Yes	$^{13}C_2$-PFOA, THPFOS	0.1–1 ng/mL	0.3 ng/mL	0.7–0.9 ng/mL	PFCA 26–100 % PFSA >70 % Other 60–82 %	Kuklenyik et al. (2004)
Human serum and whole blood	Solvent extraction + SPE	No	$^{13}C_4$- PFOA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS,	0.03–0.25 ng/g	0.007–0.2 ng/g	0.04 ng/g	PFCA 70–90 % PFSA >70 % Other >70 %	Glynn et al. (2012)

(continued)

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Table 2.3 (continued)

Matrix	Extraction mode	Matched matrix	Calibration	LOD or LOQ			Recovery	Ref.
				PFCA	PFSA	Other		
Human hair or human nail	Solvent extraction	No	$^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUdA, $^{13}C_2$-PFDoA	0.02–0.07 ng/g	0.03–0.09 ng/g		PFCA >90 % PFSA >90 %	Li et al. (2012)
Human serum	SPE	Yes	$^{13}C_2$-PFOA, $^{18}O_2$-PFOS, $^{18}O_2$-PFOSA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDeA, $^{13}C_2$-PFDoA, D_3-Me-PFOSA, D_5-Et-PFOSA-AcOH, $^{18}O_2$-PFHxS	0.1–1 ng/mL	0.1–0.5 ng/mL	0.1–0.3 ng/mL	PFCA 22–90 % PFSA >80 % Other 40–90 %	Kato et al. (2011)
Human serum	Protein precipitation	Yes	M-PFBA, M-PFHxA, M-PFOA, M-PFDA, M-PFDoA	0.0025–2.1 ng/mL			PFCA 70–100 %	Olsen et al. (2011)
Human serum	IPE	n.d.	$^{13}C_4$-PFBA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, 13C5-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnA, $^{13}C_2$-PFDoA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, D_3-MeFOSAA, D_3-Et-FOSAA	0.001–0.005 ng/mL	0.002–0.005 ng/mL	0.001–0.04 ng/mL	PFCA >90 % PFSA >80 % Others >90 %	Lee and Mabury (2011)
Human whole blood	IPE	No	$^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_4$-PFDA, $^{13}C_2$-PFUnDA, $^{13}C_2$-PFDoDA, $^{13}C_4$-6:2 and 8:2 diPAP	10–50 pg/mL	10–20 pg/mL	10 pg/mL	PFCA >80 % PFSA >90 % Others >80 %	Loi et al. (2013)

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Whole blood ski waxers	Sonication + SPE-WAX	No	$^{13}C_4$-PFBA, $^{13}C_2$-PFHXA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUNDA, $^{13}C_2$-PFDODA, $^{18}O_2$-PFHXS, $^{13}C_4$-PFOS, $^{13}C_2$-6:2 FTUCA, $^{13}C_2$-10:2 FTUCA	0.02–0.19 ng/mL	0.01–0.04 ng/mL	0.006–0.09 ng/mL	PFOA 86 % PFSA nd Others 50–90 %	Nilsson et al. (2013)
Human urine	Solvent extraction + TFC	n.d.	$^{13}C_4$-PFBA, $1^{18}O_2$-PFHxS, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDoA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUdA, $^{13}C_2$-(6:2, 8:2, 10:2 FTCA)	0.01–4 ng/mL	0.02–0.7 ng/mL	0.05–0.9 ng/mL	PFCA: 0–129 % PFSA: 30–104 % Others: 30–172 %	Perez et al. (2012)
Human serum, urine and sweat and feces	Acid digestion, SPE solvent extraction	No	$^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA	0.5 ng/mL	0.5 ng/mL		n.d.	Genuis et al. (2010)
Human nail	IPE	Yes	n.d.	0.1 ng/g	0.3 ng/g		PFCA >90 % PFSA >90 %	Xu et al. (2010)
Human liver, kidney, brain, lung, and bone	Solvent extraction + TFC	No	$^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, (13C5-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUdA, $^{13}C_2$-PFDoA	0.001–18 ng/g	0.001–14.5 ng/g	0.001–10 ng/g	n.d.	Pérez et al. (2013)
Human organs	Solvent extraction SPE C18+ SPE SAX	Yes	PFNA	0.1–0.2 ng/g	0.1–0.2 ng/g		PFCA >78 % PFCA >78 %	Maestri et al. (2006)
Human serum and liver	IPE	Yes		6.4 ng/mL 17.9–35.9 ng/g	6.1 ng/mL 4.5 ng/g	1.6 ng/mL 7.5–19.6 ng/g	PFCA: 83–86 % PFSA: 42 %–89 % PFOSA: 67–89 %	Olsen et al. (2003)

(continued)

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Table 2.3 (continued)

Matrix	Extraction mode	Matched matrix	Calibration	LOD or LOQ			Recovery	Ref.
				PFCA	PFSA	Other		
Human serum and urine	SPE HLB SPE WAX	Yes	$^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnA, $^{13}C_8$-FOSA	8–173 ng/L	9–567 ng/L	3–30 ng/L	PFCA: 80 %–144 % PFSA: 60 %–120 % PFOSA 107–114 %	Zhang et al. (2013)
Rat blood, urine and feces	Solvent extraction	n.d.	$^{13}C_4$-PFBA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFUnA, $^{13}C_2$-6:2 FTCA, $^{13}C_2$-(6:2, 8:2, 10:2 FTUCA) $^{13}C_2$-(6:2, 8:2, 10:2- FTCA)	0.3–0.5 ng/g		0.3–10 ng/g	PFCAs: 50–110 % Others >70 %	D'Eon and Mabury (2011b)
Rat plasma	SPME headspace	No	none	nd	nd	nd	nd	Fasano et al. (2006)
Rat plasma	Protein precipitation	n.d.	$^{13}C_4$-PFOA, D-8:2 FTOH, $^{13}C_2$-8:2 FTCA	0.5–1 ng/mL		0.5–20 ng/mL	73–114 %	Himmelstein et al. (2012)
Chicken serum	Acid digestion and protein precipitation	Yes	$^{13}C_2$-PFOA	<10 ng/L			nd	Jiang et al. (2012)
Rat plasma, kidney and adipose tissue	Acid digestion + solvent extraction	No	7:2 FA-iso			5 ng/mL 4–12 ng/g	>80 %	Szostek and Prickett (2004)
Blood spots from babies	Solvent extraction + online SPE	Yes	$^{18}O_2$-PFOS, $^{13}C_2$-PFOA, $^{18}O_2$-PFHxS, $^{13}C_5$-PFNA	0.1–0.2 ng/mL	0.1–0.4 ng/mL		PFOA >100 % PFNA >50 % PFSA >100 %	Kato et al. (2009)
Human serum	Online-SPE	nd	nd	0.1–0.2 ng/mL	0.1–0.2 ng/mL	0.1–0.2 ng/mL	nd	Schecter et al. (2012)

n.d. non determined

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2.10 Analytical Methods

Liquid chromatography coupled with mass spectrometry has been the preferred method for the determination of ionic PFASs such as PFCAs and PFSAs in biological tissues and fluids. An overview of current methods and their detection limits for human samples is shown in Table 2.3. Liquid chromatography coupled with triple-quadrupole (QqQ) tandem mass spectrometry with negative electrospray ionization interface is the most commonly used technique for quantitative analysis of PFASs. In the past, some studies have been performed with single quadrupole mass spectrometers (Maestri et al. 2006). Although this method can be sensitive, it remains uncertain for the analysis in complex matrixes because of lower selectivity and matrix interference, specifically for PFOS and PFHxS where known mass interferences have been reported (Benskin et al. 2007; Keller et al. 2010). MS/MS techniques allow the monitoring of two transitions while conserving good sensitivity. Transition 499–>80 for PFOS and 399–>80 for PFHxS, must be avoided, as it has interference with bile acids naturally present in biological samples; 499–>99 and 399–>99 can be used as an alternative (Benskin et al. 2007). To obtain good separation in liquid-chromatography, the use of reverse-phase C8 and C18 stationary phase has been the common practice in the analysis of PFASs. Reiner et al. (2012) compared the use of C8 column and pentafluorophenyl stationary phase for the analysis of PFCAs (C7–C11), PFHxS and PFOS. The two phases were considered comparable, although PFP was more efficient for the separation of PFOS and TDCA, making it an interesting option for analysis where TDCA is not eliminated in the extraction procedure.

In the last few years, growing interest has been brought to the isomer-specific analysis of PFASs in biological samples. The differentiation between branched and linear isomers for PFCAs, PFSAs and PFOSA, is considered important for more accurate measurements in MS/MS, but also to elucidate sources of exposure. Good separation of isomers has been achieved using a C8 or C18 reverse phase column and longer runs (>30 min) in human serum (Benskin et al. 2007; Berger et al. 2011) and urine (Zhang et al. 2014).

With the continuous new development of PFASs on the market, new analytical techniques need to be developed for the analysis of biological tissues and fluids. Although an increasing number of isotopically labeled standards and pure standards are available, their variety cannot keep pace with the analytical needs (Berger et al. 2011). Therefore, high-resolution mass spectrometry is an interesting option. There has been very little, to no use of high resolution detection of perfluorinated compounds in human samples. HPLC coupled with time-of-flight (TOF) MS has been used for the identification of a broad range of PFASs in food packaging samples (Trier et al. 2011a, b) allowing the identification of non-previously reported PFASs in these matrices. The development of HPLC-MS-qTOF analytical methods could contribute to the determination of non-previously reported PFSAs in biological samples.

LC-methods are not suitable for all PFASs. Volatile PFASs such as FTOHs are not suited for LC-MS/MS determination. Gas-chromatography coupled with mass spectrometry (GC-MS) technique has been proven useful for the determination of FTOHs. Szostek and Prickett (2004) developed a method for the analysis of 8:2 FTOH in rat plasma, liver, kidney and adipose tissue by GC-MS. This method used electron impact ionization, and did not require a derivatization step. LODs of 5 ng/mL for serum and 4–12 ng/g for rat tissues were achieved after an extraction with MTBE for serum, and hexane for tissues. Fasano et al. (2006) developed a method for the analysis of 8:2 FTOH in dosed rat plasma by headspace analysis. Headspace solid-phase microextraction analysis requires the heating of the sample to achieve volatilization of the compounds of interest. This technique is interesting as it requires a very limited sample preparation, however limits of detection were not reported in the study. Very few studies use GC-MS techniques for the monitoring of PFASs in biological samples. Although LC-MS/MS remains the method of choice for PFASs analysis in biological samples, further method developments for GC-MS/MS methods would be interesting, namely for the quantitation of FTOHs and other more volatile PFASs in biological tissues and samples in order to obtain information on human exposure to these compounds.

To determine the total load of PFASs present in a biological sample, Miyake et al. (2007), developed a method to measure total organic fluorine in a blood sample using combustion ion chromatography. The analysis revealed that only a small percentage of fluorine content in blood of general population was explained by the analysis of routine PFASs. These results suggested the presence of uncharacterized fluorine fractions in blood, demanding further studies for the identification of these unknowns.

2.11 QA/QC

In the early 2000s, quantification of PFASs was biased by the lack of proper analytical standards, isotopically labeled surrogates and reference material. For the last few years, the quantity and quality of analytical standards in the market has drastically increased, allowing for better identification and more sensitive quantifications of PFASs. A list of current and historical isotopically labeled standard used for PFASs is shown in Table 2.3. In early studies, the lack of isotopically labeled standards was overcome by the use of THPFOS for the measure of recoveries and the quantification of PFOS and PFOA (Kannan et al. 2004; Karrman et al. 2005; Kuklenyik et al. 2004). Today, many standards and isotopically labeled standards are available, not only for PFCAs and PFSAs, but also for alternative PFASs, and have been used for their identification and quantitation in serum and whole blood; for instance FTOHs, diPAPs (D'Eon and Mabury 2011b; Lee and Mabury 2011) as well as PFASs metabolites (FTCAs, FTUCAs) (Lee and Mabury 2011; Nilsson et al. 2013). Since 2003, several reviews on PFASs analysis have stressed the

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importance of isotopically labeled standards for accurate analysis (Martin et al. 2004; van Leeuwen and de Boer 2007; van Leeuwen et al. 2006; Berger et al. 2011). Authentic labeled standards allow to correct errors in quantification that can occur after recovery losses and ion suppression due to matrix effects. To overcome these biases, some studies have used matrix-matched calibration to correct for matrix effects, using for example: cow or rabbit serum, cow milk, and even cow hoof (Olsen et al. 2003; Xu et al. 2010). An inter-laboratory study determined that when matrix-matched calibration was used, an accuracy and precision of 100 % (+/– 15 %) was obtained in human serum and plasma for PFOA, PFOS and PFNA extracted with different methods (Reagen et al. 2008).

To address the issues of laboratory performance for PFASs analysis in biological tissues and fluids, inter-laboratory studies (ILS) for the analysis of human serum and milk have been conducted. These studies have compared extraction and analytical techniques. Keller et al. (2010) compared the analysis of serum standard reference material developed by the United States National Institute of Standards and Technology (NIST). This study showed RSD lower than 26 % when comparing the analysis of PFOA, PFNA, PFHxS and PFOS, in 6 different laboratory, indicating a great improvement in inter-laboratory variability in respect to previous ILS (Lindstrom et al. 2009; Longnecker et al. 2008; van Leeuwen et al. 2006). The recent availability of a serum and milk human standard reference material produced by NIST will contribute to better QA/QC practices (Reiner et al. 2011, 2012). With the development of the analysis of PFASs in other fluids, such as urine and tissues, new ILS dealing with the analysis of these matrices could be useful. Other QA/QC practices are advised, and should be followed for accurate analysis, such as the addition of procedural blanks, matrix spikes, the use of high-purity standards and when possible, the use of structurally similar and mass labeled internal standards (Keller et al. 2010).

2.12 Conclusion

Many improvements have been made over the past 10 years for the analysis of PFASs in biological tissues and fluids. Still many challenges and uncertainties remain. Because of the ubiquitous presence of PFASs in daily use, special care must be taken in the choice of laboratory equipment to avoid contamination. The addition of procedural blanks, spikes, and the use of matched matrix calibration and identical isotopically labeled surrogate standards can significantly increase the accuracy and the precision of the analysis. When analyzing PFASs in biological samples, attention must be brought to the choice of transition to avoid interferences from matrix components. Overall, new extraction and analysis techniques have allowed the detection at very low levels (<50 pg/mL) in human serum and selected tissues. New developments include the use of online-extraction methods, allowing quick and precise analysis of a greater number of samples. These high-throughput techniques are interesting for the development of large and significant epidemiological studies.

The further development of the use of high-resolution mass spectrometry will permit the detection of previously unknown PFASs, as well as PFASs metabolites that can be used as biomarkers of exposure.

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Chapter 3
PFASs in the General Population

Kayoko Kato, Xiaoyun Ye, and Antonia M. Calafat

Abstract Perfluoroalkyl and polyfluoroalkyl substances (PFASs) have been manufactured since the 1950s for use as surface protectants for textiles and leather treatment, as protection additives in food packaging and paper products, and in firefighting foams. Some PFASs are persistent in the environment and in people, and can be transported to remote regions. The main pathways of exposure to PFASs in humans include diet, drinking water, and indoor dust, but predictors of PFASs exposures are not clearly understood. Since 2002, changes in manufacturing practices appear to have reduced exposure to some of these PFASs both in the environment and in people,- but exposure to PFASs is still widespread. We review relevant research published up to the first quarter of 2014 to understand the demographic, geographic, and temporal differences that contribute to general population exposures to PFASs around the world. We also present data on exposures to PFASs in some vulnerable population groups (e.g., pregnant women, infants, young children).

Keywords Biomonitoring • Exposure assessment • PFOA • PFOS

3.1 Introduction

Polyfluoroalkyl chemicals (PFASs) have been manufactured since the 1950s (Buck et al. 2011). Because of their chemical inertness and heat stability, PFASs have been used extensively in a variety of industrial and commercial applications, such as surfactants, lubricants, paper and textile coatings, polishes, food packaging, and fire-retarding foams (Lau et al. 2007; Prevedouros et al. 2006).

Disclaimer The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

K. Kato • X. Ye • A.M. Calafat (✉)
Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30341, USA
e-mail: Aic7@cdc.gov

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_3

ED_002330_00119699-00063

Some PFASs persist in the environment and in people, and can be transported to remote locations (Paul et al. 2009; Armitage et al. 2009; Ahrens 2011; Houde et al. 2006). Because of widespread exposure to certain PFASs in wildlife and people, and the potential adverse health impacts associated with such exposures (Lau et al. 2007; Steenland et al. 2010a), in 2002, 3M, the main worldwide manufacturer of perfluorooctane sulfonic acid (PFOS), discontinued the production of PFOS precursors and related compounds in the United States. PFOS is still produced in other countries (Paul et al. 2009; Pistocchi and Loos 2009). Other PFASs including perfluorooctanoic acid (PFOA), its salts, and precursors are also produced in other countries and still manufactured in the United States (Buck et al. 2011). However, efforts from U.S. industry and government exist to limit emissions of PFOA into the environment to reduce by 2015 the global emissions of PFOA and longer chain perfluoroalkyl acids (including their relevant precursors) to 95 % of the year 2000 levels (Buck et al. 2011; Prevedouros et al. 2006; US 2006). Similarly, regulatory and other initiatives intended to reduce environmental emissions of PFASs also exist in Canada and the European Union (Buck et al. 2011). All of these efforts appear to have reduced exposure to some of these PFASs not only in the ecosystem (Butt et al. 2007; Furdui et al. 2008; Hart et al. 2008) but also in people (Calafat et al. 2007a; Olsen et al. 2008; Haug et al. 2009) as discussed later in this chapter.

The main pathway(s) of exposure to PFASs in humans include diet (Ericson et al. 2008; Fromme et al. 2007a; Tittlemier et al. 2007; Yamaguchi et al. 2013; Holzer et al. 2011; Weihe et al. 2008; Vestergren et al. 2012; Bjermo et al. 2013; Dallaire et al. 2009), drinking water (Vestergren et al. 2012; Emmett et al. 2006; Holzer et al. 2008), and indoor dust (Vestergren et al. 2012; Kato et al. 2009a; Katsumata et al. 2006; Kubwabo et al. 2005; Martin et al. 2002; Moriwaki et al. 2003; Shoeib et al. 2005; Strynar and Lindstrom 2008; Fraser et al. 2012, 2013) although sources and routes of exposure to PFASs for children and adults may differ (Calafat et al. 2007a, b; Olsen et al. 2004a). Data on the actual levels of PFASs in people (i.e., biomonitoring data) can facilitate the exposure assessment because concentrations of these compounds in biological fluids represent an integrative measure of exposure to the target chemicals from multiple sources and routes. Blood (plasma, serum, or whole blood) is a commonly used biomonitoring matrix for assessing exposure to PFASs.

Biomonitoring data in combination with indirect measures of exposure (e.g., environmental monitoring, questionnaire information) are the most appropriate tools for exposure assessment and can provide useful information about differences in exposures by geography, demographic factors (e.g., age, sex), and socio-economic status, as well as time trends. Literature on population exposures to PFASs is exhaustive and cannot be covered comprehensively in this review. In this chapter, we present an overview of environmental exposures to PFASs in human populations based on available information up to the first quarter of 2014. Specifically, we discuss demographic, geographic, and temporal differences in exposures to PFASs among the general population. We also discuss exposures to PFASs in vulnerable population groups (e.g., pregnant women, infants, young children).

3.2 PFASs in General Population Studies

Exposure to PFASs has been estimated from the concentrations of the target PFASs in serum, plasma, or whole blood in numerous PFASs biomonitoring studies conducted around the world since the early 2000s (Haug et al. 2009; Yamaguchi et al. 2013; Holzer et al. 2011; Bjermo et al. 2013; Dallaire et al. 2009; Olsen et al. 2003, 2004b, 2005, 2012; CDC 2013a; Midasch et al. 2006; Fromme et al. 2007b, 2009; Vassiliadou et al. 2010; Schroter-Kermani et al. 2013; Ericson et al. 2007; Kannan et al. 2004; Yeung et al. 2013a, b; Harada et al. 2007; Toms et al. 2009; Haines and Murray 2012; Jin et al. 2007; Audet-Delage et al. 2013; Schecter et al. 2012; Pinney et al. 2014; Frisbee et al. 2010; Ingelido et al. 2010; Zhang et al. 2010; Wan et al. 2013; Ji et al. 2012; Bao et al. 2014; Pan et al. 2010; Kim et al. 2014). In Table 3.1, we present a selection of studies with a sample size of at least 100 participants, including two national surveys: the National Health and Nutrition Examination Survey (NHANES) (CDC 2013b), conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention in the United States, and the Canadian Health Measures Survey (CHMS) (Tremblay and Gorber 2007) administered by Statistics Canada. NHANES is designed to assess the health and nutritional status of adults and children in the United States. The survey is unique in that it combines interviews, physical examinations, and analysis of biological samples for environmental contaminants (CDC 2013b), including PFASs for Americans 12 years of age and older. Similar to NHANES, CHMS provides national data on indicators of general health, chronic and infectious diseases, and environmental biomarkers; PFASs exposure data are available for Canadians 20–79 years of age (Tremblay and Gorber 2007).

For the majority of the general populations examined, the four most commonly studied PFASs have been PFOS, PFOA, perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) (Table 3.1). Generally, PFOS showed the highest serum concentrations followed by PFOA, while other PFASs are detected both at lower concentrations and frequencies. In occupational settings or in populations accidentally exposed to specific PFASs (Emmett et al. 2006; Holzer et al. 2008; Frisbee et al. 2010; Brede et al. 2010; Holzer et al. 2009; Wilhelm et al. 2009; Winquist et al. 2013; Hoffman et al. 2011; Seals et al. 2011; Shin et al. 2011a, b; Bartell et al. 2010; Steenland et al. 2009; Frisbee et al. 2009; Beesoon et al. 2013), the concentration patterns observed may differ from those reported among the general population. We will not cover occupational exposures (the main subject of Chap. 4), but will discuss some general aspects of accidental exposures later in this chapter.

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Table 3.1 Geometric mean/mean concentrations of PFOS, PFOA, PFHxS, and PFNA in serum (or plasma/whole blood) from select population studies (N > 100) around the world from 1974 to 2011

	Concentration (ng/mL)								
Year(s)	PFOS	PFOA	PFHxS	PFNA	Age	Sample size	Location	Ref	
1974	29.5	2.3	1.6	–	30–60	178	Maryland, USA	Olsen et al. (2005)	
1989	34.7	5.6	2.4	–	39–65	178	Maryland, USA	Olsen et al. (2005)	[e]
1994–1995	40.1	5.2	5.3	–	2–12	300[a]	USA	Olsen et al. (2004a)	
1994–1995	35.2	4.7	3.9	–	2–12	298[b]	USA	Olsen et al. (2004a)	
1999–2000	30.4	5.2	2.13	0.557	12–60	1,562	USA, NHANES	CDC (2013a)	
2000	31.0	4.2	2.2	–	65–96	238	Washington, USA	Olsen et al. (2004b)	
2000–2001	34.9	4.7	1.9	–	20–69	645	USA	Olsen et al. (2012)	
2002	12.9	3.0	–	–	20–36	119	Shenyang, China	Jin et al. (2007)	
2003–2004	20.7	3.95	1.93	0.966	12–60	2,094	USA, NHANES	CDC (2013a)	
2003–2004	22.3	6.8	–	–	5–84	105[a]	Northern Bavaria, Germany	Midasch et al. (2006)	[d]
2004	18.68				1>8	857	Nunavik	Dallaire et al. (2009)	
2004	10.9	–	–	–	18–39	120[b]	Nunavik	Audet-Delage et al. (2013)	[d]
2005	13.5[c]	5.7[c]	–	–	14–67	356	Southern Bavaria, Germany	Fromme et al. (2007b)	[d]
2005–2006	20.7[c]	32.6[c]	–	–	0–12	6,536	PFOA water contaminated area, USA	Frisbee et al. (2010)	
2005–2006	19.3[c]	26.3[c]	–	–	12–18	5,934	PFOA water contaminated area, USA	Frisbee et al. (2010)	
2005–2006	19.2	32.9	3.3	1.4	12–80	>65,000	PFOA water contaminated area, USA	Frisbee et al. (2009)	
2005–2006	17.1	3.92	1.67	1.09	12–60	2,120	USA, NHANES	CDC (2013a)	
2005–2007	13.2	7.8	5.1	1.4	6–8	353[b]	Great Cincinati, USA	Pinney et al. (2014)	
2005–2009	13.2	5.7	3.0	1.7	6–8	351[b]	San Francisco Bay area, USA	Pinney et al. (2014)	
2006	14.5	3.44	1.52	0.97	20–69	600	USA	Olsen et al. (2012)	[d]

2006	5.2	2.8	0.6	–	23–49	153[b]	Siegen, Germany	Holzer et al. (2008)	[d]
2006	5.8	23.4	1.1	–	23–49	164[b]	Arnsberg, Germany, PFOA water contaminated area	Holzer et al. (2008)	[d]
2006	9.7	5.8	2.2	–	18–69	103[a]	Brilion, Germany	Holzer et al. (2008)	[d]
2006	10.5	25.3	2.5	–	18–69	101[a]	Arnsberg, Germany, PFOA water contaminated area	Holzer et al. (2008)	[d]
2006–2007	26.0	11.0	2.6	–	14–88	105	Lake Mohne, Germany	Holzer et al. (2011)	[d]
2006–2008	10.6	1.39	0.57	–	18–75	233	China	Pan et al. (2010)	[e]
2007–2008	13.20	4.12	1.95	1.22	12–60	2,100	USA, NHANES	CDC (2013a)	
2007–2009	11.13	2.94	–	–	20–79	1,376[a]	Canada, CHMS	Haines and Murray (2012)	
2007–2009	7.07	2.17	–	–	20–79	1,504[b]	Canada, CHMS	Haines and Murray (2012)	
2008	8.21	3.5	1.84	1.45	>20	140	Korea	Ji et al. (2012)	
Unknown	11.5[c]	7.97[c]	2.3[c]	2.65[c]	20–71	306	Korea	Kim et al. (2014)	
2008–2010	8.5	1.8	–	–	53–79	153[a]	Sweden	Bao et al. (2014)	[e]
2009	4.10[c]	2.85[c]	1.20[c]	1.20[c]	<13	300	Texas, USA	Schecter et al. (2012)	
2009–2010	9.32	3.07	1.66	1.26	12–60	2,233	USA, NHANES	CDC (2013a)	
2009–2010	5.8[c]	2.1[c]	–	–	16–76	607	Japan	Yamaguchi et al. (2013)	
2010	8.30	2.44	1.34	0.83	20–69	600	USA	Olsen et al. (2012)	[d]
2010–2011	7.65[c]	3.24[c]	1.08[c]	0.95[c]	16–63	153	Hong Kong	Wan et al. (2013)	
2010–2011	11.20[c]	2.25[c]	1.95[c]	0.80[c]	18–80	270	Sweden	Bjermo et al. (2013)	

[a]Only males
[b]Only females
[c]Median concentration
[d]Plasma
[e]Whole blood

3.3 Determinants of General Population Exposure to PFASs

Exposure to PFASs in the general population of developed countries and many developing countries is widespread, but the extent of such exposures may vary considerably (Yamaguchi et al. 2013; Vassiliadou et al. 2010; Kannan et al. 2004; Jin et al. 2007; Audet-Delage et al. 2013; Calafat et al. 2006a; Hemat et al. 2010). Comparing PFASs concentrations among populations is difficult because of differences in study design—including age, sex, and race of the populations examined—, years of sample collection, geographical location, and analytical methodologies used (e.g., isomeric profiles). Interestingly and despite these challenges, the ranges of concentrations of PFOS, PFOA, PFHxS, and PFNA are remarkably similar worldwide. For example, NHANES data in the United States during 1999–2010 are in agreement with those from American Red Cross donors in 2000–2010 (Olsen et al. 2012); from Canada in 2007 to 2008 (Haines and Murray 2012); from several European countries in 2005 to 2006 (Fromme et al. 2009), 2005–2009 (Haug et al. 2009; Vassiliadou et al. 2010; Ingelido et al. 2010) and 2010–2011 (Bjermo et al. 2013); and from China in 2009 (Zhang et al. 2010; Wan et al. 2013).

Research is ongoing to evaluate the determinants of exposure to PFASs, but exposures to PFASs may be associated with demographic factors such as age, sex and race. Racial differences in PFASs (e.g., PFOA, PFNA, PFHxS) serum concentrations were observed in the United States (Kato et al. 2011). For instance, regardless of age, Americans of Mexican descent had lower adjusted geometric mean serum concentrations of PFNA than non-Hispanic white and non-Hispanic black Americans (Kato et al. 2011). For PFHxS, non-Hispanic whites and non-Hispanic blacks had similar concentrations, and both were higher than for Mexican Americans; at older ages, however, concentrations were different only among Mexican Americans and non-Hispanic whites (Kato et al. 2011). These differences may reflect variability in exposures as a result of differences in lifestyle, diet (Holzer et al. 2011; Zhang et al. 2010; Halldorsson et al. 2008; Rylander et al. 2010), use of products containing PFASs, physiology (e.g., elimination) (Han et al. 2008), or a combination of these factors.

Higher concentrations of PFOS, PFOA, and PFHxS among males than among females have been reported in diverse adult populations around the world (Calafat et al. 2007a; Olsen et al. 2008; Bjermo et al. 2013; Dallaire et al. 2009; Fromme et al. 2007b, 2009; Vassiliadou et al. 2010; Ericson et al. 2007; Yeung et al. 2013a, b; Haines and Murray 2012; Ingelido et al. 2010; Ji et al. 2012; Kato et al. 2011), suggesting the possibility of sex-related exposure differences, perhaps in terms of lifestyle or diet. In North America, NHANES (Kato et al. 2011) and CHMS (Haines and Murray 2012) data suggested differences in PFASs concentrations according to sex. Canadian men had higher plasma PFOS and PFOA concentrations than women (Haines and Murray 2012). In the United States, males had higher adjusted geometric mean serum concentrations of PFOS, PFOA, and PFHxS than females regardless of age (Kato et al. 2011). In addition, males had higher adjusted geometric mean serum concentrations of PFOA, PFHxS, and PFNA than females regardless of race/

ethnicity. Differences in concentrations of PFOS, PFOA, PFNA, and PFHxS by sex appeared to be more pronounced in younger than in older Americans. These concentration trends may be related to sex-related differences in exposures to these PFASs even at an early age; they may also be related to physiological differences by sex, including differences in urinary elimination due to the renal resorption of perfluoroalkyl acids by organic anion transporters (Han et al. 2008). In addition, menses (Harada et al. 2005; Taylor et al. 2014), pregnancy (Yamaguchi et al. 2013; Monroy et al. 2008) and lactation (Bjermo et al. 2013; Kubwabo et al. 2013; Karrman et al. 2007a) may affect elimination of PFASs in females and also contribute to differences in PFASs exposure between men and women (Knox et al. 2011; Harada et al. 2004).

Increasing serum concentrations as people age are common for lipophilic persistent pollutants, such as polychlorinated biphenyls, but PFASs do not partition into fat deposits in the body (Conder et al. 2008). Nonetheless, suggestive associations between age and exposure to some PFASs have been reported, although without consistent trends among studies. Geometric mean serum concentrations of PFOS, PFOA, and PFNA did not differ significantly among age groups for Americans older than 12 years from NHANES 1999–2000 (Calafat et al. 2007a), in agreement with findings from several other studies outside the United States (Olsen et al. 2008; Vassiliadou et al. 2010; Ericson et al. 2007). By contrast, geometric mean serum concentrations of PFOS and PFNA tended to increase with age regardless of sex when combining data from four NHANES cycles (1999–2008) (Kato et al. 2011). In another study, PFOS concentration in pooled serum collected from over 2000 Australian donors between 2006 and 2007 was also significantly higher in adults (>60 years) than in children (Toms et al. 2009). The increase of production of PFASs since 1970s might have resulted in increased exposure over time for persons aged >30 years at the time of blood collection in the mid 2000s (Toms et al. 2009). Other studies also reported increase of PFASs concentrations with age (Haug et al. 2009; Yamaguchi et al. 2013; Bjermo et al. 2013; Dallaire et al. 2009; Holzer et al. 2008; Fromme et al. 2007b).

For PFHxS, however, the adjusted geometric mean serum and 95th percentile concentrations were higher for adolescents than for adults in NHANES (Kato et al. 2011). Higher concentrations of PFHxS in adolescents could be related to youth's increased contact with carpeted floors because PFHxS had been used for specific postmarket carpet-treatment applications (Olsen et al. 2004a); carpets and upholstered furniture are known to trap dust, which may also contain PFHxS (Vestergren et al. 2012; Kato et al. 2009a; Katsumata et al. 2006; Kubwabo et al. 2005; Martin et al. 2002; Moriwaki et al. 2003; Shoeib et al. 2005; Strynar and Lindstrom 2008; Fraser et al. 2012, 2013). The lack of consistent age trends for PFASs may be related to differences in early life—including in-utero—exposure to these compounds, ongoing exposures being much lower than previous historical exposures when production of the chemicals peaked, poor urinary elimination due to the renal resorption of perfluoroalkyl acids by organic anion transporters (Han et al. 2008), or a combination of these factors.

Even though exposure to PFASs is widespread, differences in exposures between urban and suburban locations or among various countries also exist (Yamaguchi et al. 2013; Vassiliadou et al. 2010; Kannan et al. 2004; Jin et al. 2007; Audet-Delage et al. 2013; Calafat et al. 2006a; Hemat et al. 2010). Factors such as the environment (e.g., air and water quality), diet, and other lifestyle choices which can vary considerably among regions and even within the same country (Fromme et al. 2009; Zhao et al. 2011; Martin et al. 2010; Trudel et al. 2008; Vestergren et al. 2008; Paustenbach et al. 2007; Washburn et al. 2005) likely play a role in the observed differences. Accidental exposure to certain PFASs (Brede et al. 2010; Oliaei et al. 2013; Post et al. 2013; Weiss et al. 2012; Lindstrom et al. 2011; Wilhelm et al. 2010; Renner 2009), mainly from contaminated drinking water, is one specific example of within country differences.

In the mid–Ohio River Valley in the United States, almost 70,000 residents living near a fluoropolymer production facility had mean PFOA serum concentrations much higher than the geometric mean serum concentration in NHANES participants during the same time period (Emmett et al. 2006; Frisbee et al. 2010; Winquist et al. 2013; Hoffman et al. 2011; Seals et al. 2011; Shin et al. 2011a, b; Bartell et al. 2010; Steenland et al. 2009; Frisbee et al. 2009). The increased PFOA concentration was associated with consumption of drinking water contaminated with PFOA (Emmett et al. 2006; Winquist et al. 2013; Hoffman et al. 2011; Seals et al. 2011; Shin et al. 2011a, b; Bartell et al. 2010; Steenland et al. 2009). A similar situation occurred in Arnsberg, Germany, where about 40,000 residents were exposed to PFOA-contaminated drinking water (Holzer et al. 2008; Brede et al. 2010; Holzer et al. 2009; Wilhelm et al. 2009). In another study from Germany, blood PFOS concentrations in a group of ten people who drank contaminated water from private wells were higher than among the general population (Weiss et al. 2012).

Of interest, exposure patterns in populations accidentally exposed to specific PFASs (Emmett et al. 2006; Holzer et al. 2008, 2009; Brede et al. 2010; Wilhelm et al. 2009; Winquist et al. 2013; Hoffman et al. 2011; Seals et al. 2011; Shin et al. 2011a, b; Bartell et al. 2010; Steenland et al. 2009; Beesoon et al. 2013; Weiss et al. 2012) can differ considerably from those reported among the general population (Emmett et al. 2006; Holzer et al. 2008, 2009; Brede et al. 2010; Wilhelm et al. 2009; Winquist et al. 2013; Hoffman et al. 2011; Seals et al. 2011; Shin et al. 2011a, b; Bartell et al. 2010; Steenland et al. 2009). Studies of such populations may be useful to both evaluate associations between exposures to PFASs and potential health effects (Frisbee et al. 2010; Barry et al. 2013; Darrow et al. 2013; Vieira et al. 2013; Lopez-Espinosa et al. 2011, 2012; Savitz et al. 2012; Innes et al. 2011; Stein and Savitz 2011; Nolan et al. 2010; Steenland et al. 2010b; Nolan et al. 2009; Stein et al. 2009) as well as the efficacy of interventions to remove the PFASs from the contamination source (e.g., water) (Pinney et al. 2014; Bartell et al. 2010; Rumsby et al. 2009). For instance, certain drinking water treatments including granular activated carbon adsorption can remove PFOA and other long chain PFASs from the potable water supply (Eschauzier et al. 2012; Flores et al. 2013; Rahman et al. 2014; Takagi et al. 2011) and effectively reduced exposure to PFOA in consumers of treated drinking water (Pinney et al. 2014; Bartell et al. 2010; Rumsby et al. 2009).

Biomonitoring concentrations provide an integrated measure of exposures through all potential sources and routes of exposure (Calafat et al. 2006b), but biomonitoring data may also be useful to identify potential exposure pathways. Synthesis of PFASs has employed electrochemical fluorization (ECF) or fluorotelomerization. ECF generates linear as well as branched isomers, but telomerization exclusively generates linear isomers (Vyas et al. 2007). In a standard product after ECF, the proportion of PFOS isomers was 70 % linear and 30 % branched; ECF PFOA had a consistent isomer composition of 78 % linear and 22 % branched (Benskin et al. 2010a). The presence of PFOS and PFOA branched isomers was first noted in 2001 (Hansen et al. 2001). Limited data exist on the toxicokinetics of the various isomers (Benskin et al. 2009a, b; De Silva et al. 2009), but the structural isomer patterns in humans may be useful for understanding the routes and sources of exposure to PFASs (De Silva and Mabury 2006; Karrman et al. 2007b; Benskin et al. 2010b).

In 70 blood samples collected in 1997–2003 from Sweden, the United Kingdom, and Australia, linear PFOS was the main isomer comprising 58–70 % of the total PFOS measured, depending on the location (Karrman et al. 2007b); similarly, linear PFOS was 53 % of the total PFOS measured in 20 Canadians' blood samples collected in 2007–2008 (Zhang et al. 2013a). Differences in isomeric distributions may relate to different isomer patterns in the source products or to country-specific differences in the major human exposure pathways (Karrman et al. 2007b). The different ratio of the PFOS isomers could also indicate differential uptake of the branched and linear PFOS isomers, and also reflect different renal clearances (Zhang et al. 2013a) or tranceplacental transfer (Hanssen et al. 2010) in humans.

From 1947 to 2002, worldwide production of PFOA was mainly by ECF and exposure to both linear and branched isomers likely occurred. Branched PFOA isomers were detected in 96.9 % of NHANES 1999–2000 participants sera, with a median (25th–95th percentiles) percentage of branched PFOA isomers of 4.2 % (2.7–9.9 %) (Kato et al. 2011). By contrast, only the linear PFOA isomer was detected among NHANES 2007–2008 participants (Kato et al. 2011). Similarly, in 16 pooled sera collected across the Midwest United States during 2004 and 2005, only between 1.6 and 2.3 % of the mean concentrations of PFOA, PFNA, and another PFAS, perfluoroundecanoate, were branched isomers (De Silva and Mabury 2006). The relatively high proportion of linear PFOA in serum in these studies may be partly due to exposure to and metabolism of fluorotelomer alcohols and olefins, two classes of PFASs synthesized by the telomerization process (Benskin et al. 2010a). Linear isomers of PFASs also predominated in wildlife during 1999–2003 (Butt et al. 2010). Together, the above findings suggest that telomer products may have contributed to PFOA burden after the phase-out of ECF products (Prevedouros et al. 2006; Ellis et al. 2004).

Paired blood and urine samples (N = 86) collected from Chinese adults in 2010 were analyzed for linear and branched PFOS and PFOA isomers (Zhang et al. 2013a). PFOS and PFOA concentrations in urine and blood were correlated, but the percentage of linear and branched isomers in the two matrices differed. The mean percentage of linear PFOS in blood (53 %) was significantly lower than in the ECF

standard (70 %), but the mean percentage of linear PFOA (97 %) was higher than in the ECF standard (78 %) (Zhang et al. 2013a). Interestingly, the mean percentage of linear isomers in urine (PFOS, 45 %; PFOA, 94 %) was lower than in blood (Zhang et al. 2013a) suggesting preferential excretion of the branched isomers of PFOA and PFOS in urine (Zhang et al. 2013a). Results from this study also suggested that perfluoroalkyl carboxylates (PFCAs) were excreted more efficiently in urine than their corresponding perfluoroalkane sulfonates of the same carbon chain-length. Also, although urinary excretion was a major elimination route for short PFCAs ($C \leq 8$), other routes of excretion likely contribute to overall elimination for longer PFCAs (e.g., PFOA), PFHxS and PFOS.

3.4 Temporal Trends in Exposure to PFASs

PFASs manufacturing started in the 1950s and peaked in the 1980s–1990s (Prevedouros et al. 2006; Paul et al. 2009). Estimates suggest that the global production volumes and environmental releases of PFOS and its precursors started to decrease in the mid 1990s, but voluntary emission reduction measures were not implemented before 1997 (Paul et al. 2009). Concerns about the potential environmental and toxicological impact of certain PFASs led to (a) several major changes in manufacturing practices (Prevedouros et al. 2006; Paul et al. 2009; Pistocchi and Loos 2009; US 2006), and (b) other initiatives to reduce environmental emissions of these compounds or their precursors (Buck et al. 2011). First, 3M Company, the main global manufacturer of perfluorooctanesulfonyl fluoride (POSF)-based materials (Prevedouros et al. 2006), including PFOS, PFOA and related compounds, phased out the production of these chemicals in 2000–2002. Furthermore, the US Environmental Protection Agency and eight leading global companies participated in a stewardship agreement to reduce emissions and product content of PFOA and related chemicals by 95 % by 2010 and to work toward their elimination by 2015 (US 2006). Canadian environmental and health authorities and five companies reached a similar agreement to restrict certain PFASs in products, and a European Union Marketing and Use Directive restricted the use of "perfluorooctane sulfonates" in the European Union (Buck et al. 2011). Last, PFOS was added to the persistent organic pollutants list of the Stockholm Convention in May 2009 as an Annex B substance (i.e., restricted in its use) (Ahrens 2011). All of these changes have impacted exposure to PFASs as discussed below.

Temporal trends have been investigated in the United States (Olsen et al. 2005, 2012; Kato et al. 2011), Germany (Schroter-Kermani et al. 2013; Yeung et al. 2013a, b), Norway (Haug et al. 2009), Sweden (Glynn et al. 2012), Australia (Toms et al. 2009), Japan (Harada et al. 2007), and China (Jin et al. 2007; Chen et al. 2009). Despite differences in design among studies—pools vs individual specimens, plasma vs serum, sample size, time period—, PFASs concentrations in people follow similar increasing trends from the 1970s to the mid 1990s because of the high production and widespread use of this class of compounds and their resulting

emissions (Prevedouros et al. 2006; Paul et al. 2009). For instance, participants in two community-based cohorts from Maryland in the United States had blood concentrations of PFOS, PFOA, and PFHxS, among other PFASs, significantly higher in 1989 than in 1974 (Olsen et al. 2005). In Japan, serum concentrations of PFOS and PFOA from urban females increased 3 and 14 times, respectively, between 1977 and 1995, before plateauing between 1991 and 2003 (Harada et al. 2004). In Chinese students, faculty members and university workers, median serum concentrations of PFOA and PFOS increased significantly from 1987 until 2002 (Jin et al. 2007). Similar time trends were observed in Sweden using pooled milk samples: PFOS and PFOA concentrations increased significantly from 1972 to 2000, and showed statistically significant decreasing trends during 2001–2008 (Sundstrom et al. 2011).

Compared to the late 1990s, serum concentrations of PFOS and PFOA have shown a downward trend worldwide since the 2000s. In a Norwegian study using 57 pooled samples collected from 1976 to 2007, serum concentrations of PFOS and PFOA in men increased ninefold from 1977 to the mid 1990s, then reached a plateau before starting to decrease around the year 2000 (Haug et al. 2009); PFOA concentrations decreased by about 40 % between 2000 and 2006 in Norwegian men 40–50 years old (Haug et al. 2009). Similarly, plasma concentrations of PFOS and PFOA in 420 samples collected from residents of two German cities decreased between 2000 and 2009 (Yeung et al. 2013a, b). Sera collected from Swedish primiparous women sampled three weeks after delivery in 1996–2010 also showed decreasing concentrations of PFOS and PFOA (Glynn et al. 2012). In the period from 2002 to 2009, PFOA concentrations in serum pools from Australians older than 16 years decreased by about 50 % (Toms et al. 2009). In American Red Cross donors, PFOA geometric mean serum concentrations decreased from 4.7 ng/mL (2000–2001) to 2.44 ng/mL (2010) (Olsen et al. 2012). Similar trends were observed among the US general population with geometric mean serum concentrations decreasing from 5.2 ng/mL (PFOA) and 30.4 ng/mL (PFOS) in 1999–2000 to 3.07 ng/mL (PFOA) and in 9.32 ng/mL (PFOS) in 2009–2010 (CDC 2013a) although from 2005 to 2008, PFOA adjusted concentrations appeared to increase for males but remained the same for females (Kato et al. 2011).

Compared with PFOS and PFOA, concentrations of PFNA in NHANES participants showed an upward trend, regardless of race/ethnicity since 1999–2000 (Kato et al. 2011). The geometric mean serum concentration of PFNA in the US general population increased more than twofold between 1999–2000 and 2009–2010 (CDC 2013a). In German residents, plasma concentrations of PFNA also increased during 2000–2009 while those of PFOS and PFOA decreased (Yeung et al. 2013a, b). Because PFNA was present as a reaction by-product in POSF-based materials (Prevedouros et al. 2006) which are no longer produced in the United States since 2000–2002, the observed PFNA concentration trends may be related to the degradation of volatile fluorotelomer alcohols (Ellis et al. 2004). These human data are also in agreement with wildlife data suggesting that concentrations of PFNA and certain longer chain-length PFASs show an upward trend in the same time period (Olsen et al. 2012; Yeung et al. 2013a; Glynn et al. 2012; Dietz et al. 2008).

3.5 Exposure to PFASs in Vulnerable Populations

Biomonitoring studies among pregnant women, infants, and young children are of interest because stressors, including chemical exposures, during these critical time periods may impact health later in life. Unfortunately, these segments of the population are poorly represented in general population biomonitoring surveys such as NHANES (CDC 2006) and CHMS (Haines and Murray 2012). For instance, to date, published data on background exposure to PFASs among pregnant women in the United States general population are limited to only 180 of 1,079 women 17–39 years of age who participated in 2003–2008 NHANES (Woodruff et al. 2011; Jain 2013). Information on background exposure to PFASs exist for pregnant women or newborns in other countries including Great Britain (Maisonet et al. 2012), Denmark (Kristensen et al. 2013; Fei et al. 2009), Norway (Ode et al. 2013), Sweden (Starling et al. 2014), Canada (Monroy et al. 2008; Hamm et al. 2010), China (Wu et al. 2012), and Japan (Washino et al. 2009). In Table 3.2, we present concentrations of PFASs in women during pregnancy or at delivery, or infants shortly after birth from select studies with sample sizes of at least 30 participants (Monroy et al. 2008; Karrman et al. 2007a; Maisonet et al. 2012; Kristensen et al. 2013; Fei et al. 2009; Ode et al. 2013; Starling et al. 2014; Hamm et al. 2010; Wu et al. 2012; Washino et al. 2009; Whitworth et al. 2012; Stein et al. 2012; Liu et al. 2011; Lee et al. 2013; Kim et al. 2011a; Inoue et al. 2004; Hanssen et al. 2013; Fromme et al. 2010),

Research has also shown that PFASs can be transported across the placenta and several PFASs have been detected in cord serum (Monroy et al. 2008; Hanssen et al. 2010; Glynn et al. 2012; Ode et al. 2013; Liu et al. 2011; Lee et al. 2013; Kim et al. 2011a; Inoue et al. 2004; Hanssen et al. 2013; Fromme et al. 2010; Arbuckle et al. 2013; Lien et al. 2013; Porpora et al. 2013; Zhang et al. 2011, 2013b; Chen et al. 2012; Gutzkow et al. 2012; Llorca et al. 2012; Beesoon et al. 2011; Kim et al. 2011b; Lien et al. 2011; Apelberg et al. 2007; Midasch et al. 2007; Needham et al. 2011). Furthermore, data on paired maternal and cord blood PFASs concentrations also exist for populations around the world (Monroy et al. 2008; Hanssen et al. 2010; Glynn et al. 2012; Ode et al. 2013; Liu et al. 2011; Lee et al. 2013; Kim et al. 2011a, b; Hanssen et al. 2013; Fromme et al. 2010; Porpora et al. 2013; Zhang et al. 2013b; Gutzkow et al. 2012; Beesoon et al. 2011; Midasch et al. 2007; Needham et al. 2011). Interestingly, the ratio of concentrations between maternal and infant's samples vary depending on the compound. For example, ratios between maternal and cord serum concentration were ~1 for PFOA but ~2 for PFOS (Monroy et al. 2008; Hanssen et al. 2010; Ode et al. 2013; Lee et al. 2013; Kim et al. 2011a, b; Fromme et al. 2010; Porpora et al. 2013; Zhang et al. 2013b; Gutzkow et al. 2012; Beesoon et al. 2011; Midasch et al. 2007) suggesting differences in the partition of these compounds. Taken together, these results suggest that PFAS exposure is ubiquitous in pregnant women and their newborns.

Although infants and young children are exposed to PFASs, data in these age groups are still rather limited (Olsen et al. 2004a; Toms et al. 2009; Schecter et al.

Table 3.2 Median/geometric mean concentrations of PFOS, PFOA, PFHxS, and PFNA in vulnerable populations from select studies (N > 30) around the world from 1978 to 2011

	Concentration (ng/mL)							
Year(s)	PFOS	PFOA	PFHxS	PFNA	Sample size	Sample type	Location	Ref
1978–2001	15	2.1	0.24	–	263	Maternal serum at delivery	South Sweden	Ode et al. (2013)
1978–2001	6.5	1.7	0.2	–	263	Umbilical cord serum		Ode et al. (2013)
1988–1989	21.1	3.6	–	–	343	Maternal serum at 30 weeks	Denmark	Kristensen et al. (2013)
1991–1992	19.6	3.7	1.6	–	447	Maternal serum	Avon, Great Britain	Maisonet et al. (2012)
1996–2002	33.7	5.3	–	–	1,240	Maternal plasma	Denmark	Fei et al. (2009)
2002–2005	4.9[a]	1.2[a]	–	–	428	Maternal serum at delivery	Hokkaido, Japan	Washino et al. (2009)
2003–2004	13.03	2.25	0.60	0.39	891	Maternal plasma at middle of pregnancy	Norway	Starling et al. (2014)
2004–2005	5.94[a]	1.84[a]	–	2.36[a]	439	Umbilical cord plasma	Taiwan	Chen et al. (2012)
2004–2005	4.9[a]	1.6[a]	–	–	299	Umbilical cord serum	Maryland, USA	Apelberg et al. (2007)
2004–2005	16.6	2.13	1.82	0.73	101	Maternal serum at 24–28 weeks	Canada	Monroy et al. (2 008)
2004–2005	14.54	1.81	1.62	0.69	101	Maternal serum at delivery		Monroy et al. (2008)
2004–2005	6.08	1.58	2.07	0.72	105	Umbilical cord serum		Monroy et al. (2008)
2005–2006	1.6	1.3	0.5	0.5	71	Maternal serum at delivery	South Africa	Hanssen et al. (2010)
2005–2006	0.7	1.3	0.3	0.2	58	Umbilical cord serum		Hanssen et al. (2010)
2005–2006	7.8	1.5	0.97	–	252	Maternal serum at 15 weeks	Alberta, Canada	Hamm et al. (2010)
2005–2008	4.443[a]	1.469[a]	0.579[a]	0.359[a]	100	Umbilical cord serum	Ottawa, Canada	Arbuckle et al. (2013)
2007	–	16.95	–	–	108	Maternal serum	Guiyu, China	Wu et al. (2012)
2007	–	8.70	–	–	59	Maternal serum	Chaonan, China	Wu et al. (2012)
2007–2008	4.99	1.22	0.34	0.28	123	Maternal plasma at delivery	Norway	Gutzkow et al. (2012)
2007–2008	1.52	0.88	0.12	0.20	123	Umbilical cord plasma		Gutzkow et al. (2012)
2007–2009	3.2	2.4	0.5	0.6	44	Maternal whole blood	Munich, Germany	Fromme et al. (2010)

(continued)

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Table 3.2 (continued)

	Concentration (ng/mL)							
Year(s)	PFOS	PFOA	PFHxS	PFNA	Sample size	Sample type	Location	Ref
2007–2009	3.2	1.9	0.5	0.6	38	Maternal whole blood at delivery		Fromme et al. (2010)
2007–2009	1.0	1.4	0.2	<0.4	33	Umbilical cord blood		Fromme et al. (2010)
2008–2009	2.9	2.4	–	–	38	Maternal serum at delivery	Rome, Italy	Porpora et al. (2013)
2008–2009	1.1	1.6	–	–	38	Umbilical cord serum		Porpora et al. (2013)
2009	2.92	1.264	0.068	0.483	50	Maternal serum at delivery	Jinhu, China	Liu et al. (2011)
2009	1.47	1.115	0.055	0.315	50	Umbilical cord serum		Liu et al. (2011)
2011	9.37	2.62	1.21	–	70	Maternal serum at delivery	South Korea	Lee et al. (2013)
2011	3.18	2.08	0.57	–	70	Umbilical cord serum		Lee et al. (2013)
2003–2004	1.59	0.73	1.64	0.35	20[b]	Dried blood spot, infant (newborn screening program)	New York, USA	Spliethoff et al. (2008)
2007	2.1[a]	0.9[a]	0.4[a]	0.3[a]	98	Dried blood spot, infant	Texas, USA	Kato et al. (2009c)

[a]Geometric mean
[b]Pooled samples

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2012; Pinney et al. 2014; Kato et al. 2009b) in part because of the difficulties in obtaining blood from newborns and young children. Using dry blood spots (DBS) or residual specimens can overcome this limitation. In the United States, DBS are collected routinely from newborns within 48 h of birth for the main purposes of screening for metabolic and other health disorders. A couple of studies relied on using residual newborn DBS stored by state public health departments to demonstrate exposure to PFASs including PFOS, PFOA, PFNA, and PFHxS in Texas (Kato et al. 2009c) and New York infants (Spliethoff et al. 2008) (Table 3.2).

Three studies, two in the United States and one in Australia, used residual serum specimens collected during routine health exams to evaluate exposure to PFASs among young children (Toms et al. 2009; Schecter et al. 2012; Kato et al. 2009b). In the first study, researchers used 936 samples collected from U.S. children participants in NHANES in 2001–2002 to prepare pools that were analyzed for several PFASs. Mean concentrations of PFOS, PFOA, PFNA, and PFHxS in these pools were similar regardless of age (3–5 or 6–11 years) or sex, but were higher than the mean concentrations reported in pools from adolescents and adults NHANES 2001–2002 participants (Kato et al. 2009b). In the second US study, PFASs were detected in serum collected in late 2009 from 300 Texas children from birth through 12 years of age, several years after phasing out the manufacture of POSF-based materials (Schecter et al. 2012). Of note, serum concentrations of PFOS, PFOA, PFNA, and PFHxS did not significantly differ by sex, unlike findings from adult populations (Calafat et al. 2007a; Olsen et al. 2008; Bjermo et al. 2013; Dallaire et al. 2009; Fromme et al. 2007b, 2009; Vassiliadou et al. 2010; Ericson et al. 2007; Yeung et al. 2013a, b; Haines and Murray 2012; Ingelido et al. 2010; Ji et al. 2012; Kato et al. 2011). By constrast, concentrations appeared to increase with age, perhaps because the older children experienced higher exposures to PFASs in the late 1990s–early 2000s when environmental levels of these compounds were higher. In another study (Toms et al. 2009), investigators examined the concentrations of several PFASs in pools made from individual sera collected in 2006–2007 in southeast Queensland, Australia from 2,420 male and female donors between birth to >60 years of age. PFOS, PFOA and PFNA were detected in all pools; PFOS was detected at the highest mean concentration followed by PFOA. Concentration differences by sex were not apparent among children <12 years, in agreement with the results from the Texas children (Schecter et al. 2012), and concentration patterns by age varied depending on the compound.

The relevance of sources and routes of exposure to certain PFASs in children may differ from those in adults. For example, investigators reported higher serum mean concentrations of selected PFASs, specifically PFHxS and 2-(N-methyl-perfluorooctane sulfonamido) acetate (Me-PFOSA-AcOH), from U.S. children than from adults (Olsen et al. 2004a). Me-PFOSA-AcOH is a known oxidation product of 2-(N-methyl-perfluorooctane sulfonamido) ethanol, which was used primarily in surface treatment applications for carpets and textiles (Olsen et al. 2003). PFHxS was used as a building block for compounds incorporated in fire-fighting foams and specific postmarket carpet treatment applications (Olsen et al. 2003). One explanation for the apparent greater mean concentrations of PFHxS and Me-PFOSA-AcOH

in children than in adolescents and adults was increased exposure among children resulting from increased contact with carpeted floors and upholstered furniture coupled with hand-to-mouth activity. Carpets and upholstered furniture are known to trap dust, which may contain PFHxS. In fact, the mean concentrations of PFHxS in house dust samples collected in North America were higher than for other PFASs (Kato et al. 2009a; Strynar and Lindstrom 2008; Beesoon et al. 2013) indoor dust concentration data on Me-PFOSA-AcOH were also relatively high (Kato et al. 2009a).

Unlike lipophilic persistent organic pollutants such as polychlorinated biphenyls, PFASs bind to plasma proteins (Butenhoff et al. 2012; Wu et al. 2009; Han et al. 2003). However, PFASs have also been detected in human milk (Kubwabo et al. 2013; Karrman et al. 2007a; Sundstrom et al. 2011; Barbarossa et al. 2013; Guerranti et al. 2013; Karrman and Lindstrom 2013; Croes et al. 2012; Fujii et al. 2012; Kadar et al. 2011; Karrman et al. 2010; Liu et al. 2010; Llorca et al. 2010; Nakata et al. 2009; von Ehrenstein et al. 2009; Tao et al. 2008; So et al. 2006; Lankova et al. 2013), albeit at concentrations approximately one order of magnitude lower than in serum. Therefore, breast milk can be a source of exposure to PFASs and nursing may reduce the PFASs body burden in lactating women (Pinney et al. 2014; Loccisano et al. 2013; Haug et al. 2011; Mondal et al. 2014).

3.6 Conclusions

Diet, drinking water, and indoor dust are important sources of human exposure to PFASs; in utero and lactational exposure to PFASs are also relevant for certain segments of the population. Comparing PFASs concentrations among populations is difficult because of differences in study design (e.g., age, sex, race of the populations examined), timing of sample collection, geographical location, and analytical methodologies used (e.g., isomeric profiles). Interestingly, the concentration ranges of the most commonly studied PFASs, PFOS and PFOA, are remarkably similar in people worldwide, although important differences may exist (e.g., accidental exposures; developed vs developing countries).

Due to regulatory and voluntary efforts to reduce emissions of PFASs, human exposure to some of the PFASs appears to have decreased since the early 2000s. However, PFASs are still ubiquitously detected in people around the world. Concerns remain regarding the importance of past and present exposure sources on the human body burden of PFASs and on the potential adverse health effects of such exposures. Age; diet; route, frequency, and magnitude of exposure; potential synergistic or antagonistic interactions among chemicals; and genetic factors, among others, are critical in determining health outcomes associated with exposure to PFASs and other environmental chemicals.

Biomonitoring efforts are important to facilitate the risk assessment of PFASs. Comprehensive biomonitoring programs, such as NHANES and CHMS, provide a reliable estimate of PFASs internal dose among the general population. In addition,

future research should continue to improve our understanding of (i) determinants of exposure to PFASs, (ii) PFASs toxicokinetics with emphasis on fetal and neonatal exposures, when susceptibility to potential adverse health effects of environmental chemicals may be highest, and (iii) specific populations with known source(s) of exposure to evaluate potential health effects as well as the efficacy of intervention strategies to reduce exposures.

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ED_002330_00119699-00088

Chapter 4
PFAS Biomonitoring in Higher Exposed Populations

Geary W. Olsen

Abstract Perfluoroalkyl and polyfluoroalkyl substances (PFAS) have a wide range of applications as a result of their chemical and thermal stability of the C-F bond and their hydrophobic and lipophobic characteristics. Because of these unique physical and chemical properties there have been numerous industrial and consumer applications. These characteristics have also resulted in the widespread presence and persistence of PFAS in the environment and detection in biological tissue in humans.

In general, biomonitoring trend studies of the PFAS, in particular PFOS and PFOA, within the general population have shown marked declines in PFOS since a May 2000 phase-out announcement by a major manufacturer. The trends, however, for PFOA are more inconclusive as multiple manufacturers of PFOA and its various precursors (e.g., fluorotelomer alcohols) remain.

Higher exposed populations can be defined by identifiable exposures (e.g., environmental, occupational) that have resulted in serum, plasma, or whole blood concentrations of PFASs that are substantively larger than those reported in the general population. Although some investigators refer to these populations as 'highly exposed', this description does not sufficiently describe the magnitude of exposure that occurs within these populations and/or individuals. Thus, the term 'higher' is preferred.

For the purpose of this chapter, higher exposed populations and their serum concentrations are described into three categories: (1) PFAS manufacturing and 'downstream' production workers; (2) communities affected by specific identifiable sources of PFAS exposure (above background levels) through municipal and/or private water sources; and (3) medical, occupational, and consumer PFAS-related exposures that were targeted to a well-defined group of individuals. Each of these three higher exposure populations are reviewed separately for their biomonitoring data and then compared jointly.

G.W. Olsen, D.V.M., Ph.D. (✉)
Medical Department, 3M Company, Mail Stop 220-6W-08, St. Paul, MN 55144, USA
e-mail: gwolsen@mmm.com

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_4

ED_002330_00119699-00089

PFAS manufacturing workers had serum concentrations 2–3 orders of magnitude higher than those reported in the general population. Depending upon the communities whose drinking water sources were affected with PFAS, these populations tended to have average serum concentrations ranging between 2× and <1 order of magnitude higher than the general population. Individuals, however, within these communities may have had comparable concentrations to those at the higher levels within the manufacturing sector. Likewise, depending on the specific medical, occupational, and/or consumer exposures, there may have been substantively higher exposures to PFOS and PFOA than reported in the general population.

Keywords Perfluorochemicals • Perfluoroalkyl and polyfluoroalkyl substances • PFAS • Perfluorooctanesulfonate • PFOS • Perfluorooctanoate • PFOA • Biomonitoring

4.1 Introduction

4.1.1 Brief Review of General Population Trends

As discussed in Chap. 1 (Lau 2014), perfluoroalkyl and polyfluoroalkyl substances (PFAS) have a wide range of applications as a result of their chemical and thermal stability of the C-F bond and their hydrophobic and lipophobic characteristics. Because of these unique physical and chemical properties there are numerous industrial and consumer applications. These include surfactant (e.g., use in fluoropolymer synthesis, coatings, and aqueous film-forming-foams (AFFF)) and polymer applications (e.g., use in textiles as stain and soil repellents (e.g., carpet, clothes) and grease-proof, food contact paper) (Buck et al. 2011).

Because of their widespread presence and persistence in the environment and detection in biological tissue in humans, there has been an effort to understand PFAS exposure sources and pathways as well as potential associations with human health. In particular, this exposure research has focused on 'long chain' PFASs defined as perfluoroalkyl sulfonic acids with six or more perfluorinated carbons and perfluoroalkyl carboxylic acids with six or more perfluorinated carbons, respectively (Buck et al. 2011).

Multiple sources of potential exposure to PFAS in the environment have been identified in the general (nonoccupational) human population. These sources include food intake and packaging, drinking water (municipal and private wells), indoor (house and office) and outdoor air, and dust (Fromme et al. 2009; Domingo 2012). Dietary intake has been considered an important source of human exposure by some investigators (Fromme et al. 2009; Domingo 2012) but others have questioned this assumption noting the declining PFOS concentrations in the general

population without comparable declines of PFOS in the diet suggesting indirect sources of exposure through transformation of precursors (D'Eon and Mabury 2011; Buck et al. 2011). Although precursors of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) have not been considered a major source of exposure, subgroups of the population may be at higher risk. In general, PFAS in municipal water supplies are measured in the very low ng/L levels unless the community has been affected by specific industrial and/or environmental exposures (Fromme et al. 2009).

As detailed in the previous chapter, PFAS biomonitoring trend studies of general populations have been conducted in the United States (Kato et al. 2011; Olsen et al. 2012a), Australia (Toms et al. 2014), Germany (Yeung et al. 2013a, b), Norway (Nøst et al. 2014), and Sweden (Glynn et al. 2012; Sundström et al. 2011). Few trend studies have involved repeated measurements from the same, albeit small number, of individuals (Olsen et al. 2012a; Nøst et al. 2014). The other biomonitoring trend studies were periodic cross-sectional analyses of a general population. Collectively, these studies have analyzed and reported on 23 PFASs although only two, PFOS and PFOA, were routinely reported in these and many other studies. In general, these biomonitoring trend studies have shown declines in PFOS in the general population since the May 2000 announcement of the phase-out of perfluorooctanyl related materials by the 3M Company (3M). Results, however, for PFOA were more inconclusive as other manufacturers of PFOA remained after this phase-out including production of potential PFOA precursors (e.g., 8:2 fluorotelomer alcohol) (Buck et al. 2011; Fasono et al. 2006).

The 23 PFASs included a series of perfluoroalkyl sulfonic acids [perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), several precursors of PFOS, the homologue series of perfluoroalkyl carboxylic acids (C4 through C12), and their various precursors. The precursors of PFOS included N-ethyl perfluorooctanesulfonamidoacetate (EtFOSAA), an oxidation product of N-ethyl perfluorooctanesulfonamidoethanol (EtFOSE) which was primarily used in the building block of the phosphate ester in paper and packaging protectant applications, and N-methyl perfluorooctanesulfonamidoacetate (MeFOSAA), an oxidation product of N-methyl perfluorooctanesulfonamidoethanol (MeFOSE) that was primarily incorporated into polymer surface treatments for carpet and textile applications (Buck et al. 2011; Yeung et al. 2013b). Both compounds likely metabolize to perfluorooctanesulfonamidoacetate (FOSAA) and then to perfluorooctanesulfonamide (FOSA) (Xu et al. 2004, 2006). FOSA metabolizes to PFOS. FOSA can also be a metabolite of EtFOSE-based polyfluoroalkyl phosphate ester (di-SAmPAP) used in food contact paper. Precursors of perfluoroalkyl carboxylic acids include several polyfluoroalkyl phosphate esters which are a class of fluorotelomer-based commercial products of various fluorinated chain lengths with phosphate mono-, di-, or triesters (Yeung et al. 2013b) and the 8:2 fluorotelomer alcohol (Fasono et al. 2006, 2009).

4.1.2 Higher Exposed Populations

For the purpose of this chapter, "higher exposed" populations were defined by identifiable exposures (e.g., environmental, occupational) that resulted in larger serum, plasma, or whole blood concentrations of PFASs than those reported in the general population. Although some investigators refer to these populations as 'highly exposed', this description does not sufficiently describe the magnitude of exposure that occurs in these populations. A community may be referred to as "highly exposed" to PFASs but could be orders of magnitude lower than an occupationally exposed population, or, as will be discussed later, comprise individuals that may have exposures higher than individuals who are occupationally exposed through manufacturing. Unlike the general population, biomonitoring of higher exposed human populations have, in general, reported fewer PFAS compounds. This may be due to the specificity of exposure and therefore more targeted measurements. Nevertheless, PFOS and PFOA remain the most frequently measured compounds regardless of the type of population studied.

For the purpose of this chapter, higher exposed populations are categorized as follows: (1) PFAS manufacturing and 'downstream' production workers; (2) communities affected by specific identifiable sources of PFAS exposure (above background levels) through municipal and/or private water sources; and (3) medical, occupational, and consumer PFAS-related exposures that were targeted to a well-defined group of individuals. Each of these three categories will be reviewed separately for the biomonitoring (serum, plasma, whole blood) data reported, and then compared jointly in the chapter summary.

Provided in this chapter are summaries of PFAS concentrations measured in these higher exposed populations. The primary PFASs reviewed are PFOS and PFOA due to the frequency of measurement and reports. Not discussed in this chapter are the analytical methods and quality control procedures that were employed by the study investigators. An overview of the analytical laboratory methods that have evolved over time to measure PFASs can be found in Chap. 2. The original papers cited in this chapter should be examined by the reader to assess the precision and reliability of the analytical capabilities that were employed by the study investigators at the time of their analyses.

4.2 PFAS Manufacturing and 'Downstream' Production

Prior to its phase-out of perfluorooctanyl chemistry, the 3M Company was considered the primary manufacturer of perfluorooctanesulfonyl fluoride (POSF)-related materials but only one of several manufacturers/users of PFOA. Furthermore, there were other fluorochemical manufacturers whose product line (e.g., fluorotelomer alcohols) may degrade, to a limited degree, to PFOA. At 3M, POSF and PFOA were manufactured through electrochemical fluorination (ECF) that yielded

characteristic linear to branch isomer ratios (Buck et al. 2011). PFOA can also be manufactured by telomerization that results in a linear isomer. Because of its widespread environmental presence and persistence, in 2006 the US EPA invited eight fluoropolymer and telomer manufacturers to participate in a global stewardship program focused on the goal of working towards the elimination of PFOA and precursor chemicals as well as higher homologues by 2015 (US EPA 2006). These companies were Arkema, Asahi, BASF (successor to Ciba), Clariant, Daikin, 3M/Dyneon, DuPont, and Solvay Solexis.

Similar agreements have been obtained in other nations including perfluorocarboxylic acids in Canada (Environment Canada 2010) and PFOS in European Union countries (European Parliament 2006). PFOS was listed as an Annex B (restricted in its use) substance in the Stockholm Convention on Persistent Organic Pollutants (UN Environmental Programme 2009). Upon 3M's phase-out of PFOS production, the production of POSF–related materials in some countries, particularly China, increased (Zhang et al. 2012; Xie et al. 2013).

Among the fluorochemical, fluoropolymer and fluorotelomer manufacturers, 3M and DuPont have frequently published their biomonitoring data and therefore this information was available in peer-reviewed scientific literature and publicly accessible repositories of information (e.g., AR-226 docket of the US EPA). One other company, Miteni, had published company-specific biomonitoring (PFOA) data in the published scientific literature (Costa et al. 2009), prior to this company's phase-out of PFOA. It is possible that other PFAS biomonitoring analyses of fluorochemical and fluoropolymer manufacturing workers may have been analyzed but such information was not available in the published scientific literature.

4.2.1 3M Company

There have been four 3M Company manufacturing plants involved with the manufacture of PFAS-related materials located in the United States (Cottage Grove, Minnesota; Decatur, Alabama; Cordova, Illinois) and Belgium (Antwerp).

4.2.1.1 Cottage Grove (Minnesota)

The Cottage Grove plant manufactured the ammonium salt of perfluorooctanoic acid (APFO). APFO rapidly dissociates in the blood to PFOA (the anion) where it is bound to proteins. APFO production began at the Cottage Grove plant in 1947 and was phased-out after the May 2000 announcement by the company. Primary users included external customers (major customer was DuPont) and internal applications within the 3M/Dyneon operations. The production of APFO was a multistep process that included the following steps (Olsen et al. 2000, 2003a): electrochemical fluorination, stabilization, fractionation, distillation, purification, the addition of ammonium, drying of the salt, and packaging. The production of the

APFO could result in potential for inhalation exposure of the vapor or particulate from regular production tasks and equipment cleaning responsibilities (Raleigh et al. 2014). Exposure may also have occurred from incidental spills and background air concentration levels. Dermal and ingestion were other potential exposure pathways although deemed less likely than inhalation.

Salts of perfluorooctanesulfonate (PFOS) were manufactured at Cottage Grove but since the 1970s perfluorooctanesulfonyl fluoride (POSF), the starting material for related materials, was manufactured, through the ECF process, elsewhere (Decatur and Antwerp). Salts of shorter chain PFASs have also been manufactured at Cottage Grove (Olsen et al. 2003a, 2009).

Serum total organic fluorine (TOF) was used to estimate Cottage Grove workers' serum PFOA concentrations in the 1980s until 1993 when PFOA was then speciated by high performance liquid chromatography mass spectrometry (Gilliland and Mandel 1996; Olsen et al. 1998, 2000). TOF was considered not sufficiently specific for PFOA due to the potential for other perfluoroalkyl exposures to contribute. This includes the longer chain PFOS and more recently short-chain perfluoroalkyls at this facility (e.g., lithium bis(trifluoromethane-sulfonyl)imide, potassium salt of perfluorobutanesulfonate). Historic TOF measurements at Cottage Grove also used a higher limit of quantitation (LOQ) < 1.0 ppm (Gilliland and Mandel 1996). As a consequence, many jobs with PFOA exposures could not be quantified. For example, of the 115 Cottage Grove workers who volunteered for the 1990 fluorochemical medical surveillance program, 23 (20 %) had TOF values reported <1.0 ppm (Gilliland and Mandel 1996).

Beginning in 1993, measurement of serum concentration of PFOA was included in the periodic voluntary medical surveillance programs in the chemical division. The median PFOA concentrations reported in 1993 (n = 111), 1995 (n = 80), 1997 (n = 74), and 2000 (n = 148) were 1,100 ng/mL, 1,200 ng/mL, 1,300 ng/mL, and 810 ng/mL, respectively, with mean concentrations approximately five times higher as a consequence of the log normal distribution (Olsen et al. 2000, 2003a; Olsen and Zobel 2007). The great majority of participants were male workers. The highest PFOA concentration measured was 114,100 ng/mL in 1997 (Olsen et al. 2000). PFOA and PFOS concentrations were production process-related as seen in Fig. 4.1 for the 117 male employees who participated in 2000. These data indicate TOF would be a biased estimate for either PFOA or PFOS at the Cottage Grove facility.

Because of these limitations using TOF to estimate PFOA at the Cottage Grove site, Raleigh et al. (2014) incorporated PFOA air sampling data, both personal and environmental, in their task-based job/department exposure matrix for their Cottage Grove cohort mortality and cancer incidence study. Industrial hygiene data characterizing APFO exposure in the air within the chemical division (205 personal samples and 659 area samples) were collected between 1977 and 2000. These samples represented all processes and tasks related to APFO production. Production processes prior to 1977 involved the same procedures and tasks but exposure was less due to lower production volume. An air (mg/m^3) time-weighted average (TWA) was calculated for APFO exposure for specific department, job, work area, equipment, tasks, and year groupings to create an exposure data matrix that contained 23

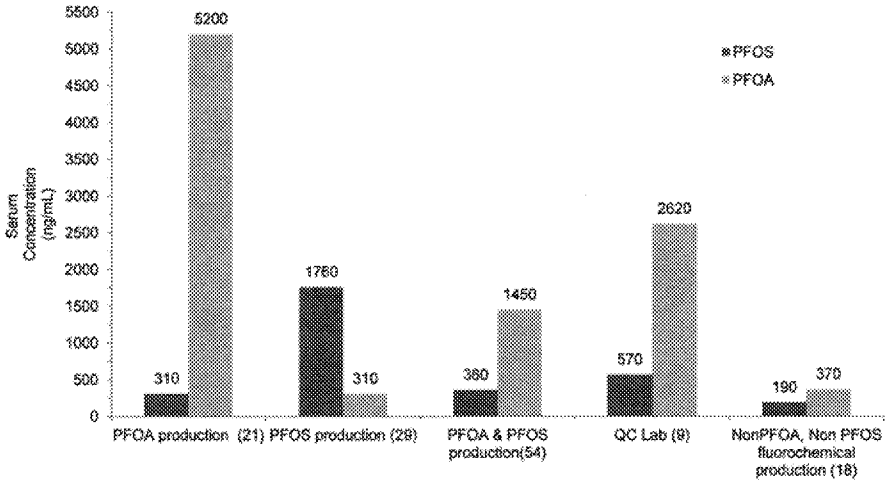

Fig. 4.1 Cottage Grove Manufacturing site, geometric mean serum PFOS and PFOA concentrations, medical surveillance program by major job classifications, (N = 117) male employees, 2000

departments and 45 job titles within the chemical division for all production years (1947–2002). This task-based exposure model incorporated the amount of time spent during an 8 h shift in up to three predefined work task areas: (1) exposure-associated tasks in the production area, (2) nonexposure associated tasks in the production area, and (3) tasks outside the production area. TWAs for jobs in APFO production ranged from 1×10^{-4} mg/m^3 to 4.0×10^{-1} mg/m^3. TWAs estimated for non-APFO production areas within the chemical division were estimated to range between 1×10^{-8} mg/m^3 and 3×10^{-5} mg/m^3. Exposures within the non-chemical division were considered to be between 1×10^{-8} mg/m^3 and 1×10^{-6} mg/m^3.

As a measure of external validity, Raleigh et al. (2014) reported PFOA concentrations for the year 2000 fluorochemical medical surveillance program's 50 participants who worked only in the APFO-related manufacturing area had a geometric mean serum PFOA concentration of 2,538 ng/mL (95 % CI 1,626–3,961 ng/mL). Those who partially worked in the APFO production area had a geometric mean PFOA of 979 ng/mL (95 % CI 565 ng/mL–1,695 ng/L). Those who never worked in the PFOA manufacturing area but still within the chemical division had a geometric mean PFOA of 282 ng/mL (95 % CI 194–410 ng/mL).

Materials derived from perfluorobutanesulfonyl fluoride (PBSF) have been introduced by 3M as replacement chemistry for some PFOS-related materials. The N-alkyl derivatives of perfluorobutanesulfonamides are used in various applications including fabric, carpet, and upholstery protectants as well as surfactant applications. Atmospheric degradation of N-methyl perfluorobutanesulfonamidoethanol has been shown to produce among other degradation products, PFBS (D'eon et al. 2006). Six Cottage Grove employees who had finished a semi-annual batch production of the potassium salt of PFBS participated in a 6-month follow-up pharmacokinetic-related study (Olsen et al. 2009). At study onset, shortly after

production was completed, the employees' mean serum PFBS concentration was 397 ng/mL (range 92–921 ng/mL). At study completion the geometric mean serum elimination half-life was calculated at 25.8 days (95 % CI 16.6–40.2) as compared to 4.8 years (95 % CI 4.0–5.8) for PFOS in a study of 26 retired fluorochemical production workers (Olsen et al. 2007). Urine was a major route of PFBS elimination as concentrations early in the study ranged from 5 to 173 ng/mL and declined during the study such that all measurements by end-of-study were less than the LOQ (5 ng/mL). ECF production of PBSF occurs at the 3M Antwerp and Cordova plants. Production of PFBS-related materials has occurred at all four 3M plants (Cottage Grove, Decatur, Cordova, Antwerp).

4.2.1.2 Decatur (Alabama)

The 3M Decatur manufacturing site consists of two plants: Specialty Film (film plant) and Specialty Materials (chemical plant) (Olsen et al. 1999b, 2003b). PFASs were not significantly used in the film plant except for one product line. On the other hand, hundreds of different manufacturing processes were run in the chemical plant with the majority batch operations that occurred in several buildings. The three major product groups were: protective chemicals, performance chemicals, and fluoroelastomers. Raw materials and intermediates for each product group went through several different production buildings before packaging and shipment. Perfluorooctanesulfonyl fluoride (POSF) was the base chemical until its phase-out announced in 2000. Essentially, octyl mercaptan reacted with chlorine and ammonium to produce octanesulfonyl fluoride (OSF). OSF became the cell feed for ECF to produce POSF, the precursor to the production of a variety of perfluorinated amides, alcohols, acrylates, and other fluorochemical polymer materials manufactured as protective and performance chemicals. PFOA was also manufactured by ECF for limited time periods at Decatur. This production occurred in the majority of months in 1969, 1977, 1978, 1999, and 2000 and a few months in 1967, 1970, and 1972–1974 (personal communication, David Courington). PFOA was also produced as a by-product (residual) of POSF ECF. Also manufactured were intermediate products and surface active chemicals (e.g., AFFFs). PFHxS was produced via the ECF process for use primarily in performance chemicals such as fire suppression liquids. Fluoroelastomers were manufactured from combinations of tetrafluoroethylene, chlorotrifluoroethylene, hexafluoropropylene and vinylidene fluoride. Upon the phase-out of PFOS, the shorter chain PBSF replaced POSF as the basic building block for protective and performance chemicals. ECF production of PBSF occurs at the Cordova, Illinois and Antwerp, Belgium manufacturing plants. The ECF operations at Cottage Grove and Decatur were demolished after the company's phase-out of perfluorooctanyl products (Olsen et al. 2012 b).

Prior to 1995, serum TOF was measured in the Decatur voluntary medical surveillance examinations. Beginning in 1995, PFOS was specifically measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) during these examinations (Olsen et al. 1999a), as well as PFOA. In 1998 a random sample of the

chemical and film plant employee population was examined for several PFASs with statistical analyses stratified by the workers' major job categories (Olsen et al. 1999b, 2003b). A total of 126 chemical plant employees participated in the random sample (80 % of targeted sample). Presented in Table 4.1 are measures of central tendency from this study. The geometric mean serum concentrations for PFOS and PFOA were 941 ng/mL and 899 ng/mL, respectively. The highest PFOS and PFOA concentrations were 10,600 ng/mL and 6,760 ng/mL, respectively. [Note: The highest PFOS concentration ever reported at Decatur was 12,830 ng/mL in an employee in the 1995 voluntary medical surveillance examinations (Olsen et al. 1999a).] Presented in Fig. 4.2 are the geometric mean (GM) concentrations reported for five PFASs stratified by seven major job categories from this Decatur random sample of employees. Findings from this study showed that combined serum concentrations of PFOS and PFOA accounted for 86 % of the serum TOF that was measured. This suggested historical TOF measurements could not be considered specific to any one PFAS.

In 2000, at the onset of the company's announced phase-out, a total of 263 Decatur employees voluntarily participated in the medical surveillance program (Olsen et al. 2001a, 2003a; Olsen and Zobel 2007). Except for PFOA, the serum concentrations were comparable to those measured in the 1998 random sample (Table 4.1). The geometric mean serum PFOA concentration had increased due to the start-up of ECF production of PFOA in 1999.

A total of 24 of the 26 retirees that were followed for 5 years to estimate their serum elimination half-life were from the Decatur plant (Olsen et al. 2007). The other two retirees were from the Cottage Grove plant. This study calculated geometric mean half-lives of PFOS and PFOA of 4.8 years (95 % CI 4.0–5.7) and 3.5 years (95 % CI 3.0–4.1). Russell et al. (2014) estimated the half-life for PFOA among those retirees (n = 7) whose initial concentrations were >500 ng/mL was 3.0 years (95 % CI 2.4–3.8). This would result in minimal bias (1 %) in the calculation of an intrinsic half-life for PFOA due to the highly elevated initial concentrations and long sampling duration of almost two half-lives. Analysis of the apparent half-lives of retirees whose initial concentrations were less than 500 ng/mL had biased upward estimates up to 13 % with the lowest initial concentrations (~70 ng/mL) (Russell et al. 2014).

4.2.1.3 Cordova (Illinois)

3M Cordova manufactures specialty chemicals, adhesives, and fluorinated chemicals for the company's internal and external customers. As part of this product-mix, the plant manufactures PBSF, C3/C4 acid fluorides, and hydrofluoroethers. Unlike Cottage Grove or Decatur, the Cordova plant was not a primary manufacturer of APFO or PFOS-related materials, One production that did occur was that of a post-market carpet protectant. Voluntary biomonitoring of Cordova employees was conducted in 1997 (n = 66). Median concentrations of PFOS and PFOA were 151 ng/mL and 100 ng/mL, respectively.

Table 4.1 PFAS measures of central tendency, Decatur biomonitoring random sample (1998) and medical surveillance results, Decatur and Antwerp, 2000

	Decatur 1998 random sample (N = 126 employees)				Decatur 2000 medical surveillance (N = 263 chemical employees)				Antwerp 2000 medical surveillance (N = 258 employees)			
	AM	Range	GM	95 % CI	AM	Range	GM	95 % CI	AM	Range	GM	95 % CI
PFOS	1,505	91–10,600	941	790–1,130	1,320	60–10,060	910	820–1,020	800	40–6,240	440	380–510
PFOA	1,536	21–6,760	899	720–1,120	1,780	40–12,700	1,130	990–1,300	840	10–7,040	330	270–400
PFHxS	345	5–1,880	180	150–220	300	7–1,670	180	160–210	170	1–1,430	70	60–80
N-Me-FOSE-AcOH	150	8–992	81	70–100	250	8–992	110	100–130	150	3–1,060	60	50–70
N-Et-FOSE-AcOH	23	1–269	8	6–10	60	3–440	40	10–40	20	3–140	9	8–10

AM = Arithmetic Mean
GM = Geometric Mean

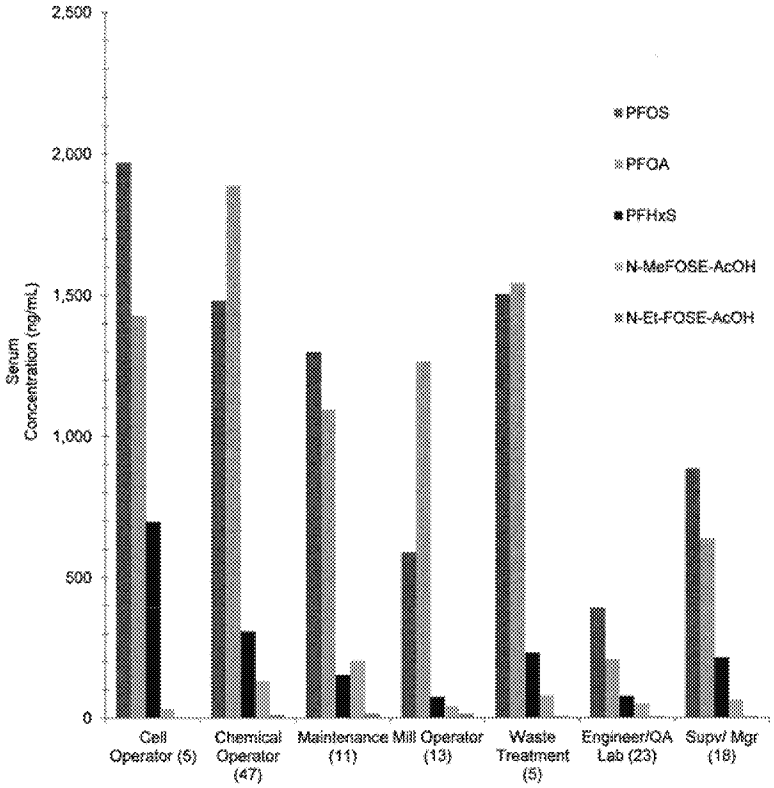

Fig. 4.2 Decatur manufacturing site, geometric mean serum PFOS and PFOA serum concentrations (ng/mL) by major job classifications, random sample (N = 128), 1998

4.2.1.4 Antwerp (Belgium)

Beginning in the mid-1970s and similar to Decatur production processes, POSF was the base material produced by ECF at the Antwerp plant that was the precursor to the production of PFOS-based protective and performance materials (Olsen et al. 2001b). Also manufactured were intermediate products, end-products (PFOA), inerts, and surface active chemicals (fire fighting foams). Synthetic fluoroelastomers were manufactured from hexafluoropropylene and vinylidene fluoride. Nonfluorochemical production involved phenolic resins, acrylate polymers and adhesives, polyurethane polymers, and acrylates.

Beginning in 1995, voluntary participation by employees in medical surveillance activities involved LC-MS/MS measurement of PFOS and PFOA (Olsen et al. 1999a, 2001b, 2003c; Olsen and Zobel 2007). These analyses showed serum PFOS,

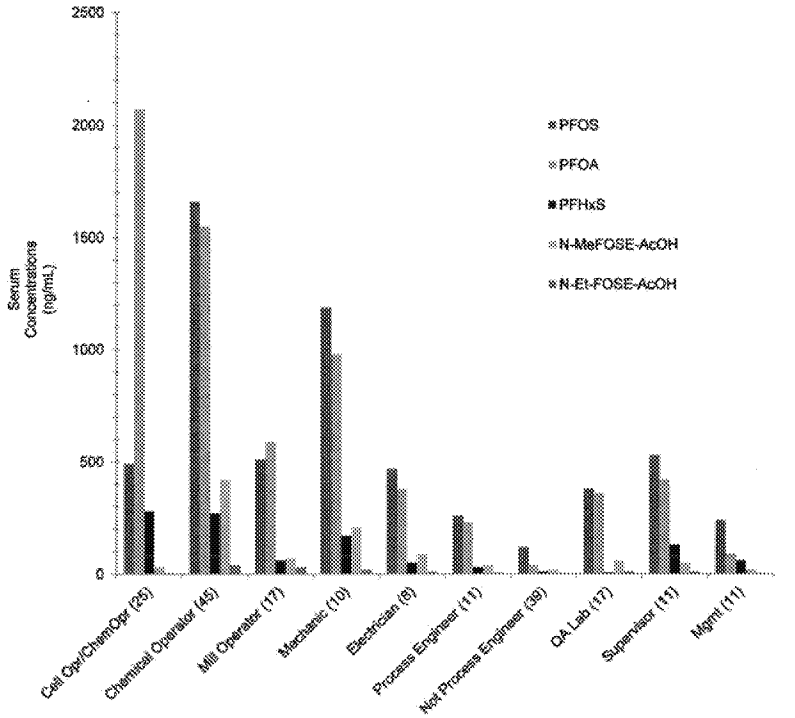

Fig. 4.3 Antwerp manufacturing site, geometric mean serum PFOS and PFOA concentrations (ng/mL), by major job classifications, medical surveillance program (N = 258), 2000

PFOA, and other PFAS-related serum levels were generally lower than those observed among Decatur employees. For example, in 1995 and 1997 the mean PFOS concentrations in Antwerp workers were 1,930 ng/mL and 1,480 ng/mL compared to the Decatur workforce at 2,440 ng/mL and 1,960 ng/mL, respectively (Olsen et al. 1999a, b).

In the 2000 voluntary medical surveillance examinations, the Antwerp mean serum PFOS and PFOA concentrations were 800 ng/mL and 840 ng/mL, respectively. The distributions were log normal as the geometric means were 440 ng/mL and 330 ng/mL, respectively (Table 4.1). Highest PFOS and PFOA concentrations measured in 2000 at Antwerp were 6,240 ng/mL and 7,040 ng/mL, respectively. Figure 4.3 shows the geometric mean concentrations for five PFASs stratified by seven major job categories from the 2000 Antwerp medical surveillance data.

4.2.1.5 Decommission, Demolition and Disposal Activities (3M Cottage Grove and Decatur)

PFAS biomonitoring was conducted among workers who decommissioned, demolished and disposed of former PFAS manufacturing facilities (Olsen et al. 2012b). These facilities included the APFO manufacturing buildings at Cottage Grove and

the ECF cell building operations at Decatur. Decommission work involved pipe and equipment removal and disposal. Because of the uniqueness of this work, the extent of potential exposures was unknown. Therefore, as part of the decommissioning work, an exclusion work zone was established that required anyone entering the area to have appropriate training, personal protective equipment, and participate in a medical monitoring program. The latter included baseline and end-of-project blood collections for the determination of PFOA and PFOS serum concentrations. A total of 204 individuals (primarily contract workers) completed the baseline and end-of-project assessments. Of these individuals, 120 (59 %) had PFOA and PFOS baseline concentrations comparable to that measured in the general population (Kato et al. 2011; Olsen et al. 2012a). Among these 120 individuals their matched pair median change and interquartile range for PFOA was 5.3 ng/mL (0.5–32.6) and 0.7 ng/mL ((−1.0)–4.7), respectively. Their mean matched-pair changes were PFOA 44.2 ng/mL ($p<0.0001$) and PFOS 4.2 ng/mL ($p<0.0001$). These biomonitoring results suggested an increase in serum PFOA concentrations, and lesser so for PFOS, among workers who were engaged in this decommissioning, demolition, and disposal work.

4.2.2 DuPont Washington Works (Parkersburg, WV)

Fluoropolymer production began at the DuPont Washington Works plant in 1951 (Woskie et al. 2012). APFO was used as a surfactant in the emulsion polymerization of tetrafluoroethylene (TFE) to make polytetrafluoroethylene (PTFE) whose variety of products, included use in non-stick pans and water repellent clothing. Brand names were sold under the labels Teflon® and Gore-tex®. PFOA was not considered incorporated into the final product of these fluoropolymers (Kreckmann et al. 2009). Other production applications included wire and cable, inert tubing, and semiconductors. APFO was also used in co-polymer production of fluorinated ethylene propylene (FEP) and perfluoroalkoxy (PFA) polymer. Depending on time periods, PFOA was used as either a powder or premixed liquid form for these polymer and co-polymer production processes.

TOF was measured as a surrogate for PFOA in the blood prior to laboratory capabilities to speciate for PFOA. Between 1972 and 1981 whole blood analysis for TOF used the Wickbold torch method followed by a spectrophometric method of detection (Woskie et al. 2012). Beginning in 1981, the whole blood analyses were converted to a gas chromatography with electron capture detector method (GC-ECD) that specifically quantified PFOA. Beginning in 2003, PFOA measurements were subsequently analyzed using high pressure LC/MS-MS methods using a serum matrix. Based on an analysis of 114 samples by both the Wickbold and GC-ECD methods, a correction factor was applied to the TOF data to convert to GC-ECD PFOA analysis equivalents and then these whole blood PFOA measurements were subsequently adjusted to serum PFOA equivalents.

Kreckman et al. (2009) used actual serum PFOA measurements from 1,025 Washington Works workers that were obtained in a cross-sectional health study in 2004. They combined these data with current job titles to derive three relative APFO similar exposure categories. The mean of the serum PFOA measurements for each job exposure category served as an intensity factor. The job exposure categories were applied to historical job titles and validated with PFOA measurements collected among voluntary participants between 1979 and 2002. A cumulative exposure matrix was derived.

Woskie et al. (2012) expanded on this methodology to predict serum PFOA levels over time at this DuPont Washington Works site for the following eight plant workplace categories:

1. Fine powder/granular polytetrafluoroethylene (PTFE) job categories (direct exposure to PFOA).
2. Fluorinated ethylene propylene (FEP) and perfluoroalkoxy fluoropolymer (PFA) job categories (direct exposure to PFOA).
3. Non-PFOA use in Teflon polymer and co-polymer production job category (intermittent direct or plant background PFOA exposure). Included two subcategories:

   (3a) Teflon polymer co-polymer production department category (intermittent direct PFOA exposure). This included ethylene-tetrafluoroethylene fluoropolymer and fluorotelomer co-polymer operations.
   (3b) Tetrafluoroethylene (TFE) monomer operation category (plant background PFOA exposure).

4. Maintenance job category (intermittent direct or plant background PFOA exposures). Included two subcategories:

   (4a) Assigned to Teflon/co-polymer maintenance job category (intermittent direct PFOA exposure).
   (4b) Not assigned to Teflon/co-polymer maintenance job category (plant background PFOA exposure).

5. Non-Teflon/co-polymer production division with no PFOA-use job category (plant background PFOA exposure). Included two subcategories:

   (5a) Jobs considered potentially exposed to PFOA in either (1), (2), (3), or (4) above (intermittent direct PFOA exposure).
   (5b) Jobs not exposed to PFOA (plant background PFOA exposure).

Linear mixed models were developed to predict serum PFOA concentrations for each DuPont plant employee and year of their work history for the above eight job category/job group combinations. These retrospective models took into account individual and repeated measures from a collective sample of 2,125 serum measurements from 1,308 workers collected between 1979 and 2004. Of the 2,125 samples, the median and mean serum PFOA concentrations were 580 ng/mL and 2,050 ng/mL, respectively. The highest PFOA measurement was 59,400 ng/mL that was measured in a worker involved with PTFE production. Figure 4.4 shows the median

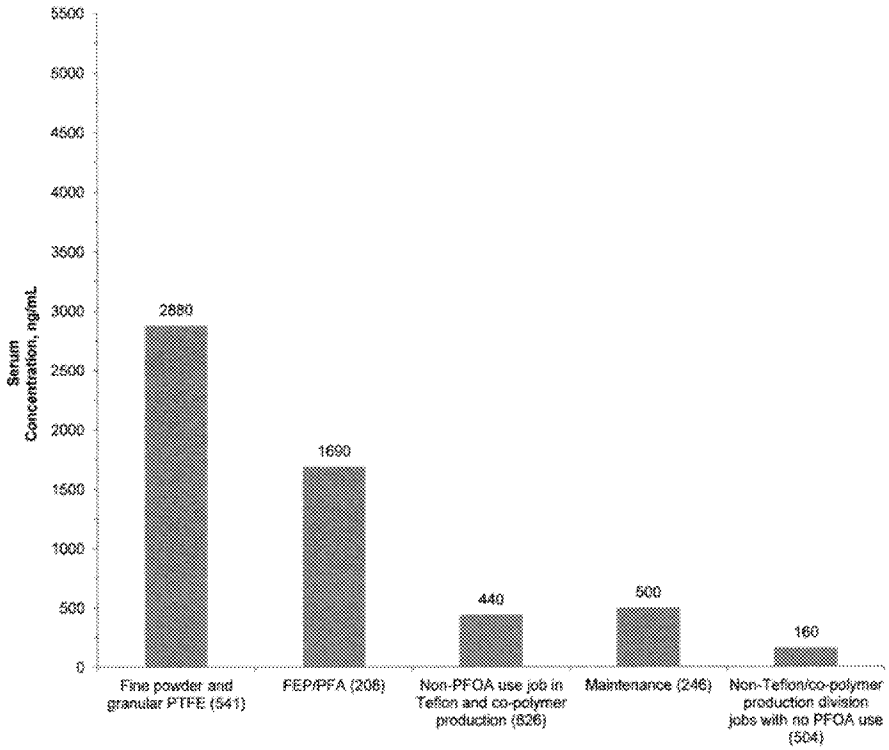

Fig. 4.4 DuPont Washington Works plant, median serum PFOA concentrations, (2,125 Samples) by five major job classifications, 1979–2004

serum PFOA concentrations from the samples for the five major job groups described above.

Covariates considered in the linear mixed models included workers' cumulative years worked, historical production of APFO used or emitted by the plant (Fig. 4.5) and the time where major process changes may have influenced workers' exposure. Predicted serum PFOA concentrations prior to 1979 were largely influenced by either the amount of PFOA used (direct or indirect exposure) or amount emitted (plant background exposure). From these linear mixed models, estimated weighted predicted annual median serum PFOA concentrations for all workers with potential exposure to PFOA reached the highest level of approximately 0.8 ppm (800 ng/mL) in the late 1980s (Fig. 4.6a). Among workers unexposed to PFOA, the highest predicted annual median concentration was in the 0.2 ppm (200 ng/mL) range (Fig. 4.6b). Data from these annual prediction serum PFOA models were used in an epidemiologic cohort mortality analysis of this plant population (Steenland and Woskie 2012).

The modeled estimated median serum PFOA levels, by year, are shown for workers in job groups with direct exposure (Fig. 4.7). [Note: Figs. 4.7 and 4.8 are

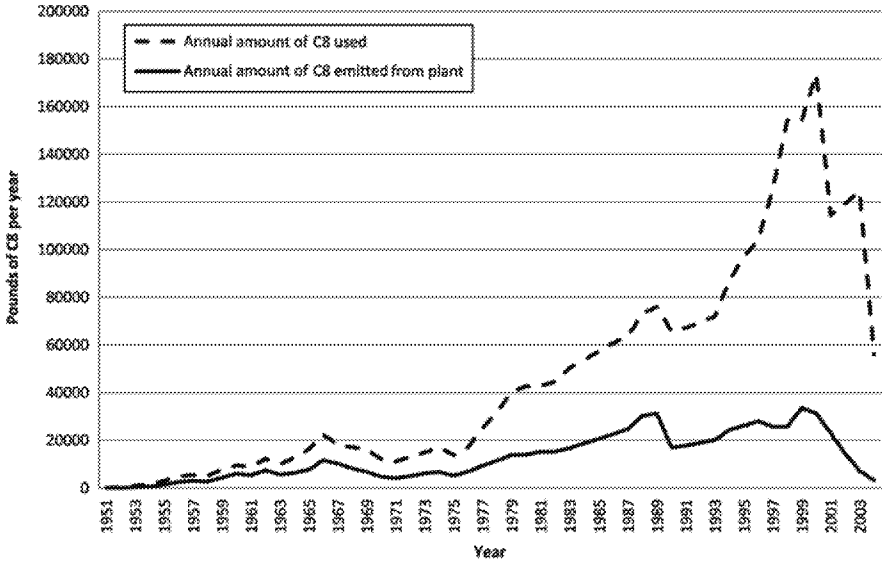

Fig. 4.5 Annual amount of PFOA used at the DuPont plant (lbs/year) and the estimated annual PFOA emissions from the plant (Reprinted from Woskie et al. 2012, by permission of Oxford University Press)

adapted from Woskie et al. (2012) who presented the data in ppm (μg/mL) units. [Note: Multiply by 1,000 to derive ppb (ng/mL) levels.] The increase in serum PFOA concentrations until 1980 among workers with direct exposure reflected the increase in annual PFOA use (Fig. 4.5). Although PFOA use increased substantially after 1980, there was a decline in estimated median PFOA concentrations from these models among the chemical and finish operators in the Fine Powder/Granular PTFE category. This was the consequence of the implementation of several exposure controls including the replacement of weighing powdered PFOA with premixed liquid PFOA, a dryer scrubber, and use of personal protective equipment. Estimated median serum concentrations of the FEP/PFA operator were also influenced by operational changes over time. Annual production use also influenced predicted median PFOA concentrations among job groups with intermittent direct or plant background PFOA exposure (Fig. 4.8).

4.2.3 Other APFO and Fluoropolymer Manufacturers

Besides 3M and DuPont plants discussed above, few other APFO manufacturing, fluoropolymer, and/or fluorotelomer manufacturing companies have published employee PFAS biomonitoring data in the scientific literature. This does not necessarily infer that biomonitoring analyses of these company workers have not been

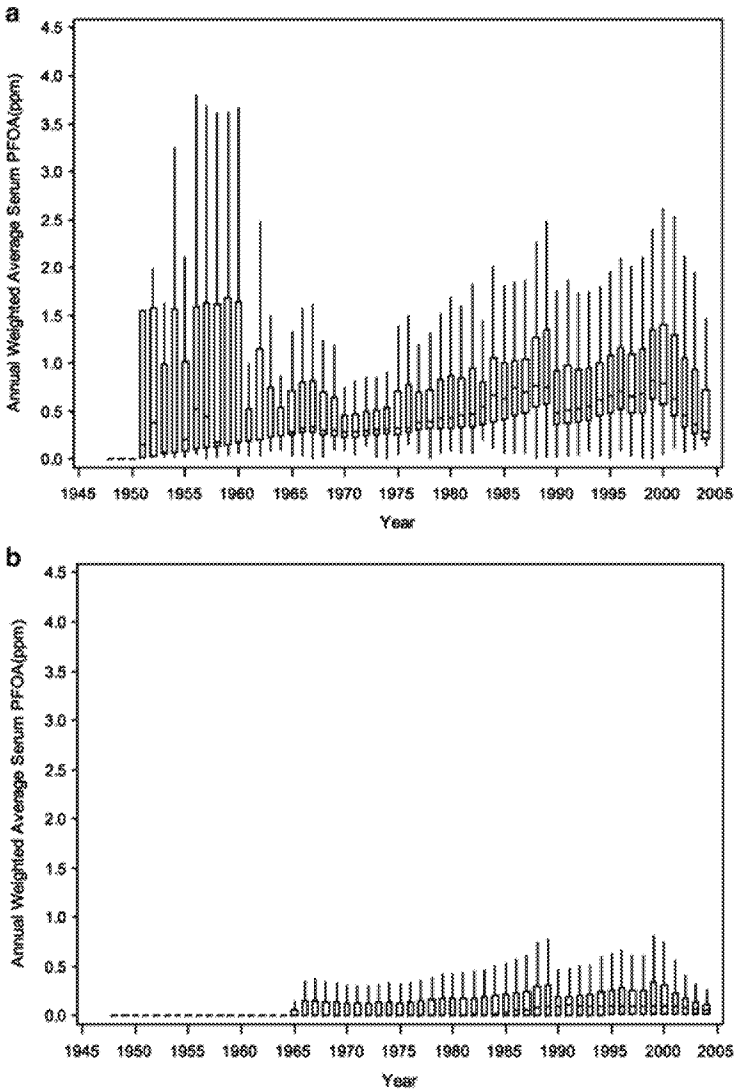

Fig. 4.6 Stem and leaf plots of the model estimated weighted annual median serum PFOA levels (ppm) for the full cohort of DuPont workers for those in job groups (see text) with potential PFOA exposure during the calendar year (**a**) and without potential (**b**). *Box plots* show the minimum, maximum, 25th and 75th percentiles and median (Reprinted from Woskie et al. 2012, by permission of Oxford University Press)

performed – just that nothing was found available in the scientific literature. The US EPA's 2010/2015 PFOA Stewardship Program (US EPA 2006) identified eight major fluoropolymer and telomer manufacturers: Arkema, Asahi, BASF (formerly Ciba), Clariant, Daiken, 3M/Dyneon, DuPont, and Solvay Solexis.

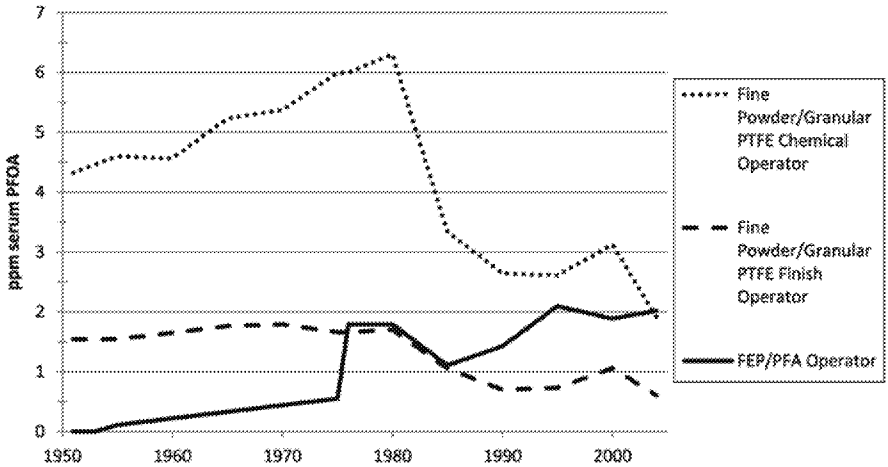

Fig. 4.7 Median serum levels estimated from models for workers in job groups with direct exposure to PFOA during the year (Reprinted from Woskie et al. 2012, by permission of Oxford University Press)

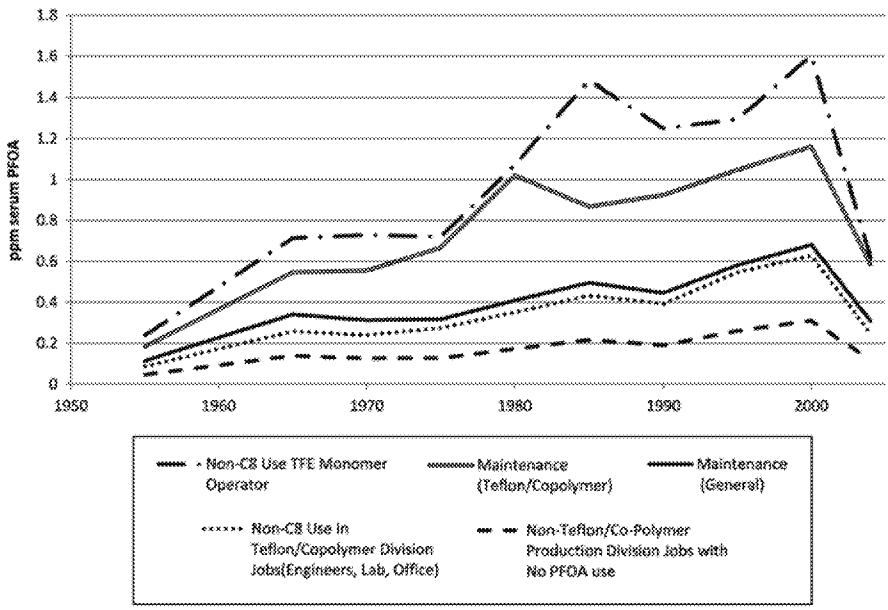

Fig. 4.8 Median serum levels estimated from models for workers in job groups with intermittent direct or plant background PFOA exposure to PFOA during that year (Reprinted from Woskie et al. 2012, by permission of Oxford University Press)

After the 3 M phase-out of PFOS-related materials, it appears many small scale POSF-based Chinese manufacturers expanded their production capabilities. Several PFOS-based companies have been identified to operate in Hubei and Fujian provinces.

Besides PFOS, APFO, fluoropolymer and telomer manufacturers, there are 'downstream' users of PFAS-related materials that need to be considered for biomonitoring of their workforce (Butenhoff et al. 2006). These 'downstream' users include the carpet, textile, mill, paper (food-protectant applications), semi-conductor, and metal plating industries.

4.2.3.1 Polytetrafluoroethylene (PTFE) Plants (DuPont, Dyneon, Asahi, Solvay Solexis)

Consonni et al. (2013) reported a cohort study of PTFE production workers from four companies consisting of six manufacturing sites: Dyneon (Gendorf, Germany); Solvay Solexis (Spinetta Marengo, Italy); Asahi (Hillouse UK; and Bayone, NJ, USA); and DuPont (Dordrecht, Netherlands and the Washington Works plant located near Parkersburg, WV, USA). A total of 5,879 workers were in this cohort which included 2,379 from the DuPont Washington Works plant discussed above (this was approximately 40 % of the DuPont Washington Works overall cohort reported by Steenland and Woskie 2012). Consonni et al. developed a job exposure matrix with yearly semi-quantitative estimates (in arbitrary units) of both TFE and APFO exposure at each of the six plant sites. These semi-quantitative arbitrary units of TFE and APFO exposure were highly correlated ($r^2 = 0.72$, $p < 0.0001$).

The PTFE production processes are closed systems because of TFE's explosive nature. The potential for TFE exposure, however, could still occur through opening autoclaves in the polymerization area or decomposition of PTFE. Consonni et al. (2012) estimated historic TFE exposure in this cohort could have been up to a few parts per million. Overall findings of this multi-plant cohort included modestly elevated standardized mortality ratios (SMRs) for cancer of the liver and kidney, and leukemia (see reviews in Chap. 14 and Chang et al. 2014). [Note: the International Agency for Research on Cancer (IARC) recently evaluated PFOA as a group 2B (possible) human carcinogen. They evaluated TFE as a group 2A (probable) human carcinogen (Benbrahim-Tallaa et al. 2014).] Consonni et al. stated that these effects could not be disentangled between the highly correlated TFE and APFO exposure based on their semi-quantitative arbitrary units of analysis.

This inability to disentangle the reported associations with APFO and TFE, however, differed from the conclusion by Steenland and Woskie (2012) who reported APFO exposure response trends for liver and kidney cancer and leukemia in the DuPont Washington Works cohort study. Steenland and Woskie discounted the potential for TFE exposure in their DuPont Washington Works cohort study because they believed appreciable exposures to TFE were unlikely during normal operations because of its explosive nature. As a point of clarification, it should be noted that the lower explosion limit for TFE is 110,000 ppm (ACGIH 1997). The

8 h time-weighted average (TWA) for TFE is 2 ppm. Therefore, it is likely that low level TFE exposures could have occurred given the disparity between exposure levels between the TWA and the lower explosion limit. It should also be noted that the 3M Cottage Grove plant (discussed above) that manufactured APFO was not a PTFE production plant and therefore the exposure at this 3M plant was done in near isolation of any TFE exposure (Raleigh et al. 2014).

4.2.3.2 Miteni Plant (Trissino, Italy)

PFOA had been produced by ECF since 1968 at the Miteni plant in Trissino, Italy (Costa et al. 2009). It was not stated whether ECF production of POSF occurred at this facility. Worker biomonitoring for PFOA began in 2000 and continued annually through 2007 (Costa et al. 2009). For the 25–50 PFOA production workers who had serum measurements evaluated between 2000 and 2007, their geometric mean serum concentrations were 11,700 ng/mL, 10,200 ng/mL, 9,300 ng/mL, 6,900 ng/mL, 6,500 ng/mL, 5,800 ng/mL and 5,400 ng/mL, respectively. Maximum serum PFOA concentrations measured were >45,500 ng/mL (upper limit of quantitation), >45,500 ng/mL (upper limit of quantitation), 91,900 ng/mL, 74,700 ng/mL, 46.300 ng/mL, 41,900 ng/mL, and 47,000 ng/mL, respectively. Declining concentrations of PFOA were observed after 2002 following several workplace exposure reduction activities. PFOA is no longer manufactured at this facility.

4.2.3.3 Arkema Plant (Pennsylvania)

A series of cross-sectional 1976, 1989, 1995, 1998, 2001) medical surveillance analyses were conducted of workers (number ranging between 163 and 323 per year) from a manufacturing facility that used perfluorononanoic acid (PFNA or C9) blend in the production of high-performance polymers (Mundt et al. 2007). Other fluorinated hydrocarbons in this blend were the perfluorocarboxylic C11 and C13 congeners. No specific biomonitoring measurements of PFNA were reported. PFNA serum elimination half-life data in humans is unknown but would be expected to be several years, similar to PFOA.

4.2.3.4 Manufacturers in China

Manufacturing of POSF-based materials increased in China after the announced phase-out by the 3M Company (Fig. 4.9) (Zhang et al. 2012). The trend in PFOS-related production declined slightly in 2008 after the European Union directive to not import textiles treated with POSF-related materials (Zhang et al. 2012; Xie et al. 2013). PFOS-based production has remained relatively steady since 2009. Three major PFOS-based applications exist in China: metal plating; fire fighting foams; and insecticides (sulfuramid).

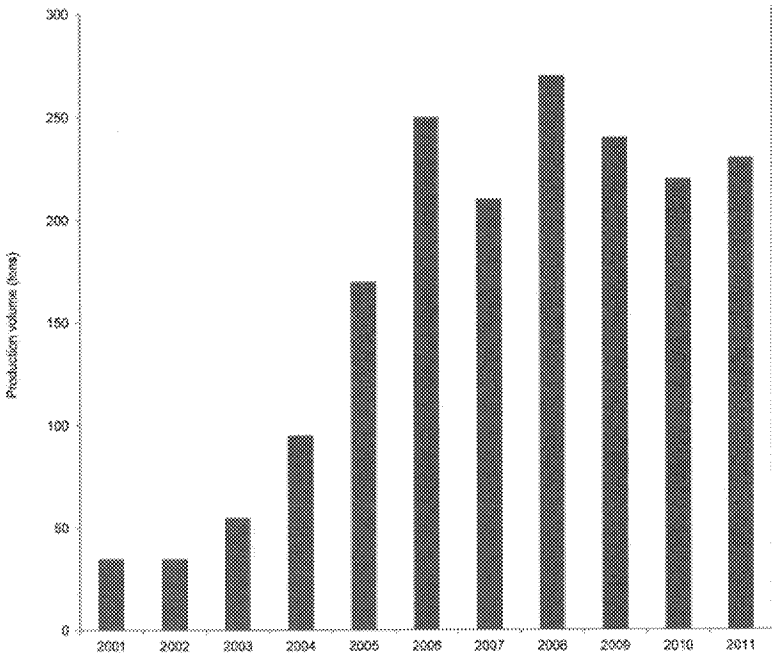

Fig. 4.9 Approximate annual production of PFOS (tons) in China, 2002–2008 according to Xie et al. (2013)

Twelve companies were identified as manufacturers of POSF-related materials that are located in Hubei and Fujian provinces (Table 4.2) but worker biomonitoring data were not presented (Huang et al. 2013). Wang et al. (2012) described an environmental assessment at a manufacturing facility near Wuhan, (Hubei Province) China. PFOS and PFHxS were found in dust, water, soil and chicken eggs. Concentrations were dependent on distance from the plant. Although dust was a major exposure, biomonitoring of the workers was also not reported. High serum concentrations of PFOS were reported among commercial fishermen in Tangxun Lake (Wuhan, China) that receives waste water treatment plant discharges originating from an industrial park that included a POSF-related manufacturing plant(s) (Zhou et al. 2014). See a detailed description of Tangxun Lake in the community studies section of this chapter. A fluorine chemical industrial zone was identified near Changshu City, in the Jiangsu province (Wang et al. 2012). A total of 27 fluorochemical plants were located in this industrial zone but Wang et al. did not identify company names or products manufactured were not identified. At one of these fluorochemical plants a biomonitoring study was performed on 55 male workers and 132 nearby residents (Wang et al. 2012). Results showed both occupational as well as residential exposure to PFOA compared to other general population levels reported in China (Wang et al. 2012). PFOA values differed between workers

Table 4.2 POSF- related chemical manufacturers (China)

Hubei Province (n = 9)
1. Hubei Hengxin Chemical Co., Ltd
2. Wuhan Chemical Industry Institute Co., Ltd.
3. Changjiang Fluorochemical Co., Ltd
4. Yingcheng Sanwei Chemical Co. Ltd
5. Hubei Xiaochang Xiangshun Chemical Co., Ltd.
6. Wuhan Jinfu Economic Development Co., Ltd
7. Wuhan Jiangrun Fine Chemical Co., Ltd.
8. Hubei Kaie Printing Ink Manufacture Co., Ltd
9. Wuhan Defu Economic Development Co., Ltd
Fujian Province
1. Shaowu Huaxin Chemical Co., Ltd
2. Shaowu Jintang Anshengqi Chemical Co., Ltd
3. Jianyang Tianfu Chemical Co., Ltd

Presentation given by Jun Huang[a], Gang Yu[a], and Shengfang Mei[b], at the 5th International Workshop on Per- and Polyfluorinated Alkyl Substances. Helsingör, Denmark. October 2013
[a]School of Environment, Tsinghua University, China
[b]China Association of Fluorine and Silicone Industry (CAFSI)

and residents: [workers 1,636 ng/mL (range 95–7,737 ng/mL); residents [378 ng/mL (range 10–2,427 ng/mL)]. Unlike PFOA, the other PFASs measured, including PFHpA, PFNA, PFDA, PFDoA, PFBS, PFHxS, and PFOS were not substantively different between workers and nearby residents.

4.2.3.5 'Downstream' PFAS Production

Specific PFASs applications occur in the carpet, textile, and leather industries, paper mills, the semiconductor industry, and metal plating. Although environmental exposure data was reported related to emissions from various industrial settings, biomonitoring data were not available related to the workers at these 'downstream' PFAS user facilities. Although PFAS biomonitoring data were not found in the scientific literature, it is possible such analyses could have been conducted but never published. For example, at Dalton, Georgia, known as the "carpet capital of the world," Konwick et al. (2008) conducted an environmental assessment of PFASs in surface water and reported high concentrations at a location in the Conasuga river near a wastewater land application site. A US EPA assessment of the drinking water in the area did not find PFOA or PFOS concentrations exceeding the Agency's provisional guidelines (US EPA 2009).

Similar to the above investigation at Dalton, Georgia, perfluorinated chemical analyses occurred in a river near the location of major Taiwanese semiconductor and electronics industries (Lin et al. 2009) as well as near the largest science park in

Taiwan (Lin et al. 2014). However, there was no indication that PFAS biomonitoring occurred among employees or residents living near these industries.

4.3 Biomonitoring in Communities Affected by Environmental Exposures of PFASs

Several communities have been affected by environmental releases of PFASs that reached surface and/or groundwater sources for drinking water. Depending on location, environmental releases were from three major types of sources: (1) industrial emissions (air, water) from nearby PFAS manufacturing plants; (2) landfill leachate where PFAS materials had been legally deposited with subsequent finding into aquifers; and (3) run-off into water ways (creeks, rivers, lakes) from agricultural fields where treated sewage sludge had been applied as soil conditioner.

Biomonitoring for PFAS in each affected community demonstrated concentrations above levels reported in the referent general population. The largest population (approximately 69,000) studied in a series of epidemiologic investigations, to date, resided in a mid-Ohio River valley community encompassing six water districts in either Ohio or West Virginia. In another community, increased serum PFAS concentrations were associated with fish consumption resulting in the highest serum PFOS serum concentration ever reported in the literature.

Brief reviews of six affected community studies are provided below.

4.3.1 United States

4.3.1.1 Minnesota ("East Metro" Study of Minneapolis-St. Paul)

The Minnesota Department of Health (MDH) (2008, 2012a), under cooperative agreement with the U.S. Agency for Toxic Substances and Disease Registry (ATSDR), issued public health assessments regarding emissions of PFASs from the 3M Cottage Grove manufacturing facility as well as several local landfills where the plant had legally disposed of wastes in the 1950s, 1960s, and 1970s. Several PFASs were detected in public and private wells in east metro communities of the Minneapolis-St. Paul metropolitan area. Exposure was attributed to landfill leachate with PFOA entering the aquifer. PFASs levels in the drinking water in some of these wells were above the state's health risk limits of 0.3 μg/L for PFOA and/or PFOS (see Chap. 17). Remediation efforts included installing granular activated charcoal filtration systems in municipal water systems, connecting residential users of affected private wells to municipal water systems, and placement of whole-house activated carbon filters in other rural residences. In addition remediation efforts at the affected landfills occurred to mitigate further leachate mediated PFOA entry into the groundwater.

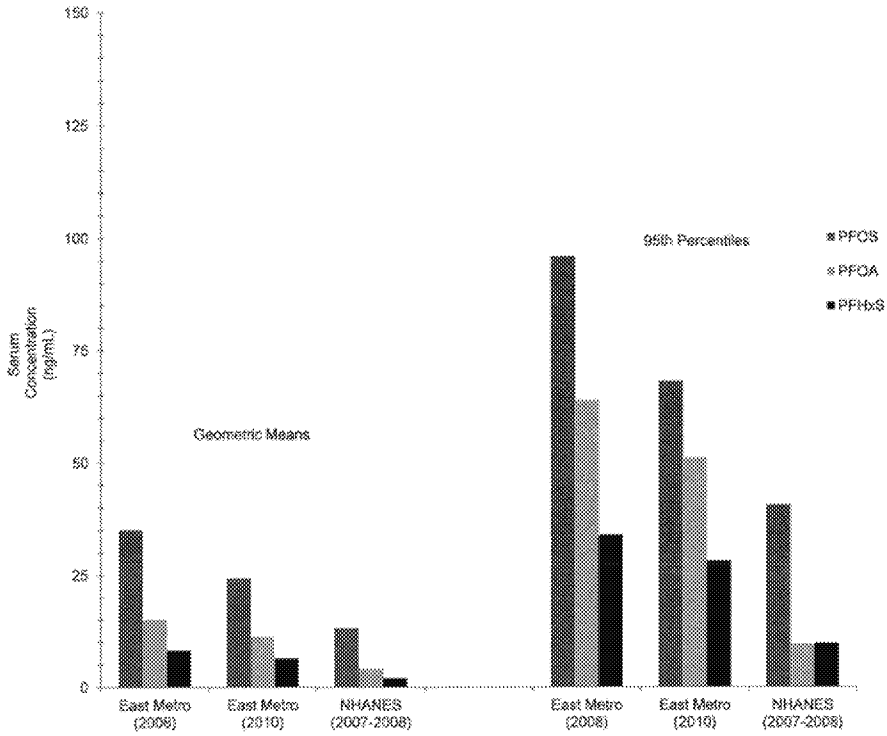

Fig. 4.10 Geometric mean and 95th percentile serum PFOS and PFOA concentrations in east metro residents of Minneapolis St-Paul, compared to NHANES

As part of the MDH's effort to assess citizens' exposure, a random sample of east metro citizens was contacted who lived in affected areas (MDH 2012b, c). In 2008 the geometric mean serum concentrations for PFOS (35. 1 ng/mL), PFOA (15.1 ng/mL), and PFHxS (8.2 ng/mL) were approximately three to four times higher than the 2007–2008 NHANES data (Fig. 4.10). In a resampling of this population 2 years later, the geometric mean serum concentrations for the East Metro area were 24.3 ng/mL, 11.3 ng/mL, and 6.4 ng/mL, respectively. This represented 2-year percentage declines of 26 %, 21 %, and 13 %, respectively. These percentage reductions approximate what might be the expected serum elimination half-lives that have been reported for these three PFASs (Olsen et al. 2007). Based on these percentage declines, the MDH concluded the exposure reduction efforts appeared to be working but continued biomonitoring was warranted (MDH 2012c). A third biomonitoring sampling of this population is being conducted in 2014.

4.3.1.2 Alabama (in the Vicinity of Decatur, AL and Morgan, Lawrence, and Limestone Counties)

Between 1996 and 2008, the Decatur, Alabama waste water treatment plant (Decatur Utilities) processed permitted wastewater effluent from local industries involved in the manufacture and/or use of PFASs (Lindstrom et al. 2011). It was estimated 34,000 tons of impacted biosolids from the wastewater treatment plant were applied as treated sludge to approximately 5,000 acres of agricultural fields in Lawrence, Morgan, and Limestone Counties. In 2007 the U.S. EPA was notified by a PFAS manufacturer, Daiken Corporation, in Decatur, Alabama that it had unknowingly discharged large amounts of perfluorocarboxylic acids into the Decatur Utilities wastewater treatment plant (Lindstrom et al. 2011; Decatur Daily 2009). The US EPA conducted a series of investigations sampling biosolids, surface water, ground water, drinking water, and soils in the area around these agricultural fields to determine PFAS concentrations. PFOA was detected (57 %) in surface waters near these fields and 4 (22 %) of 19 private wells had PFOA concentrations above the EPA's Provisional Guideline of 0.4 μg/L.

As reviewed by the US Agency for Toxic Substances and Disease Registry (ATSDR 2013), between 2008 and 2011 the US EPA conducted a series of municipal water testing involving five other public water distribution systems in the area (ATSDR 2013). One of the five municipal public drinking water systems, West Morgan/East Lawrence (WM/EL), had detectable PFOA and PFOS concentrations but both were below the EPA's Provisional Health Advisory levels. The WM/EL obtained its water from the Tennessee River 13 miles downstream from an industrial area with PFAS manufacturing and use that was located in the Decatur area. Studies of PFAS concentrations in the river had been previously reported (Hanson et al. 2002; Weston 2012).

The ATSDR (2013) subsequently conducted a human exposure investigation that resulted in letters sent to 519 eligible households. A total of 85 households (16 %) participated (153 people volunteered from these households). A primary objective was to measure PFASs in these participants' serum that lived and worked in the affected WM/EL public water system. Figure 4.11 provides the geometric mean serum concentrations for PFOS, PFOA, and PFHxS for the three different water sources reported by the ATSDR (2013): WM/EL public water system; water sources without detectable PFAS levels; and private drinking wells with detectable PFAS levels. NHANES general population data are also presented for comparison purposes. Geometric means serum PFAS concentrations were approximately two to five times higher in individuals residing within the WM/EL or having an affected private well than those individuals living in residences without detectable water levels (Fig. 4.11). The range of serum PFOS concentrations for these three water categorizations were (in parentheses): (5.6–248 ng/mL), (38.6–472 ng/mL), and (5.4–201 ng/mL), respectively. The range of serum PFOA concentrations were (in parentheses): (2.2–78.8 ng/mL), (7.6–144 ng/mL), and (2.8–50.4 ng/mL),

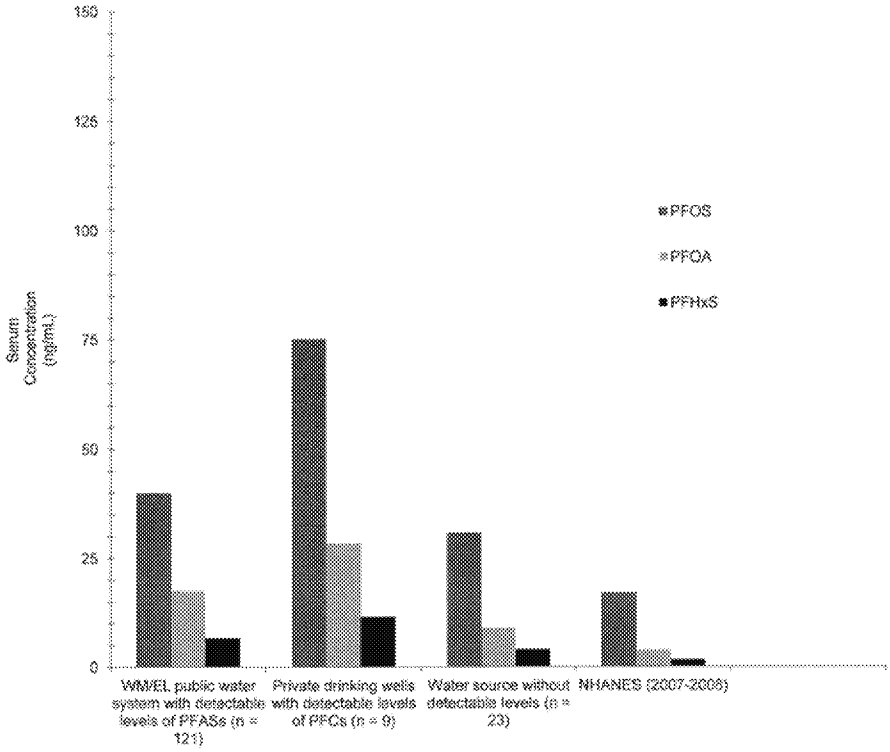

Fig. 4.11 Geometric serum PFOS, PFOA, and PFHxS concentrations in residents of affected communities through drinking water exposure near Decatur, AL compared to NHANES

respectively. The range of PFHxS concentrations for these three water sources were (in parentheses): (0.6–32.3 ng/mL), (6.1–59.1 ng/mL), and (1.2–24.8 ng/mL), respectively. Additional epidemiologic analyses and interpretation is included in this ATSDR Health Consultation (2013) (See Chap. 14).

4.3.1.3 Mid-Ohio River Valley (West Virginia/Ohio)

As discussed above, APFO (ammonium salt of PFOA) was used as an emulsifier in the polymerization of TFE to polytetrafluoroethylene (PTFE) at the DuPont Washington Works plant (near Parkersburg, West Virginia). Cumulative evidence demonstrated PFOA entered water supplies along this mid-Ohio River Valley area which led to a certified class action between plaintiffs (the 'Class') and DuPont (Frisbee et al. 2009). The PFOA water exposure was attributed to industrial emissions of APFO from the nearby DuPont Washington Works plant (Paustenbach et al. 2007; Shin et al. 2011a, b, 2012, 2014). A pretrial settlement between the Class and DuPont provided: (1) funds for health and education projects (subsequently known as the C8 Health Project); (2) provisions by the company to remove PFOA

from the water supply of six affected water districts; and (3) establishment of an independent panel of three scientific experts (the C8 Science Panel) to determine the presence or absence of "probable links" defined as the weight of the available scientific evidence that it is more likely than not there is a link between exposure to PFOA and a particular human disease among the Class. To arrive at these conclusions the C8 Science Panel initiated 12 epidemiologic investigations of 55 diseases and reached 6 probable link conclusions (Steenland et al. 2014). These 'probable links' are reviewed in Chap. 14.

Several papers provide insight into the chronology of some of the exposure research activities that were conducted in this community.

Emmett et al. (2006) initially described the highest PFOA drinking water concentrations were from the Little Hocking Water District (LHWD). The average PFOA concentration in the LHWD from January 2002 until May 2005 was 3.55 μg/L (range 1.5–7.2 μg/L). This is approximately tenfold higher than the current US EPA Provisional Advisory for PFOA (0.4 μg/L). Emmett et al. suggested residential water, and not air, was the likely pathway of exposure of PFOA among 161 households that participated in their study. Self-reported number of glasses of water consumed per day was associated with increased serum PFOA concentrations. The median serum PFOA concentrations for 0, 1–2, 3–4, 5–8, and >8 glasses of tap water drinks per day were 301 ng/mL, 265 ng/mL, 370 ng/mL, 373 ng/mL, and 486 ng/mL, respectively (trend $p<0.0001$). Households that used bottled water had significantly lower serum PFOA concentrations. Also, residents who used household carbon water filters had lower serum PFOA levels (318 ng/mL) than those who did not (421 ng/mL) ($p=0.008$).

Published a few years later were the Class findings from the settlement's large cross-sectional health survey and exposure study (i.e., the C8 Health Project) (Frisbee et al. 2009). Under the court settlement, an independent company, Brookmar, Inc., designed and implemented the C8 Health Project. The Class eligibility was defined by exposure to contaminated water, a combination of geographic, and concentration criteria and exposure duration (Frisbee et al. 2009). The final C8 Health Project enrollment was 69,030 individuals who were individually compensated for their study participation per terms of the settlement (Frisbee et al. 2009). Both clinical laboratory tests and measurement of serum PFASs were included in the study. Serum PFASs included the perfluorocarboxylate homologue series C5 to C12 (PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, and PFDoA) and the perfluorosulfonates PFHxS and PFOS. Individual blood samples were collected over a 13 month time period (August 2005–August 2006).

The overall geometric mean serum PFOA concentration was 32.9 ng/mL (mean = 82.9 ng/mL; SD = 240.8) compared to 3.9 ng/mL for NHANES (2003–2004) (Frisbee et al. 2009). The range of concentrations in each of these water districts was not reported by Frisbee et al. Figure 4.12 provides the age- and sex-adjusted mean serum PFOA and PFOS concentrations for the six water districts and private well users (West Virginia and Ohio) of the C8 Health Project cross-sectional study. The adjusted mean PFOA concentrations ranged from fourfold higher (Pomeroy, Mason County) to >50-fold higher (Little Hocking Water Association) than comparable NHANES data. The age- and sex-adjusted mean for the Little

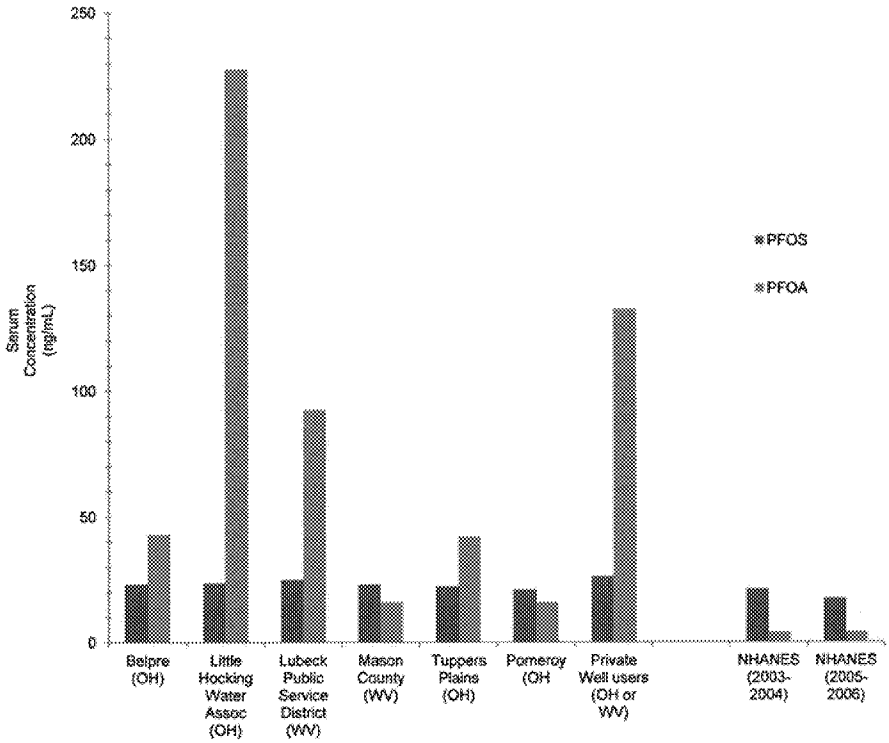

Fig. 4.12 Age-, sex- adjusted mean serum PFOS and PFOA concentrations for the C8 health project by six water districts and comparison with NHANES data

Hocking Water Association was 227.6 ng/mL for PFOA. The next highest adjusted mean PFOA concentration was for private well users (132.6 ng/mL). The overall mean serum PFOS concentration in this mid-Ohio river community (19.2 ng/mL) and was similar to NHANES data for 2003–2004 (20.7 ng/mL). This is to be expected as occupational exposure to PFOS was not present at this DuPont plant.

The Little Hocking Water Association residents studied by Emmett et al. had a 40 % higher average serum PFOA than those shown for this group in the C8 Health Project (in Fig. 4.11). This difference has been attributed to the fact that by 2005–2006 Little Hocking Water Association households had already reduced their exposure to PFOA because bottled drinking water had been supplied to their residences as part of the Class settlement (Frisbee et al. 2009). Subsequent to the 2005–2006 C8 Health Project, serum PFOA concentrations declined 26 % in a subset of residents from the Little Hocking and Lubeck water districts during the first year after charcoal activated filters were installed resulting in a serum elimination half-life of 2.3 years (Bartell et al. 2010).

Serum PFOA concentrations were higher among males than females in the C8 Health Project cross-sectional data (Fig. 4.13). The overall geometric mean for males (39.4 ng/mL) was higher than females (27.9 ng/mL) (means 98.2 vs. 68.8 ng/mL,

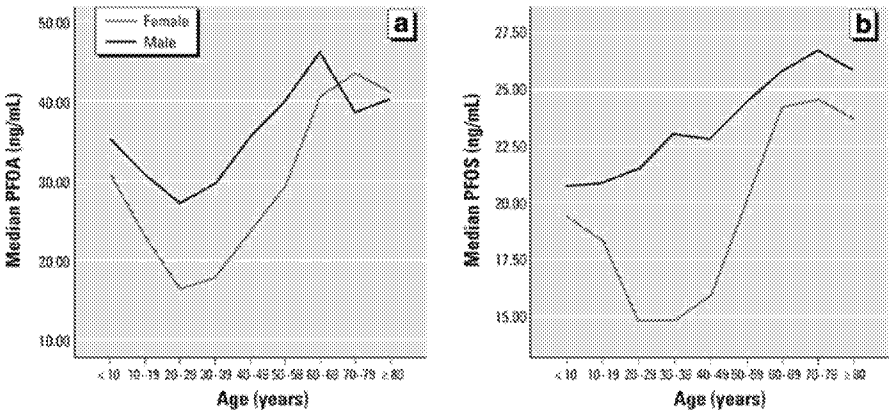

Fig. 4.13 Geometric mean PFOA and PFOS serum concentrations by age and sex, C8 health project, 2005–2006 (From Frisbee et al. 2009)

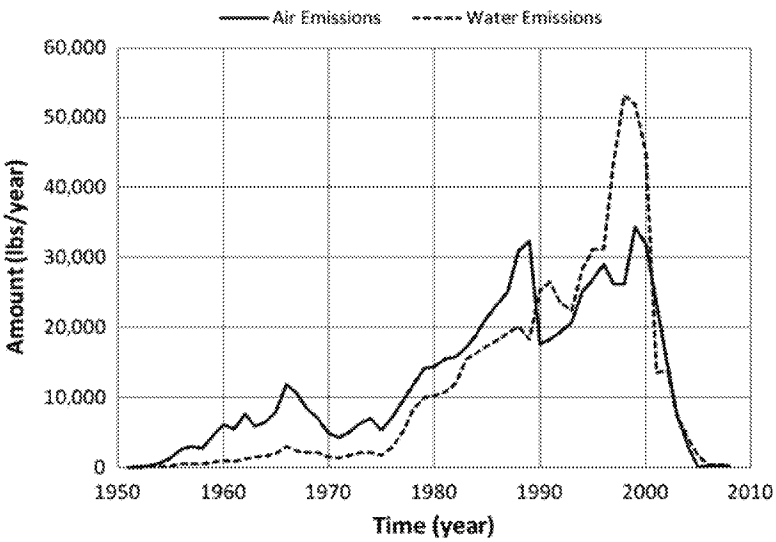

Fig. 4.14 Estimated historical PFOA releases into C8 health project area (Reprinted with permission from Shin et al. 2011a. Copyright 2011, American Chemical Society)

respectively). Several pharmacokinetic factors could explain, at least partially, these sex-related differences as PFOA is bound to serum protein. These factors may include pregnancy (Kato et al. 2014), parity (Berg et al. 2014; Brantsæter et al. 2013), lactation (Brantsæter et al. 2013; Fei et al. 2010; Sundström et al. 2011); and menstruation (Taylor et al. 2014).

Historic air and water emissions data were modeled by Shin et al. (2011a) (Fig. 4.14). These data, along with absorption, distribution, metabolism, and excretion (ADME) modeling, were used to predict annual serum PFOA exposures

(1951–2008) for the C8 Health Project participants based on their individual residential histories and water sources (municipal, well) (Shin et al. 2011b). For the six water districts' combined populations, the predicted median serum PFOA concentrations were within 0.5 orders of magnitude of the observed median concentrations measured in 2005/2006 during the course of the C8 Health Project. For example, for the C8 Health Project participants who had the same residence and workplace in one of the six water districts from 2001 to 2005 and assumed water consumption had a predicted median serum PFOA concentration in 2005–2006 of 32.2 ng/mL compared to 40.0 ng/mL observed. Predictions were less reliable for bottled water drinkers (10.5 ng/mL predicted versus 27.5 ng/mL observed) and those individuals not having had residences and workplace in one of the water districts from 2001 to 2005 (5.7 ng/mL predicted versus 15.3 ng/mL observed).

For the purpose of exposure reconstruction of PFOA in the C8 Science Panel's series of epidemiologic studies of a combined cohort of community participants and DuPont workers Winquist et al. (2013) were able to target 40,145 community members from the 54,457 C8 Health Project participants aged 20 years of age and older and 3,713 DuPont workers from the original 6,026 DuPont cohort. Of these 3,713 DuPont workers, 1,890 were also in the community cohort resulting in a total cohort of 30,431 participants that were contacted during the course of their epidemiologic studies. This combined cohort's historic annual estimated serum PFOA concentrations between 1951 and 2011 are presented in Fig. 4.15. Among only the community cohort, their median and interquartile ranges were 24.2 ng/mL and 12.3–58.9 ng/mL, respectively. Among the DuPont workers in the community cohort, their respective serum concentrations were 109.8 ng/mL and 55.9–256.2 ng/mL. The combined cohort's trend in serum PFOA concentrations (Fig. 4.15) mirrored the plant air and water emissions (Fig. 4.14).

Bartell et al. (2010) sampled the blood (up to six samples) of 200 residents of the Little Hocking and Lubeck water districts over an 18 month time frame. Their initial sampling serum PFOA concentration was 54.5 ng/mL. After water filtration was implemented, these investigators estimated the average serum PFOA decline was 26 % per year resulting in a median serum PFOA half-life of 2.3 years. This estimate was minimally biased from background exposures (Bartell et al. 2012).

4.3.1.4 Paulsboro, New Jersey

PFNA was detected at approximately 150 pg/mL (parts per trillion) in drinking water from the Paulsboro water department municipal well (New Jersey Health Department 2014). A potential source of exposure may be a nearby Solvay polymer plant that used to manufacture PFNA until 2009. No serum concentrations of area residents have been reported, to date.

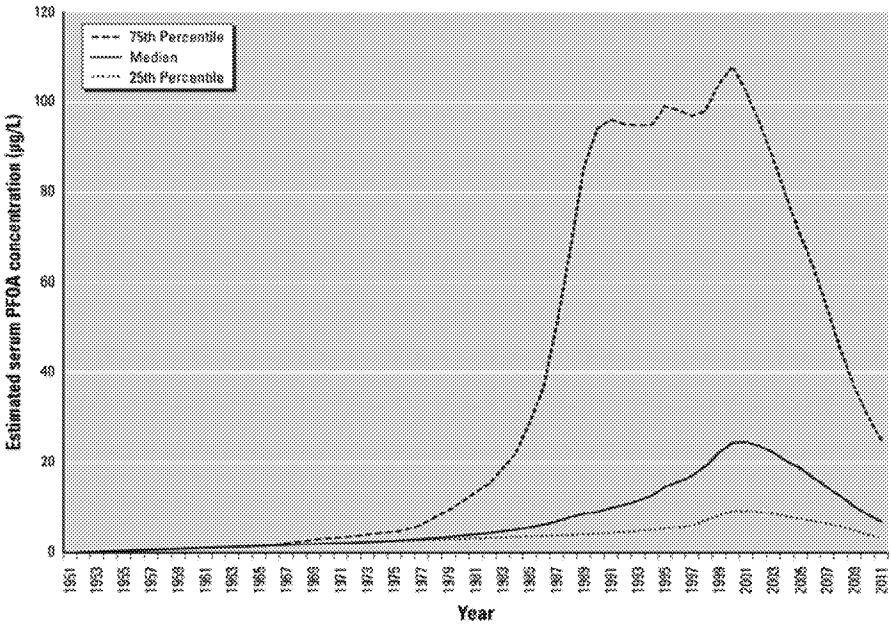

Fig. 4.15 Estimated historical serum PFOA concentrations by year for the community worker cohort study (From Winquist et al. 2013)

4.3.2 Germany

4.3.2.1 Arnsberg, Germany (Möhne Lake, Möhne River, Tributary of Ruhr River, Sauerland North Rhine-Westphalia Region)

A large environmental sampling study was conducted of surface and drinking water samples taken from multiple sites of the Rhine river and its main tributaries (Skutlarek et al. 2006). Sampling activity showed the summed PFAS concentrations in the water below 0.1 µg/L and that PFOA represented approximately 10 % of this total of PFAS. One tributary of the Rhine river, the Ruhr river located in northwest Germany, had at its mouth (near the city of Duisburg), a summed concentration of 0.94 µg/L with PFOA surprisingly representing 50 % of this amount. Investigators sampled further upstream on the Ruhr river focusing on one of its tributaries, the Möhne river. In a reservoir on this tributary, Lake Möhne, the summed PFAS concentrations reached 0.82 µg/L (PFOA = 0.65 µg/L). Upriver from this lake, the summed PFAS concentrations on the Möhne river reached 4.39 µg/L at Heidberg (PFOA = 3.64 µg/L). Investigations localized the main source of contamination between two parallel creeks where at the mouths of these creeks the summed PFAS concentrations reached 8.3 µg/L and 43.4 µg/L. PFOA represented more than 75 % of this amount at each mouth. Other perfluoroalkyls measured included PFBS, PFOS, PFBA, PFPeA, PFHxA, and PFHpA.

The investigators determined that between these creeks in a 10 ha-wide area (near Brilon-Scharfenberg) soil improvers were applied to the fields that had incorporated industrial wastes impregnated with high PFAS concentrations by a recycling company. Upon discovery of this affected area, installation of special drainage and water treatment was applied to reduce exposure to the upper Möhne river as well as charcoal filters installed in the Möhnebogen water works with frequent monitoring and change-out (Wilhelm et al. 2008).

Concentrations between the PFAS levels in the Möhne river and public drinking water were comparable for four boroughs of the city of Arnsberg which is situated near the mouth of the Möhne river. The majority of PFAS concentrations measured in the drinking water (total PFAS 0.60 μg/L) was due to PFOA (0.52 μg/L).

A biomonitoring study was conducted of a sample of residents from this Arnsberg population (men and mothers/children) with referent populations selected from the city of Brilon (men from the upper reaches of Möhne river before contamination site) and mothers and children in the Siegen area (not located on the Ruhr river or its tributaries) (Hölzer et al. 2008). Baseline blood samples were taken in 2006 and repeated in 2007 (Hölzer et al. 2008) and 2008 (Hölzer et al. 2009).

Provided in Fig. 4.16 are the results for the participants in the 2006 and 2008 surveys (Brede et al. 2010). In the baseline year (2006), the geometric mean and range of PFOA plasma concentrations (in parentheses) were: children (23.4 ng/mL, 95 % CI 19.2–28.5); mothers (23.6 ng/mL, 95 % CI 19.2–29.0); and men (30.3 ng/mL, 95 % CI 25.3–36.3). The 95th percentiles were 45.7 ng/mL, 53.5 ng/mL, and 49.2 ng/mL, respectively. These geometric mean and 95th percentile concentrations were approximately fivefold higher than the referent populations. Two years later after the remediation efforts, the geometric mean PFOA plasma levels declined by 39 % (children and mothers) and 26 % (men) in the Arnsberg population compared to 13–15 % in the reference groups (Fig. 4.16). The higher percentage declines among mothers and children were considered likely due to this population undergoing a greater initiative to reduce their drinking tap water consumption upon discovery of this environmental issue. The small decline of PFAS concentrations in the reference populations likely reflected the similar decreases observed in the general population. Geometric means of PFOS and PFHxS plasma concentrations were similar between the Arnsberg and referent populations.

Based on this 2 year study, Brede et al. calculated an estimated serum elimination geometric mean half-life of PFOA at 3.3 years assuming only background exposure. However, due to the fact this background exposure (approximately 4 ng/mL) approached the Arnsberg exposure data, Bartel (2012) calculated this 3.3 year half-life estimate was biased upwards by 26 %. Similarly, Russell et al. (2014) adjusted for background exposure in the Arnsberg database and estimated the PFOA geometric mean intrinsic half-life of 2.5 years (95 % CI 2.4–2.7) instead of the apparent half-life calculation of 3.3 years.

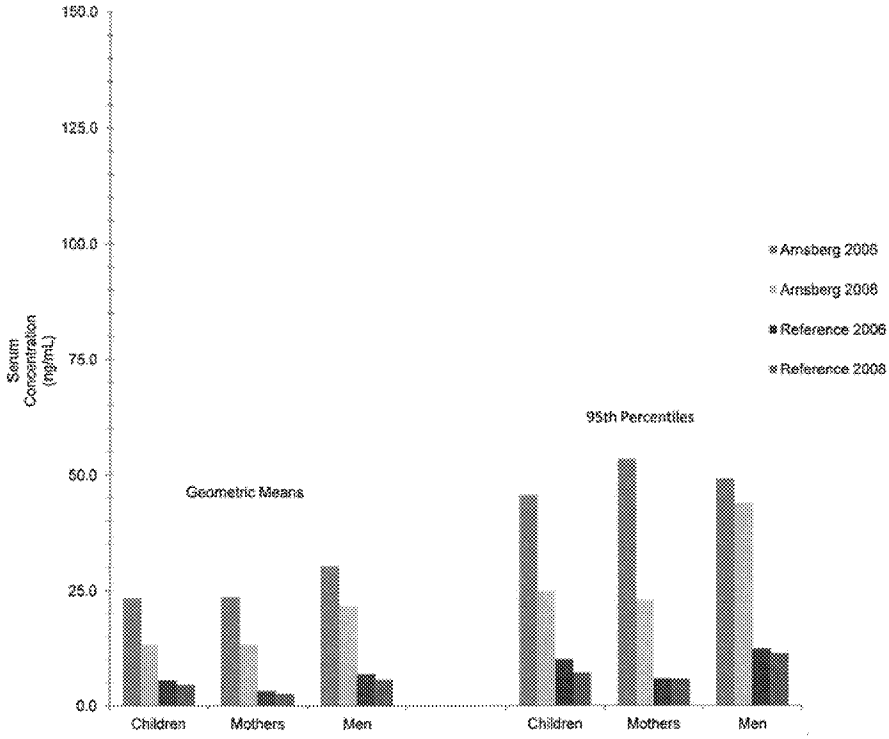

Fig. 4.16 Geometric mean and 95th percentile serum concentrations (ng/mL) of PFOA among children, women, and men from the City of Arnsberg, Germany and referent populations (Brilon, Siegen), 2006 and 2008

4.3.3 China

4.3.3.1 Tangxun Lake, Wuhan, China

Tangxun Lake is a relatively shallow body of water 36 km^2 area wide located in Wuhan, China (population ten million). Tangxun Lake water drains to the nearby Yangtze river. Elevated serum concentrations of PFASs were reported in the surface water and sediments of Tangxun Lake, its aquatic biota samples, and serum from commercial fishermen on the lake (Zhou et al. 2013, 2014). Fluorochemical manufacturing plants were identified in an industrial park upstream from the wastewater treatment plant situated on the upper reaches of the lake. As previously mentioned, Wang et al. (2010) described an environmental assessment at a manufacturing facility near Wuhan, (Hubei Province) China. The main products produced by ECF were perfluoroalkyl sulfonic acid, perfluorocarboxylic acids, and perfluorotertiary amines and their derivatives. Whether this plant is part of this industrial park is uncertain.

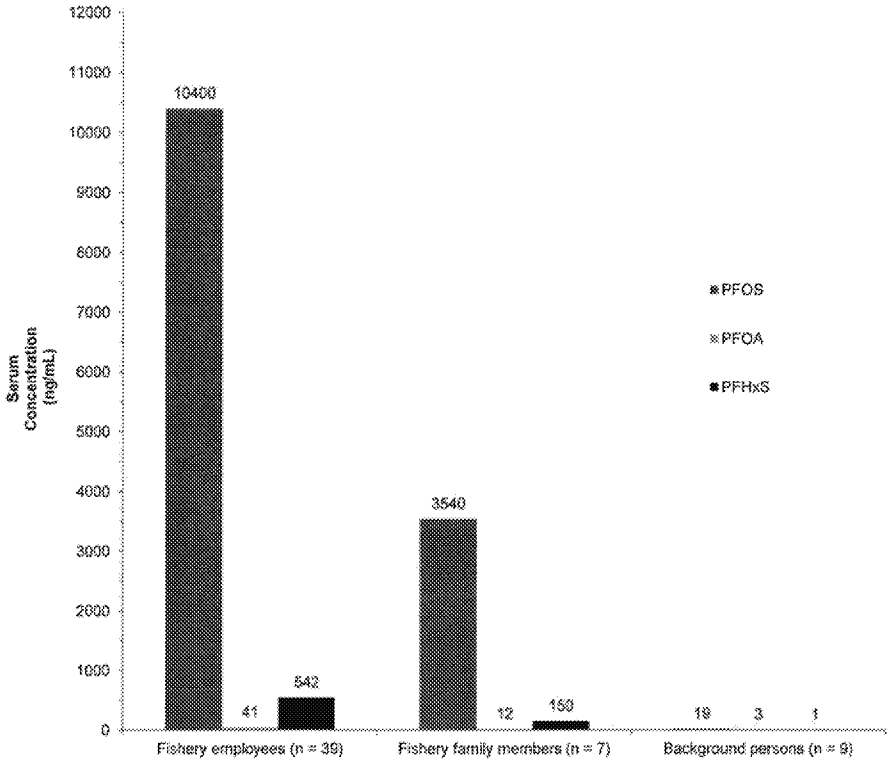

Fig. 4.17 Median serum PFOS and PFOA concentrations (ng/mL) among fishery employees and their family members, Tangxun Lake, Wuhan, China

Upstream from this Tangxun Lake wastewater treatment plant were water samples that had the following concentrations measured: PFBA 47.8 μg/L; PFOA 2.6 μg/L; PFBS 15.3 μg/L; PFHxS 0.49 μg/L; and PFOS 2.14 μg/L. Effluent from the wastewater treatment plant had similar mean concentrations of PFOS and PFOA but mean concentrations of PFBA and PFBS declined to 6 μg/L and 5 μg/L, respectively. Surface lake water concentrations of PFOA and PFOS ranged between 0.1 and 0.3 μg/L. For PFBA and PFBS, the average surface lake water concentrations were 4.8 μg/L and 3.7 μg/L, respectively. For perspective, the US EPA Provisional Advisory for Water for PFOS and PFOA are 0.2 μg/L and 0.4 μg/L (See Chap. 17 for other advisory values from other states and countries.)

Figure 4.17 presents the median PFAS serum concentrations for 37 Tangxun Lake fishermen, 7 family members, and 9 reference individuals. The median PFOS concentrations were 10,400 ng/mL, 3,540 ng/mL, and 19 ng/mL, respectively. The linear/branch PFOS ratio was 3.6:1 which approximates the ratio to be expected with the ECF manufacture of POSF. The highest serum PFOS concentration measured was 31,400 ng/mL in a commercial fisherman. This concentration is threefold higher than the next highest value ever reported – a worker engaged in

POSF production workers at the 3M Decatur plant (Olsen et al. 2003b). PFHxS concentrations were also considerably higher in the commercial fishermen (median 542 ng/mL) but lower for PFOA (41 ng/mL) (Fig. 4.16). Several species of Tangxun lake carp, shrimp and snail had PFOS concentrations ranging between 200 and 600 ng/g/ww (Zhou et al. 2013). Exposures to commercial fishermen on Tangxun Lake were considered likely due to their fish consumption (Zhou et al. 2014). Zhou et al. concluded the population identified around Tangxun Lake may offer an excellent research opportunity to resolve controversial PFAS findings in the published epidemiologic studies.

4.4 Other Biomonitoring Data (Medical, Occupational, and Consumer)

4.4.1 Phase I Clinical Trial of Cancer Patients

APFO has been shown to cause endoplasmic reticulum stress, inhibit PIM kinases, and exhibit anti-cancer activity in multiple xenograft models (MacPherson et al. 2010, 2011). The tolerability, safety and pharmacokinetics of APFO were reported in an update of a phase I clinical trial in 41 human patients with advanced (solid) cancer (MacPherson et al. 2010). Sequential cohorts of three patients were enrolled in this dose escalation trial that followed a standard 3+3 design until dose-limiting toxicity was observed in two or more of six patients at a given dose. The protocol-defined maximum tolerated dose was not reached. The recommended Phase 2 dose was 1,000 mg weekly based on the common cumulative drug-related toxicity of fatigue, nausea, vomiting, and diarrhea at weekly 1,200 mg doses. Based on a poster presentation (McPherson et al. 2010), highest plasma level of PFOA achieved in a patient approached 1250 μM (approximately 515,000 ng/mL). To date, this would be the highest PFOA concentration known to have been reported in a human.

4.4.2 Professional Ski Waxers

In a series of papers, Nilsson et al. (2010a, b, 2013) investigated PFAS exposures among professional ski waxers. Two types of ski wax are used depending on the race: grip and glide, but only the latter contains fluorinated additives. Glide ski waxes are applied using a petroleum based product that contains various linear hydrocarbons with the formula $(CH_3(CH_2)_nCH_3$ [where n is between 10 and 80 carbon atoms] and semifluoroalkanes with the formula $(CH_3(CH_2)_m(CF_2)_nCF_3$ [where m varies from 14 to 20 and n from 2 to 16]. Specific formulas are usually not disclosed by the manufacturers.

Nilsson et al. (2010a) conducted a seasonal biomonitoring trend study of eight professional ski waxers employed by the Swedish and US national cross-country ski teams. During the professional racing season, these technicians applied fluorinated ski wax approximately 30 h per week. A total of 57 blood samples were examined before, during, and after the International Federation Ski (FIS) World Cup season in 2007–2008. Nilsson et al. compared the ski wax technicians' blood levels to unexposed men of similar age. Among the professional ski waxers, their median PFOA whole blood concentration was 112 ng/mL compared to the unexposed group's 2.7 ng/mL level. Their PFNA levels (range 10.1–163 ng/mL) were between 15 and 270 times that of the referent group. Other perfluorocarboxylate concentrations reported higher than expected concentrations included perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorodecanoate (PFDA), and perfluoroundecanoate (PFUnDA). The sulfonated PFASs were not above background levels because these compounds are not incorporated in ski waxes.

Nilsson et al. (2010b) examined inhalation exposure to fluorotelomer alcohols as application of the ski waxes frequently occurs in small cabins. Air monitoring of perfluorocarboxylates, perfluorosulfonates, and fluorotelomers (6:2FTOH, 8:2 FTOH, and 10:2 FTOH) were analyzed. The 8:2 FTOH (range 830–250,000 ng/m^3) was the highest measured and ranged 8–32 times higher than PFHxA (57–14,000 ng/m^3) and 10–800 times higher than PFOA (80–4,900 ng/m^3). The average concentration of telomer alcohols 6:2 FTOH, 8:2 FTOH, and 10:2 FTOH were 240 ng/m^3, 92,800 ng/m^3, and 370 ng/m^3 in the air, respectively. Mean levels of PFOA and PFNA were 1,200 ng/m^3 and 30 ng/m^3 compared to 4,900 ng/m^3 for PFHxA. Air monitoring data were not correlated to serum concentrations due to the long serum elimination half-life of PFOA.

Nilsson et al. (2013) studied whether the PFOA measured in these professional ski wax technicians came from direct exposure to PFOA in the air or from biotransformation of the 8:2 FTOH. Their data indicated metabolism of FTOHs to PFOA and PFNA was the likely biotransformation pathway because 5:3 fluorotelomer carboxylic acid (5:3 FTCA) and 7:3 FTCA metabolites were also measured in the whole blood of these ski wax technicians (median concentrations 1.7 ng/mL and 0.92 ng/mL), respectively.

Freberg et al. (2010) examined PFAS blood concentrations and air samples among 13 professional ski waxers from the Norwegian ski team. They monitored serum concentrations after one season (March 2008), obtained a second sample just before the next season (November 2008), and collected final samples obtained after the second season (March 2009). At the end of the second season, median serum PFOA (57 ng/mL, range 20–162), perfluorononanoate (PFNA) (6.8 ng/mL, range 2.3–27), and PFDA (0.9 ng/mL, range 0.2–3.3) concentrations were approximately 25-fold, tenfold and tenfold higher, respectively, than the Norwegian general population. Serum PFOA, PFNA, and PFDA concentrations were correlated with the number of years exposed to ski waxes. C4–C14 chain lengths were determined in the air monitoring data; with PFOA (C8), PFDA (C10), and PFDoDA (C12), and perfluorotetradecanoate (PFTrDA) (C14) having the highest concentrations.

4.4.3 Firefighters

Because of their surface-tension properties, aqueous film-forming foams (AFFF) are chemical mixtures developed to extinguish and prevent re-ignition of hydrocarbon fuel-based fires. AFFFs were formulated with proprietary fluorinated surfactants (D'Agostino and Mabury 2014). AFFF differed by their multiple manufacturers and year of production (Houtz et al. 2013). In general, AFFFs contained fluorosurfactants, hydrocarbon surfactants, cosolvents and solvents (Weiner et al. 2013). PFOS was a commonly used fluorosurfactant in AFFFs (before phase-out) with 6:2 fluorotelomer chain length products subsequently becoming a dominant source in AFFFs with 6:2 fluorotelomermercaptoalkylamido sulfonate (FTAS) and 6:2 fluorotelomersulfonamide alkylbetaine (FTAB) as common components. D'Agostino and Mabury (2014) recently identified 12 novel and 10 infrequently reported PFAS classes in AFFFs with fluorinated chain lengths ranging from C3 to C15 that represented 103 total compounds. Investigations have examined AFFF training locations, including airport fire fighting training facilities whether they were military or civilian operations. Several of these sites have now been characterized for soil, ground water and other environmental assessments of PFASs and the reader is directed elsewhere for such analyses as findings are site-specific (Awad et al. 2011; Place and Field 2012; Weiss et al. 2012; Weiner et al. 2013).

Studies were not identified that reported biomonitoring data obtained from individuals who were trained with AFFF formulations that were applied to specific hydrocarbon fuel-based fires, whether actual or ignited for training based purposes. In Cologne, Germany ten nearby community residents were sampled via biomonitoring whose private drinking wells contained PFASs likely from a nearby fire training area (Weiss et al. 2012). For well "A", plasma concentrations for five of the individuals ranged for PFOS from 19.4 to 295 ng/mL, for PFOA from 4.0 to 18.0 ng/mL, and for PFHxS from 18.9 to 205 ng/mL. Serum concentrations were somewhat lower for individuals who belonged to well "B".

Large scale perfluoroalkyl-related biomonitoring studies of fire fighters have not been published. A subset of 36 individuals from the mid-Ohio River valley C8 Health Project cross-sectional study conducted in 2005–2006 (see community exposure) self-identified their single employment category as firefighters (Jin et al. 2011). These individuals' median PFHxS concentration was 4.6 ng/mL compared to those who reported other employment (3.6 ng/mL) or no job reported (3.5 ng/mL). Likewise, the median PFOS serum concentrations were 27.9 ng/mL, 23.0 ng/mL, and 20.9 ng/mL, respectively. Although Jin et al. suggested the PFHxS difference was likely the result of exposure to fire-suppression foam and/or fire conditions in households with stain resistant carpet applications, their small sample size, the absolute difference (1 ng/mL), and the lack of detailed occupational history suggests the authors' inference was rather speculative.

4.4.4 Fishermen

As reported in several northern European countries, diet, particularly that of fish, is considered an important source to the PFOS serum concentrations measured in Scandinavian general populations (Falandysz et al. 2006; Haug et al. 2010; Rylander et al. 2010), although this association has not been consistently observed in these countries (Eriksen et al. 2011). A sample of 196 Greenlandic Inuits, whose traditional diet consists of consumption of fish and marine mammals, had a mean PFOS concentration of 51.9 ng/mL in 2002–2003 (Lindh et al. 2012). This was approximately 50 % higher than NHANES data (Kato et al. 2011). Among these Inuits, higher concentrations were reported for those who lived in more isolated regions than those residing in Nuuk, the capital of Greenland, where purchased foods were a greater source of PFASs.

In other parts of Europe, geometric mean serum PFOS and PFOA concentrations of 478 freshwater fish anglers from six regions in France were comparable to U.S. and Canadian general population levels (Denys et al. 2014). This finding may be the result of the relatively low frequency of fresh water fishing in this population. However, the top 10th percentile of these anglers fished at least ten times during the year. At the 95th percentile of these fresh water anglers, their geometric mean concentration was 56.7 ng/mL for PFOS compared to 40.4 ng/mL in NHANES suggesting fresh water fish consumption contributes to PFOS concentrations among those who fish often.

It should therefore not be unexpected that fishermen may have higher serum concentrations of PFASs when consuming fish from lakes affected with higher PFAS concentrations as a consequence of industrial PFAS releases (e.g., Tangxun Lake, Wuhan, China) or from agricultural run-off into rivers where soil conditioner containing perfluoroalkyl compounds had been applied to the land (Lake Möhne, Germany). Commercial fishermen on Tangxun Lake, as well as their family members, had serum PFOS concentrations in the higher ranges of occupational manufacturing workers (Fig. 4.17). The highest serum PFOS concentration (31,400 ng/mL) reported, to date, in the scientific literature, was measured in one of these Tangxun Lake commercial fishermen (Zhou et al. 2014). At Lake Möhne (discussed above) near Arnsberg, Germany, Hölzer et al. (2011) reported two to threefold higher serum PFOS concentrations among individuals consuming at least three fish per month than those who did not consume fish (Fig. 4.18).

4.4.5 Post-market Consumers

Because diet was considered to represent a much larger source contribution, Trudel et al. (2008) estimated exposure to consumer products would result in minor exposures to PFOS and PFOA. For example, consumer-related exposures may occur through treated carpets and coated food contact material but exposures could change over time based on different formulations (Liu et al. 2014).

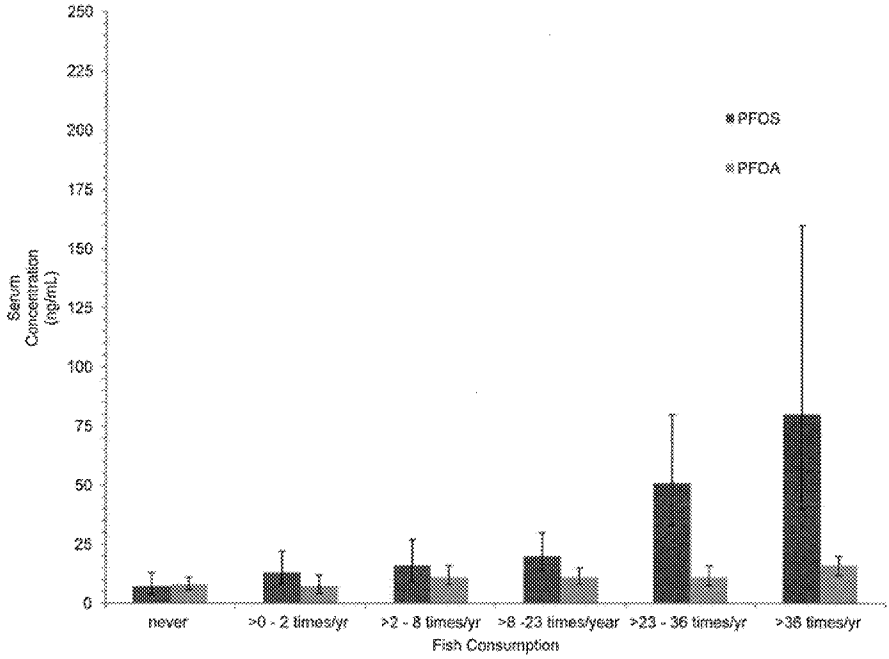

Fig. 4.18 Geometric mean PFOS and PFOA plasma concentrations (95 % confidence intervals) by fish consumption in 105 Anglers, Lake Möhne, Germany

Although human exposure to treated products is considered low, subgroups of the general population could obtain higher exposures (Herzke et al. 2012). In this regard, Beeson et al. (2012) reported unusually high serum perfluorohexanesulfonate (PFHxS) concentrations (range 27.5–423 ng/mL) in a family whose carpets had been commercially cleaned eight times over 15 years. As a reference, PFHxS concentrations in the Canadian general population ranged between 1 and 3 ng/mL. Measurement of vacuum dust in the home found high concentrations of PFHxS (2,780 ng/g) and PFOS (1,090 ng/g). In this particular family's case, their Edmonton residence had an in-floor radiant heating system. Wall-to-wall carpeting had been installed on top of these heated floors. Beeson et al. believed the high PFHxS to PFOS ratio reflected two historical 3M Scotchgard™ products that might have been commercially applied to provide such an exposure ratio. For reasons yet understood, the shorter chain PFHxS has been reported to have a longer serum elimination half-life in humans (approximately 7–8 years) than the longer chain PFOS (approximately 4 years) (Olsen et al. 2007) or the shorter chain PFBS (approximate half-life of 30 days) (Olsen et al. 2009). This is unlike the C4 (PFBA), C6 (PFHxA), and C8 (PFOA) perfluorocarboxylates whose human serum half-lives have been approximately 3 days (Chang et al. 2008), 32 days (Russell et al. 2013), and 2.3–3.5 years (Bartell 2012; Olsen et al. 2007), respectively.

Although general population serum concentrations of PFHxS in the United States and Canada generally range between 1 and 3 ng/mL, Olsen et al. (2004a) observed PFHxS concentrations were slightly higher among subsets of 598 children and hypothesized it may be due to their activity exposure patterns (e.g., related to playing more on carpets) (Olsen et al. 2004a). They reported 95 % of the adult (Olsen et al. 2003d) and elderly (Olsen et al. 2004b) populations had serum PFHxS concentrations less than 10 ng/mL but this percentage was 73 % in children. Furthermore, only 1 of 645 adults and 1 of 238 elderly individuals had measured serum PFHxS concentrations greater than 30 ng/mL compared to 11 % (N=67) of the 598 children samples. Similar distributions were observed for N-methyl perfluorooctanesulfonamidoacetate, found in PFOS-based products used primarily in surface treatment applications (e.g., carpets, upholstery, textiles), and therefore considered a possible marker for consumer-related exposure. Subsequent NHANES data reported by Kato et al. (2009) supported such a hypothesis in pooled analyses of children's serum. In addition, other sampling exposure regimens continued to show household dust as a source for PFASs, including PFHxS and fluorotelomer alcohols (Strynar and Lindstrom 2008; Haug et al. 2011) It should also be noted that N-methyl perfluorooctanesulfonamidoacetate (N-MeFOSA-AcOH) has declined in NHANES data between 1999 and 2000 when the geometric mean serum concentration was 0.97 ng/mL (95 % CI 0.84–1.11) and 2007–2008 (geometric mean 0.35 ng/mL, 95 % CI 0.32–0.38).

4.5 Summary

Provided in Fig. 4.19 (PFOS), Fig. 4.20 (PFOA), and Fig. 4.21 (PFHxS) are comparative analyses of the magnitude of concentrations measured across the three types of higher exposed populations that were reviewed in this chapter: (1) PFAS manufacturing and 'downstream' production workers; (2) communities affected by specific identifiable sources of PFAS exposure (above background levels) that have affected municipal and/or private water sources; and (3) medically-, occupationally- and consumer-related.

Until recently, the highest serum PFOS, PFOA, and PFHxS concentrations measured involved PFAS manufacturing workers. While this remains true for average concentrations, this is not the situation on an individual basis. A commercial fisherman in Tangxun Lake (Wuhan, China) has the highest concentration reported, to date, for PFOS (31,400 ng/mL). This is approximately threefold greater than the highest PFOS measured in a manufacturing worker and 2,500 times larger than the average in the general population. As for PFOA, again from a population standpoint, the highest exposed populations with PFOA concentrations are in the occupational setting (3M, DuPont, and Miteni). However, the highest serum PFOA concentration ever reported was from a cancer patient enrolled in a phase I clinical trial of APFO. This patient's serum PFOA concentration exceeded 515,000 ng/mL. This is fivefold greater than the highest PFOA concentration measured in a

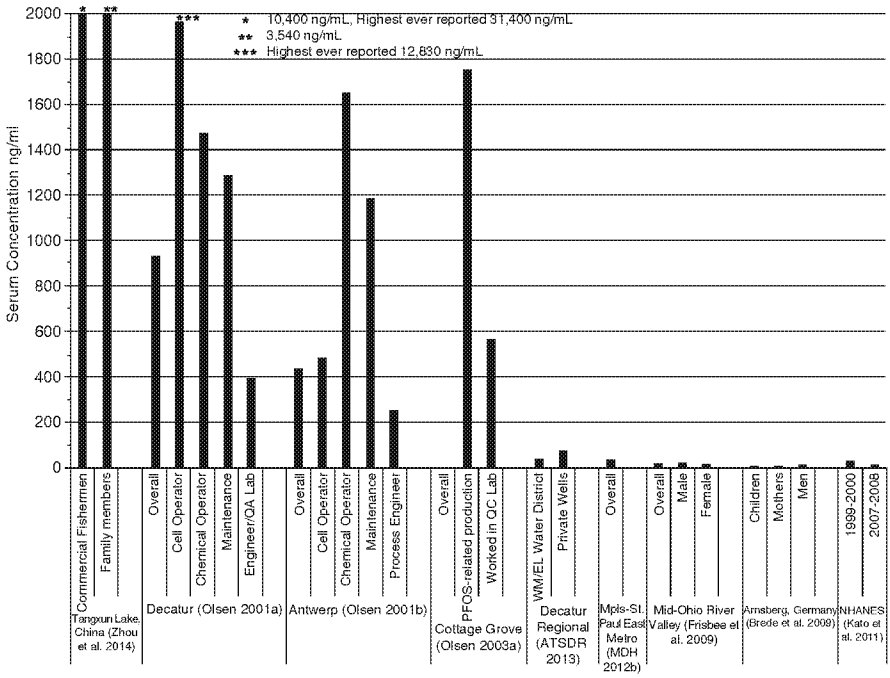

Fig. 4.19 Geometric mean (or median) serum PFOS concentrations in higher exposed populations. See text for descriptions of studies

manufacturing worker and 100,000 times higher than the average person in the general population.

Although affected communities are sometimes referred to as 'highly exposed', such terminology is always relevant to a baseline. Although individuals in these communities' populations may have concentrations comparable to manufacturing workers, as shown in Figs. 4.19, 4.20, and 4.21, the higher average exposed populations remain those in PFAS manufacturing and application jobs. The higher exposed former manufacturing populations have PFOS, PFOA, and PFHxS average serum concentrations 2–3 orders higher than the NHANES general population (Figs. 4.19, 4.20, and 4.21). Events unfolding with the very high PFOS concentrations being measured among commercial fishermen in Tangxun Lake, Wuhan, China may provide further opportunities for understanding PFAS concentrations in higher exposed populations (Zhou et al. 2014).

There are several areas of investigation among higher exposed populations that may be of interest to PFAS biomonitoring researchers. First, have the PFAS concentrations declined among the former manufacturing industrial workers? Have their PFAS blood concentrations declined at a rate to be expected? Unlike several biomonitoring trend studies of the general population where PFOS concentrations have declined by approximately two-thirds since 2000, such trends have not been reported among manufacturing workers. Second, have affected communities who had PFAS

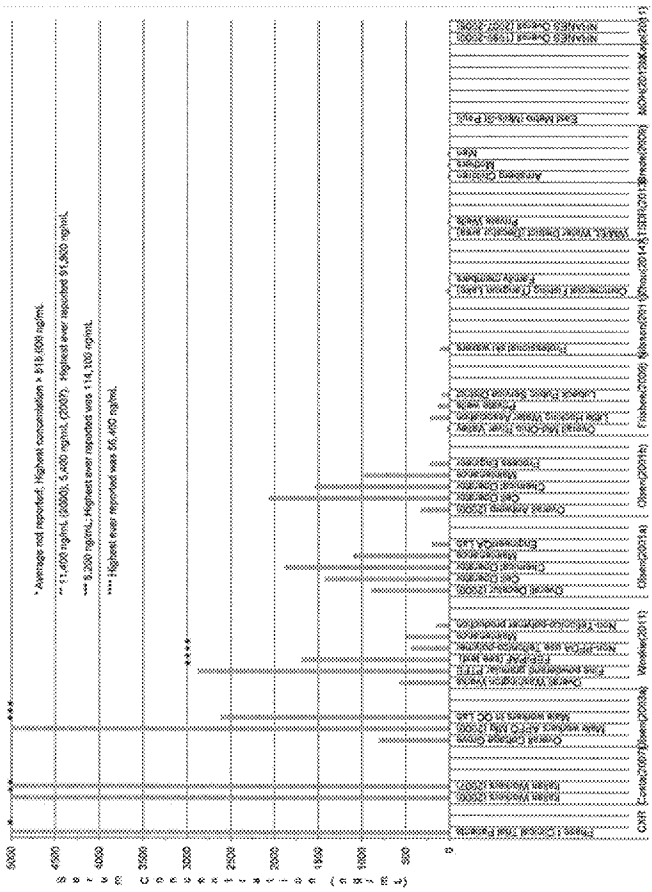

Fig. 4.20 Geometric mean (or median) serum PFOA concentrations in higher exposed populations. See text for descriptions of studies

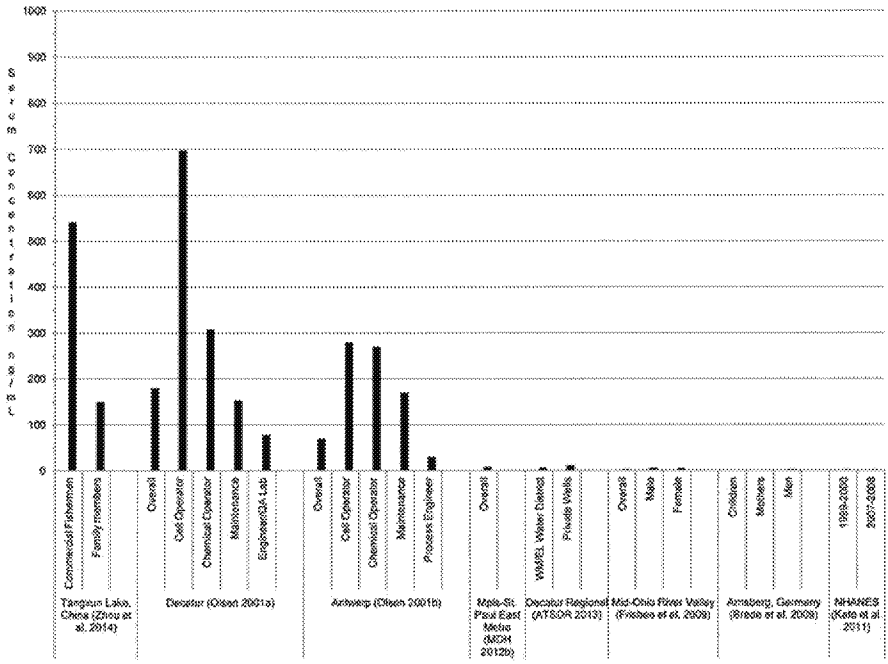

Fig. 4.21 Geometric mean (or median) serum PFHxS concentrations in higher exposed populations. See text for descriptions of studies

mitigation activities applied to their water supplies, continued to experience declining PFAS concentrations? Third, research should be directed at measuring and reporting PFAS concentrations in workers in the burgeoning Chinese perfluorochemical industry. Although concentrations in the Chinese general population appear to have increased (Jin et al. 2007), there is a virtual absence of reporting of biomonitoring data in these manufacturing workers. Fourth, although the study population was small, epidemiologic analyses of the clinical chemistry data of the patients in the Phase 1 clinical trial of APFO could answer critically important epidemiologic questions. These answers may provide substantial insight into the various epidemiologic associations reported about PFOA that have been inconsistently reported at much lower concentrations, often reported at general population levels (see Chap. 14). Finally, this chapter primarily focused on PFOS and PFOA. The replacement chemistry for the higher chain PFASs are shorter chain perfluorosulfonate and perfluorocarboxylate compounds that have considerably faster serum elimination rates in humans. Whereas there have been some biomonitoring data reported on the short-chain PFASs in the general population (Kato et al. 2011; Olsen et al. 2012a), understanding higher exposed populations to these shorter chain compounds in the occupational setting will provide additional perspectives.

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Chapter 5
Perfluorinated Alkyl Acids in Wildlife

Jessica L. Reiner and Benjamin J. Place

Abstract The first measurements of perfluorinated alkyl acids (PFAAs) in wildlife from the aqueous and terrestrial environment showed their ubiquitous presence. Since the initial studies in 2001 their presence, distribution, and fate in the environment has broadly been studied. PFOS, perfluorooctane sulfonate, is the dominant and most frequently detected PFAA in wildlife from around the world. Additionally long-chain (greater than eight carbons long) perfluoroalkyl carboxylates (PFCAs) have also been measured in biota, with some of the highest concentrations being measured in wildlife from the Arctic. The majority of temporal studies have shown PFOS concentrations increasing over time; however, more recent observations have started showing a decline in PFOS in some wildlife. Long-chain PFCA (greater than eight carbons) concentrations have shown an increase in recent temporal studies, with PFCA concentrations comparable to those of PFOS measured in the same animals. Many food-web studies have examined bioaccumulation and biomagnification of PFAAs. Most studies have shown biomagnification of PFAAs, with PFOS especially having a higher biomagnification potential compared to the PFCAs. While much work has been done during the 15 years PFAAs have been examined in wildlife, there are still gaps. There has been limited work on wildlife from terrestrial ecosystems. There are also many geographical locations that have very limited studies or lack them altogether. Additional monitoring of the terrestrial environment and the inclusion of new geographical locations are needed to help understand the global distribution of PFAAs in wildlife.

Keywords Biomonitoring • Temporal trends • Food-web • Fish • Mammals

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Disclaimer Certain commercial equipment or instruments are identified in the paper to specify adequately the experimental procedures. Such identification does not imply recommendations or endorsement by the NIST nor does it imply that the equipment or instruments are the best available for the purpose.

J.L. Reiner (✉)
Chemical Sciences Division, Hollings Marine Laboratory, National Institute of Standards and Technology, 331 Fort Johnson Road, Charleston, SC 29412, USA
e-mail: jessica.reiner@nist.gov

B.J. Place
Chemical Sciences Division, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899, USA

© Springer International Publishing Switzerland 2015

J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_5

ED_002330_00119699-00138

5.1 Introduction

The first reports of the global distribution of a perfluorinated compound (PFC) in wildlife, mainly perfluorooctane sulfonate (PFOS), were in 2001 (Giesy and Kannan 2001; Kannan et al. 2001a, b). These studies included a wide variety of organisms spanning many trophic levels and from many different regions (North America, Europe, Arctic, etc.) and concluded that PFOS was a widespread, persistent pollutant. The initial studies on PFAAs focused on PFOS and perfluorooctanoic acid (PFOA), but expanded quickly to include perfluorinated sulfonic acids (PFSAs), perfluorinated carboxylic acids (PFCAs), and PFAA precursors (fluorotelomer alcohols, sulfonamide ethanols, perfluorsulfonamides, and fluorotelomer saturated and unsaturated carboxylic acids). While the list of PFAAs has increased significantly, PFOS is still the dominant compound analyzed for and found in wildlife.

The majority of initial studies focus on measurement of PFAAs in wildlife coming from North America and Europe, two of the major manufacturing locations until 2002 (Prevedouros et al. 2006). As the years have progressed other regions around the world have started providing measurements in their wild populations. Currently there are numerous studies from the Northern Hemisphere, especially from wildlife in North America, Europe, and Asia. Although few, there have been studies looking at PFAAs in wildlife from the Southern Hemisphere.

This chapter summarizes monitoring data for PFAAs in wildlife throughout the world. There is a brief discussion of the challenges associated with wildlife sampling, laboratory measurement, and analytical issues. After describing the current knowledge of PFAAs in wildlife, we will go into more detail about distribution, sex and age trends, temporal trends, and food-web information. Finally we will summarize and discuss some data gaps which should be addressed in future studies.

5.2 Challenges

Most wildlife samples are collected opportunistically and chemical analysis of an emerging compound class like PFAAs have been done on archived samples initially collected for another purpose. Since the majority of samples were initially collected for other purposes, the contamination during sampling, storage, and processing before chemical analysis should be carefully considered. Most studies do not describe the sampling methods used during the acquisition phase of the experiment; furthermore measurements of PFAAs from field blanks are rarely described, possibly not even considered during sample analysis.

There are many studies examining PFAAs in all different types of wildlife (invertebrates, reptiles, fish, birds, mammals). A major issue with looking at all wildlife is making comparisons in different tissue samples. The majority of tissue samples that have be investigated for PFAAs has included the protein rich matrices such as liver, blood, serum, plasma, kidneys, eggs, and whole animals. However, in order to understand the body burden in an animal species, some studies which examine

PFAAs in other, less protein rich tissues like blubber are needed. The majority of studies have focused on liver, blood, serum, and plasma because of the predisposition for PFAAs to accumulate in these matrices. When discussing PFAAs in wildlife in this chapter we will mainly focus on these matrices.

Since PFAAs differ from the traditional lipophilic persistent organic pollutants (POPs), the analytical methods for the analysis of PFAAs in a variety of matrices remain a topic of active investigation. Hansen et al. (2001) developed one of the first methods which extracted PFOA and PFOS from tissues using the ion-pairing method. Other methods using basic methanol for alkaline digestion and acetonitrile for protein precipitation have been used extensively for measurements of PFAAs from biological matrices (Taniyasu et al. 2005; Reiner et al. 2011b, 2012; Sinclair et al. 2006). Having methods adapt to produce better limits of detection and better recovery is beneficial to the analytical community; however, there are issues with comparability among studies using different analysis techniques. There have been numerous interlaboratory studies looking at the reproducibility of measurements in a variety of matrices (van Leeuwen et al. 2006; Longnecker et al. 2008; Lindstrom et al. 2009; Keller et al. 2010; Reiner et al. 2012). In general chemical analysis has improved throughout the years, but historically laboratories have used in-house control materials to make measurements that have limited interlaboratory comparability. With the amount of wildlife studies from many different laboratories and the different extraction and cleanup methods being used in different laboratories, reference materials are ideal to help understand the quality and comparability of that data originating from all of these laboratories. Only recently have reference materials been available for the measurement of PFAAs in biological matrices (Keller et al. 2010; Reiner et al. 2011b, 2012) and are now being incorporated into biological measurements on a routine basis (Reiner et al. 2011a).

5.3 Zooplankton and Invertebrates

There have been few studies looking at the accumulation of PFAAs in zooplankton and invertebrates (Tomy et al. 2004; Powley et al. 2008; Haukås et al. 2007; Houde et al. 2008; Gulkowska et al. 2006; Haug et al. 2010; van Leeuwen et al. 2009; Yeung et al. 2009). These studies have focused in the Arctic, North America, Europe, and Asia. PFOS has been the most frequently detected PFAA in zooplankton and invertebrate studies; however, most studies showed concentrations of PFOS and other PFAAs very close to the limits of detection.

5.4 Amphibians and Reptiles

Although infrequently studied, there are a number of reports that have examined the presence of PFAAs in amphibians and reptiles across the planet, focused primarily on frogs and turtles. Most studies have included amphibians and reptiles as part of

a larger set of animal classes, in order to evaluate the distribution of PFAA contamination across species in regions of Canada (de Solla et al. 2012), the United States (Giesy and Kannan 2001; Kannan et al. 2005a), and China (Shi et al. 2012a; Wang et al. 2011). Notably, there have been a few studies that have focused solely on the PFAA contamination of turtles on the Italian coast (Guerranti et al. 2013) and the southeast coast of the United States (Keller et al. 2005; O'Connell et al. 2010).

As with the other animal classes, PFOS was the dominant PFAA detected in most tissues (Kannan et al. 2005a), with some of the long-chain PFCAs and PFSAs regularly detected (de Solla et al. 2012; Keller et al. 2005; O'Connell et al. 2010; Shi et al. 2012a). In a study of freshwater biota nearby an international airport in Canada, de Solla et al. (2012), reported very high concentrations of PFOS in snapping turtle plasma (mean PFOS concentration: approximately 2,380 ng/g). PFOA and PFOSA were not regularly detected in many reptile and amphibian tissues (Guerranti et al. 2013; O'Connell et al. 2010; Kannan et al. 2005a). In contrast, Keller et al. (2005) did find detectable quantities of PFOA in loggerhead and Kemp's ridley sea turtle plasma (range 0.493–8.14 ng/mL).

PFAA exposure to reptiles and amphibians was regularly identified as coming from areas of high human population; O'Connell et al. (2010) found a statistically significant positive correlation between human populations near sampling sites and the PFOS and PFUnA concentrations in juvenile loggerhead turtles. While few studies have included amphibian and reptile tissues as part of the sample collection, the studies that have been done shown similar PFAA contamination to other classes of animals and give us a broader view of overall PFAA contamination in wildlife.

5.5 Fish

Unlike most wildlife, fish occupy solely aquatic environments of both marine and freshwater nature. PFAA contamination in fish can indicate point sources of PFAA pollution through water discharge or can be due to dietary intake of biota in lower trophic levels. Early studies concentrated specifically on PFOS and PFOA, but subsequent studies have included additional short- and long-chain PFCAs and PFSAs, as well as PFAA precursors. Fish studies have occurred around the globe, with most studies focusing on the fish collected in the Arctic, North America, Europe, and eastern Asia.

5.5.1 Regional Observations

Arctic regions of fish studies include Iceland and the North Sea (Ahrens and Ebinghaus 2010), Swedish waters (Berger et al. 2009), the Barents Sea (Haukås et al. 2007), and the Canadian Arctic (Kelly et al. 2009; Martin et al. 2004a; Powley et al. 2008; Tomy et al. 2004, 2009). In North America, PFAAs have been measured

in fish from freshwater lakes and rivers (de Solla et al. 2012; Delinsky et al. 2010; Moody et al. 2001; Sinclair et al. 2006; Ye et al. 2008a, b) and coastal seawater (Senthil Kumar et al. 2009; Houde et al. 2006b). Similar to other classes of animals, important areas of North America that have been studied are the Great Lakes region (Giesy and Kannan 2001; Kannan et al. 2005a). Fewer studies have been performed regarding PFAAs in fish from South American waters (Quinete et al. 2009).

The Mediterranean coast of Europe provides a source of marine fish, such as swordfish (Corsolini et al. 2008; Kannan et al. 2002b) and bluefin tuna (Giesy and Kannan 2001; Kannan et al. 2002b), which can be measured for PFAAs. In addition, saltwater and freshwater fish throughout central and northern (non-Arctic) Europe have been studied for PFAA contamination (Falandysz et al. 2007; Fernández-Sanjuan et al. 2010; Hoff et al. 2003; Holzer et al. 2011; Kwadijk et al. 2010; Rüdel et al. 2011; Schuetze et al. 2010).

Fish derived from the waters of eastern Asia have been frequently studied for PFAA contamination, including the waters of China (Gulkowska et al. 2006; Loi et al. 2011; Peng et al. 2010; Shi et al. 2010, 2012b; Zhao et al. 2011), Japan (Murakami et al. 2011; Nakata et al. 2006; Senthilkumar et al. 2007), and Korea (Naile et al. 2013; Yoo et al. 2009). In addition, more widespread studies across Asia include the measurement of PFAAs in skipjack tuna from the mid-North Pacific and Indian Oceans by Hart et al. (2008b) and over many countries of Asia by Murakami et al. (2011).

5.5.2 Relevant PFAAs Identified

PFOS is the PFAA most frequently analyzed and detected in fish tissues, a characteristic in common with many other classes of wildlife. Even in studies where other PFAAs were detected in fish, PFOS remained the most dominant compound by concentration (Moody et al. 2001; Martin et al. 2004a; Haukås et al. 2007; Rüdel et al. 2011; Senthil Kumar et al. 2009; Shi et al. 2012b; Taniyasu et al. 2003). Concentrations of PFOS in various fish tissues ranged from below detection limits to 72,900 ng/g wet weight. The highest concentrations were reported by Moody et al. (2001) in livers from common shiner collected from freshwater bodies adjacent to a Canadian international airport, which was the site of an accidental release of PFC-containing firefighting foams in June 2000. Additional frequently-detected PFAAs in fish include long-chain PFCAs (Loi et al. 2011; Kwadijk et al. 2010; Houde et al. 2006c; Falandysz et al. 2007), perfluorohexane sulfonate (PFHxS) (Falandysz et al. 2007; Kwadijk et al. 2010, Taniyasu et al. 2003), and perfluorooctane sulfonamide (PFOSA) (Martin et al. 2004a, b). Although PFOS has been frequently detected, studies by Tomy et al. (2009), Martin et al. (2004a), and Delinsky et al. (2010) did not detect PFOS as the dominant PFAA in the analyzed fish tissues. In a study of fish around the Ariake Sea in Japan, Nakata et al. (2006) detected higher levels of PFOA than PFOS in fish located in tidal flats, but the levels of PFOS were higher than PFOA in fish sampled from the shallow waters.

More uncommon PFAAs have also been detected in fish tissues, including PFAA precursors, some sulfonamide ethanols (Ahrens and Ebinghaus 2010) and some perfluorosulfonamides (Loi et al. 2011; Peng et al. 2010; Tomy et al. 2004; Yoo et al. 2009). The PFCAs with a carbon length between six and nine were also detected in the fish tissue of some studies (Senthil Kumar et al. 2009; Tomy et al. 2009; Nakata et al. 2006). A few studies have successfully separated and measured the linear and branched isomers of PFAAs in fish. Powley et al. (2008) detected both linear and branched PFOS in Arctic cod with 50 % of the total PFOS being branched isomers. In contrast, Kwadijk et al. (2010) did not detect branched PFOS or PFOA isomers in eel samples although the branched isomers were detected in corresponding water samples.

5.5.3 Routes of Exposure

A notable difference found within, and between, many studies of PFAAs in fish is the finding that freshwater fish often have higher concentrations of PFAAs in their analyzed tissues compared to fish from marine ecosystems (Berger et al. 2009; Zhao et al. 2011). This observation could be due to the more frequent presence of PFAA contamination sources located on freshwater bodies. As with other classes of wildlife, higher concentrations and more frequent detections of PFAAs are often detected near more industrialized areas, such as Charleston Harbor in the United States (Houde et al. 2006b), the confluence of the Yamuna and Ganges Rivers (Yeung et al. 2009), and along the Mississippi River in Minnesota (Delinsky et al. 2010). Hart et al. (2008b) found significant correlations between PFAAs in open ocean water and skipjack tuna livers. Outside of the aforementioned airport release of PFAAs (Moody et al. 2001), the highest detected concentrations of PFOS include fish sampled from the Belgian North Sea (Hoff et al. 2003), Japanese coast (Taniyasu et al. 2003), and United States freshwater rivers (Delinsky et al. 2010; Ye et al. 2008a), with maximum concentrations reported as 7,760 ng/g wet weight, 7,900 ng/g wet weight, and 2,000 ng/g wet weight, respectively.

Differences in dietary intake have also been indicated as a possible route for PFAA exposure. More specifically, statistically significant differences of PFAA concentrations in fish tissues were observed between piscivorous and non-piscivorous fish (Holzer et al. 2011; Ye et al. 2008b; Zhao et al. 2011). Sinclair et al. (2006) studied both largemouth and smallmouth bass, but detected no significant difference in PFOS concentrations, which was expected due to similarities in dietary habits.

5.6 Bird

PFAA studies of birds benefit from having species in nearly every region of the planet, including both aquatic and terrestrial ecosystems, representing a broad range of PFAA sources (both from point-source contamination and long-range transport).

The majority of studies focus on birds coming from the Arctic, North America, and Europe, while there are limited studies from the Southern Hemisphere (Antarctica and the Southern Ocean). Initial studies focused on PFOS and PFOA, but the number of PFAAs examined quickly expanded to precursor compounds, PFCAs, and PFSAs.

5.6.1 Regional Observations

As with other classes of wildlife, the dominant location of most bird studies is in the Arctic region including Norway (Verreault et al. 2005, 2007; Herzke et al. 2009; Miljeteig et al. 2009; Löfstrand et al. 2008; Knudsen et al. 2007), Sweden (Löfstrand et al. 2008; Holmström et al. 2010), Canada (Butt et al. 2007a; Martin et al. 2004a; Tomy et al. 2004; Kelly et al. 2009; Holmstrom and Berger 2008), Russia (Miljeteig et al. 2009), Greenland (Bossi et al. 2005b), and the Faroe Islands (Bossi et al. 2005b; Löfstrand et al. 2008).

Another region of frequent bird studies is Asia, including studies from China (Wang et al. 2008a, b; Zhang et al. 2010), Japan (Taniyasu et al. 2003; Nakata et al. 2006; Kannan et al. 2002a; Senthilkumar et al. 2007), and South Korea (Kannan et al. 2002a); with studies on both aquatic and terrestrial species of birds. Studies regarding PFAAs in birds provide a unique insight on not only PFAA occurrence in wildlife, but also human exposure through the consumption of birds and eggs. A few studies have focused on bird and egg samples acquired from Chinese farms (Wang et al. 2010) or directly from Chinese food markets (Wang et al. 2008a; Zhang et al. 2010), which represent a direct source of PFAA-contaminated food.

Multiple studies have reported concentrations PFAAs in birds around the Laurentian Great Lakes regions (Sinclair et al. 2006; Kannan et al. 2001a, 2005a; Gebbink et al. 2009). Kannan et al. (2001a) studied a wide range of region around the United States, collecting piscivorous (fish eating) birds from across the continental United States and the Midway Atoll (Hawaii). Non-Arctic European studies included birds and eggs from Belgian (Meyer et al. 2009; Dauwe et al. 2007) and German locations (Rubarth et al. 2011; Rüdel et al. 2011). Giesy and Kannan (2001) reported the results of a study focused on a global evaluation of PFOS in various species of birds (as well as reptiles, fish, and mammals). It is important to note that birds in the Antarctic and Southern Oceans have been studied; however less frequently (Schiavone et al. 2009; Tao et al. 2006).

5.6.2 Relevant PFAAs Identified

The PFAAs measured in most of the bird studies are similar to those measured in other classes of wildlife. The most frequently studied, and detected, PFAA in birds, across tissues, species, and regions of study, was PFOS. The concentrations of PFOS in bird tissues typically ranged from below the detection limit to 1,000 ng/g,

although multiple studies reported concentrations 1,000–11,000 ng/g in specific bird samples (Dauwe et al. 2007; Giesy and Kannan 2001; Kannan et al. 2001a, 2005a; Meyer et al. 2009; Rüdel et al. 2011; Taniyasu et al. 2003; Holmström et al. 2004). The other more common PFAAs include PFOA, PFOSA, perfluorobutane sulfonate (PFBS), PFHxS and the long-chain PFCAs. Of the frequently studied PFAAs, PFOS and the long-chain PFCAs are the most frequently detected in birds.

Less frequently studied in birds are the PFAA precursors, including the fluorotelomer (both saturated and unsaturated) acids (Butt et al. 2007a; Loi et al. 2011; Verreault et al. 2007; Miljeteig et al. 2009), perfluorodecane sulfonate (PFDS) (Zhang et al. 2010; Loi et al. 2011; Verreault et al. 2007; Kelly et al. 2009; Holmstrom and Berger 2008), and perfluorosulfonamides (Loi et al. 2011; Löfstrand et al. 2008; Tomy et al. 2004). For many of the less frequently studied compounds, the percentage of quantifiable detections was low. In the studies measuring PFDS in birds, a majority of the studies found quantifiable concentrations of PFDS.

5.6.3 Routes of Exposure

From the analysis of datasets containing a number of samples within and outside of specific classifications of birds (e.g., diet, age, gender, and region), patterns can be observed that may elucidate the routes of PFAA exposure for birds. For instance, Wang et al. (2010) and Kannan et al. (2002b) found higher concentrations of PFAAs in birds from regions closer to industrial and urban communities. In addition to vicinity, Wang et al. (2010) identified that farm-raised chickens had lower concentrations of PFAAs than free-range chickens even though the farm was near a PFAA manufacturing plant. The authors suggested that the difference in PFAA contamination may be due to a more controlled diet for the farm-raised chickens, rather than the scavenging diet of free-range chickens. In a study by Sinclair et al. (2006), concentrations of PFOS was 2.5 times greater in piscivorous birds than in non-piscivorous birds, indicating that fish consumption poses a significant route of PFOS exposure in some birds. In the same study (Sinclair et al. 2006), the PFOS concentrations in adult mallard livers was significantly greater than those in juvenile mallards, which reflects the bioaccumulative nature of PFOS.

5.7 Mammals

For almost 15 years several studies looking at PFAAs in mammals from around the world have been conducted. Mammals, similar to birds, span many geographic areas and environments, aquatic, semiaquatic, and terrestrial. Initially, studies were focused on mammal samples located in the Arctic, Asia, Europe, and North America; however, more recent assessments have expanded measurements of PFAAs to mammals from Antarctica and South America. Similar to previous studies

in other wildlife, original studies started only looking at PFOS and PFOA, but have since expanded to include a suite of precursor compounds, PFCAs, and PFSAs.

5.7.1 Regional Observations

Similar to bird studies, mammals from the Arctic region have been the subject of the majority of mammal studies. Many whale species, including beluga whale, narwhal, minke whale, pilot whales, and harbor porpoises, from the United States (Reiner et al. 2011a), Canada (Tomy et al. 2004), Greenland (Bossi et al. 2005a), and Iceland (Van de Vijver et al. 2003) have been examined for PFAAs. Pinnipeds (walrus, seal, and sea lions) from the United States (Giesy and Kannan 2001; Hart et al. 2009), Canada (Tomy et al. 2004; Martin et al. 2004a; Butt et al. 2007b, 2008), Greenland (Bossi et al. 2005a), Norway (Kannan et al. 2001b), and Russia (Ishibashi et al. 2008) have also been examined for PFAAs. Blood and liver samples from the apex Arctic predator, the polar bear, have been examined for PFAAs (Giesy and Kannan 2001; Kannan et al. 2001b, 2005a; Bossi et al. 2005b; Martin et al. 2004a; Dietz et al. 2008; Smithwick et al. 2005, 2006; Riget et al. 2013). Polar bears from Canada, Greenland, and the United States have all been shown to have high concentrations of PFOS in their liver tissues (Bentzen et al. 2008; Braune et al. 2005; Butt et al. 2010). In addition to the aquatic and semi aquatic mammals, the terrestrial Arctic fox has been measured for PFOS and PFCAs (Martin et al. 2004a). Ostertag et al. (2009) looked at PFAAs in the livers of caribou from Canada to help understand potential dietary exposures to PFAAs. Additionally Muller et al. (2011) has examined PFAAs in caribou livers in a terrestrial food web study. In this same terrestrial food-web study Muller et al. (2011) looked at the concentrations of PFAAs in the liver of wolves. The mammal studies from the Arctic give a unique perspective about human exposure because some arctic mammals used in these existing studies were collected as part of Native subsidence hunts (Reiner et al. 2011a; Ostertag et al. 2009).

Studies of PFAAs in tissues and blood from whale and pinniped species from North America, Europe, and Asia have dominated the literature (Shaw et al. 2009; Kannan et al. 2006; Law et al. 2008; Ahrens et al. 2009a). Mink liver samples from the United States and Canada have been examined for PFOS (Kannan et al. 2002c, 2005a; Persson et al. 2013; Giesy and Kannan 2001; Martin et al. 2004a). In Poland measurements of PFOS and long-chain PFCAs have been determined in the livers of beavers (Falandysz et al. 2007). Two studies have been conducted examining the concentrations of PFAAs in river otter from the United States (Kannan et al. 2001b, 2002c). In Belgium, liver samples from wood mice have detected relatively high concentrations of PFOS (Hoff et al. 2004). Blood samples from wild rats and mice from Japan were examined for PFAAs (Taniyasu et al. 2013). Far less frequently have been studies of PFAAs in mammals from South America and the Antarctic (Tao et al. 2006; Dorneles et al. 2008; Quinete et al. 2009; Schiavone et al. 2009).

5.7.2 Relevant PFAAs Identified

By far the dominant PFAA identified in mammals is PFOS; however there has been a noticeable increase in the detection and reporting of long-chain PFCAs, particularly the odd chain PFCAs perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUnA), in mammals. The concentration of PFOS in liver tissues range from below the limit of detection to greater than 2,000 ng/g wet mass (Houde et al. 2006b, 2011). PFHxS has been routinely identified in liver samples from mammals (Dietz et al. 2008; Galatius et al. 2013; Hart et al. 2009; Moon et al. 2010; Reiner et al. 2011a; Smithwick et al. 2005; Taniyasu et al. 2013).

In mammal studies PFAA precursors (fluorotelomer alcohols, sulfonamide ethanols, perfluorosulfonamides, and fluorotelomer saturated and unsaturated carboxylic acids) have been studied much less frequently. When studied, these compounds are not as frequently detected as the PFCAs and PFSAs (Houde et al. 2011). Some perfluorosulfonamides have been detected in harbor seals (Ahrens et al. 2009b) and belugas from the Arctic (Reiner et al. 2011a; Tomy et al. 2004). Interestingly there is a high prevalence of PFOSA in beluga samples from the high latitude Arctic area. It was hypothesized by Houde et al. (2006c) that elevated levels of PFOSA in Arctic beluga whales may be due to the belugas low biotransformation potential of organohalogenated compounds. Further validating her hypothesis, a study by Reiner et al. (2011a) showed beluga samples from the Chukchi Sea, a high Arctic location, have significantly higher concentrations of PFOSA compared to beluga samples from Cook Inlet, AK (a much lower latitude). In the few studies measuring PFDS, it has been found in mammal samples (Ahrens et al. 2009a; Shaw et al. 2009).

5.7.3 Routes of Exposure

Similar to the fish and bird studies, when looking at the mammal samples as one large group, patterns can be observed that help to understand routes of exposure to PFAAs. Population density has been linked to higher concentrations of PFAAs using wild rats in Japan (Taniyasu et al. 2013). Additionally, wood mice samples from an industrialized area with a known fluorochemical plant have shown some of the highest concentrations of PFAAs in mammals (Hoff et al. 2004). Trophic levels play an important role in the accumulation of PFAAs. Studies have shown that apex predators have higher concentrations of PFAAs compared to lower trophic level mammals (Muller et al. 2011; Tomy et al. 2004; Butt et al. 2010). Maternal transfer of PFAAs to fetuses has been shown as a significant route of exposure in different mammal species (Reiner et al. 2011a; Bytingsvik et al. 2012; Hart et al. 2008a; Ishibashi et al. 2008).

5.8 Trends

5.8.1 Tissue Distribution

Since PFAAs are found in protein rich matrices, the majority of wildlife studies analyzed liver tissue, blood, serum, and plasma. However, as stated earlier, this can lead to issues when comparing data among studies. In smaller wildlife the whole body has been used for chemical analysis, but in large animals (fish, birds, and mammals), different organs, whole blood, serum, plasma, and even eggs are used for PFAA analysis. Additionally using only one matrix (liver, blood, etc.) for PFAA analysis does not give a complete assessment of the animal's body burden. In limited studies the tissue distribution of PFAAs has been examined.

5.8.1.1 Fish

Multiple tissue compartments within fish have been studied for PFAA contamination, including bile (Ahrens and Ebinghaus 2010), eggs (Giesy and Kannan 2001; Kannan et al. 2005a), and other organs (Murakami et al. 2011; Quinete et al. 2009; Peng et al. 2010), but the most common tissues are liver, muscle (fillets), or whole body analysis. The highest concentrations of PFOS were reported in liver (Hoff et al. 2003; Moody et al. 2001; Taniyasu et al. 2003), muscle (Delinsky et al. 2010), or whole body (Ye et al. 2008b) of the studied fish. When the muscle of sardines and anchovies were analyzed for PFAAs in a study by Fernandez-Sanjuan et al. (2010), no detectable levels of PFAAs were measured, but when whole body fish were analyzed, PFOS was found. The lack of accumulation of PFOS in fish muscle was also reported by others (Murakami et al. 2011; Quinete et al. 2009).

Other studies have examined the PFAA profiles of fish from the various tissues available for analysis. Murakami et al. (2011) identified the highest concentrations of total PFAAs in carp kidneys, followed by livers, ovaries, and muscles, in decreasing order. Peng et al. (2010) measured the highest concentrations of total PFAAs in eggs and liver of sturgeon. In the same study, perfluorosulfonamides were detected in different compartments, such as the intestine, stomach and gills, with PFOSA being the dominant PFAA in these tissues (Peng et al. 2010). PFOSA was detected in higher concentrations than PFOS in swordfish blood, but was not above the limit of quantification in corresponding livers of the fish (Kannan et al. 2002b).

Analyses of fish muscle and whole body for PFAA contamination can present insight on the chemical body burden of the fish and the PFAA exposure to humans via consumption. Multiple studies have specifically focused on measuring PFAAs in fish collected from local fish markets and fisheries (Murakami et al. 2011; Luque et al. 2010; Gulkowska et al. 2006; Senthilkumar et al. 2007; Yeung et al. 2009; Zhao et al. 2011).

5.8.1.2 Bird

Multiple tissue components of birds have been studied for PFAA contamination, including blood (including whole blood, plasma and serum), egg (including yolk and whole egg), livers, kidneys, feathers, muscle, gall bladder, spleen, and brain. The dominant bird tissue for PFAA detection, as with other classes of wildlife, is the liver, where the highest concentrations of PFAAs over other tissues were often reported (Herzke et al. 2009; Taniyasu et al. 2003; Tao et al. 2006; Meyer et al. 2009; Zhang et al. 2010). When looking at less invasive tissues, Meyer et al. (2009) found a positive correlation between PFAA levels in livers and feathers. Dauwe et al. (2007) findings of extremely high PFOS levels (over 11,000 ng/g in a sample) in the livers of great tits, roosting near a large fluorochemical plant, suggested that they could be used as indicators of potential point-source environmental PFAA contamination. Bird blood and blood-based components have been frequently used in contamination studies. Kannan et al. (2001a) found higher concentrations of PFOS in plasma than those found in whole blood. Taniyasu et al. (2003) and Tao et al. (2006) found higher concentrations of PFOS in liver than in blood samples.

A few studies focused on evaluating the concentrations of various bird tissues within a specific set of species. Holmstrom and Berger (2008) found the highest PFOS concentrations were located in guillemot eggs, followed by chick liver, adult liver, kidney, and muscle, in decreasing order. Alternatively, the total distribution of the higher-order PFCAs was highest in chick livers. Herzke et al. (2009) identified similar levels of PFOS, PFOSA, PFHxS and perfluorodecanoic acid (PFDA) in the plasma and eggs of the European shag, but higher levels of PFOSA and PFDA in the liver. Total PFAAs was also higher in the liver of the species (Herzke et al. 2009). Of the ten different tissues studied by Rubarth et al. (2011), livers had the highest mean concentration of total PFAAs, followed by kidneys, lungs, gall bladder, and blood.

Birds provide a unique compartment of measurement for contaminant exposure studies; bird eggs are an easily accessible wildlife material that can represent both the exposure of PFAAs to mothers and the subsequent contamination transfer to progeny. In studies that evaluated PFAA contamination in different bird tissues, eggs contained both the highest (Holmstrom and Berger 2008) and some of the lowest concentrations (Verreault et al. 2005; Herzke et al. 2009) of PFAAs when compared to other tissues. Eggs have also been suggested to be a mode of PFAA excretion; this conclusion was suggested by the observed lower concentrations of PFOS in adult mother livers than those in chick livers by Holmstrom and Berger (2008). In addition to their environmental relevance, eggs represent a human food source in some cultures and therefore a source of PFAA exposure. Wang et al. (2008a) and Zhang et al. (2010) studied PFAA contamination in bird eggs purchased from markets in China. Additionally, Wang et al. (2008a) found that nearly all of the PFOS contamination in market chicken eggs was located in the egg yolk.

5.8.1.3 Mammals

Similar to fish and bird studies, by far the majority of mammal studies examined PFAAs in liver tissue. These studies tend to use liver as the matrix of examination because it is more readily available compared to blood, serum, and plasma samples. Many marine mammal samples are acquired opportunistically during strandings or native subsistence hunts, processes that make it very hard to collect blood, serum, or plasma samples.

There have been a few studies focusing on the tissue distribution in mammals. Ahrens et al. (2009b) and Van de Vijver et al. (2005) examined the distribution of PFAAs in harbor seals. These studies looked at different matrices, including liver, kidney, spleen, lung, heart, blood, brain, muscle, thyroid, thymus, and blubber to help understand the tissue distribution and the overall body burden in harbor seals. Liver tissue had the highest concentrations of PFAAs compared to the other tissues measured in the studies (Ahrens et al. 2009b; Van de Vijver et al. 2005). In harbor porpoises the liver, kidney, brain, muscle, and blubber tissues were examined for PFAAs (Van de Vijver et al. 2007). Liver, kidney, and muscle tissue for tucuxi dolphin samples have been examined for PFAAs (Quinete et al. 2009). In the studies, PFOS tended to be the dominant PFAA detected in the tissues followed by long-chain PFCAs (Ahrens et al. 2009b; Van de Vijver et al. 2005, 2007; Quinete et al. 2009).

5.8.2 Sex and Age

Generally speaking, there have been few studies showing any sex related differences in reptiles, fish, and birds. Significant differences in PFOS concentrations between genders of snapping turtles was reported by Kannan et al. (2005a), with male snapping turtles having a higher mean PFOS concentration than females. The proposed rationale for this difference was due to the transfer of PFOS contamination to eggs (Kannan et al. 2005a). Male bass fish from a remote lake in New York, United States had higher concentrations compared with female fish (Sinclair et al. 2006). Lower levels of PFAAs were seen in female birds from the United States and Norway compared to their male counterparts (Sinclair et al. 2006; Butt et al. 2007a; Bustnes et al. 2008).

Mammal studies give less clear conclusions for sex related similarities and differences. In the majority of marine mammal studies there are no sex related differences. However, there have been differences noted in some studies. In beluga whale liver samples from Alaska, males tended to have higher concentrations of most PFAAs compared with female samples; however, female liver samples had significantly higher concentrations of PFNA compared to the male samples (Reiner et al. 2011a). PFOS measured in sea otter livers (Kannan et al. 2006) and grey seal livers (Kannan et al. 2002b) showed significantly higher concentrations of PFOS in the

male liver samples compared with the female samples. In contrast, female porpoise livers from the North Sea showed significantly higher mean concentrations of PFOS compared to the male porpoises (Van de Vijver et al. 2003).

Age related trends are not commonly seen in reptiles, fish, and birds. There are some exceptions reported in the literature. Chinese alligator sera samples showed higher concentrations of long-chain PFCAs in juvenile animals (2–9 years) compared to adults ages 10–15 and adults over 15 years of age (Wang et al. 2013). Conversely, Chinese sturgeon showed an increase in long-chain PFCAs with age (Peng et al. 2010).

Some mammal studies have reported age related differences. Most studies report an age related decrease in PFAA concentrations. Whales and pinnipeds from Antarctica, Canada, Europe, Russia, and the United States have all showed higher concentrations of PFAAs in younger animals compared to adults (Tao et al. 2006; Butt et al. 2007b; Van de Vijver et al. 2003; Ishibashi et al. 2008; Houde et al. 2005, 2006a; Fair et al. 2010; Ahrens et al. 2009a; Hart et al. 2008a; Smithwick et al. 2006). Interestingly, there are studies done in the same mammals which indicate no significant difference in age (Kannan et al. 2002b, 2005b; Van de Vijver et al. 2007; Tomy et al. 2009; Butt et al. 2008).

5.8.3 Temporal Trends

There have been quite a number of retrospective, temporal studies examining PFAA concentrations. The temporal studies in fish, birds, and mammals have shown mixed results for PFAAs over time. There is a general trend of increase concentrations of most PFAAs until the 2000s, but beginning in the current decade the trends start to become more specific to wildlife location and species.

Two studies on lake trout from Lake Ontario, Canada have shown temporal increases in PFOS and PFCA concentrations from 1979 to 2004 (Martin et al. 2004b; Furdui et al. 2008). The temporal studies looking at bird liver and egg samples have shown similar results to the fish studies. Livers from thick-billed murre and northern fulmar from Canada collected at three time points from 1975 to 2004 showed an increase in PFOS and PFCAs (Butt et al. 2007a). Guillemot eggs collected in the Baltic Sea from 1968 to 2003 showed an increase in PFOS concentrations during the time period, with maximum PFOS concentrations between 1997 and 2000 (Holmström et al. 2004). After 2000, there was a decrease in the PFOS concentrations measured in the guillemot egg samples (Holmström et al. 2004). Peregrine falcon egg samples from Sweden, collected between 1974 and 2007, showed an increase in PFOS from 1974 until 1984, with a leveling off effect starting after 1984 (Holmström et al. 2010). The long-chain PFCAs exponentially increased in the peregrine falcon eggs over the whole study period (Holmström et al. 2010).

There are many more mammal temporal studies compared to temporal studies in fish and birds. Current reviews from Butt et al. (2010), Houde et al. (2006c, 2011), and Sturm and Ahrens (2010) have discussed temporal trends in detail. This section

will only briefly summarize information provided from those studies and discuss temporal trend studies since the last review in 2011. Most temporal studies showed a temporal increase of PFOS concentrations over the study time periods. However, more recent long term temporal studies that encompass samples collected after the phase out of PFOS have indicated some variations, with decreases in PFOS concentration in some Arctic samples (Houde et al. 2006c, 2011; Butt et al. 2010; Sturm and Ahrens 2010). Long-chain PFCAs have shown an increase in most temporal studies (Houde et al. 2006c, 2011; Butt et al. 2010; Sturm and Ahrens 2010).

Beluga whale liver samples collected in the Alaskan Arctic showed an increase in most PFAAs, specifically PFOS and long-chain PFCAs from 1989 to 2006 (Reiner et al. 2011a). In an assessment of grey seal liver tissues collected in the Baltic Sea between 1969 and 2008 Kratzer et al. showed an increase of PFOS from 1974 to 2008 (2011). However, the authors note that the data was much more variable after 1997 (Kratzer et al. 2011). Additionally in the grey seal liver samples, the long-chain PFCAs showed a significant increase from 1974 to 1997 and then tended to decrease or level off after 1997 (Kratzer et al. 2011). Harbor porpoises liver samples collected from the North Sea between 1980 and 2005 initially did not show any temporal trends for PFAAs (Galatius et al. 2011). However, after the removal of neonates and lactating females, they found an increase in the levels of PFOS and long-chain PFCAs. Marine mammal samples, including ringed seals, pilot whales, and white-sided dolphins, collected in the Arctic and North Atlantic oceans showed increases of long-chain PFCAs from 1984 to 2009 (Rotander et al. 2012). The data for PFOS in these same samples tells a very different story. PFOS concentrations in the pilot whale samples showed an increase from 1984 until 2002; however after 2002 there was no temporal trend, even a slight decrease in PFOS concentrations (Rotander et al. 2012). Conversely the concentrations of PFOS in ringed seal and hooded seal liver samples did not increase during the sampling period (Rotander et al. 2012). Roe deer livers collected from 1989 to 2010 in Germany showed an increase in PFOS until 2000 (Falk et al. 2012). From 2000 until 2010 there was a significant decrease in PFOS concentrations (Falk et al. 2012). Temporal trends were investigated in liver tissues from harbor porpoises collected in the Baltic and North Sea from 1991 to 2008 (Huber et al. 2012). PFOS did not significantly change during the sampling period; however eight of the other compounds measured showed significant temporal trends (Huber et al. 2012). Concentrations of PFHxS, PFHpS, and PFOSA showed a significant decrease while the long-chain PFCAs increased from 1991 to 2008 (Huber et al. 2012).

5.8.4 Food-Web Studies

There have been a few food-web studies of perfluorinated compounds. Most of these food-web studies focus on bioaccumulation and biomagnification in the aquatic environment (Haukås et al. 2007; Houde et al. 2006b, 2008; Kelly et al. 2009; Loi et al. 2011; Renzi et al. 2013; Tomy et al. 2004, 2009; Powley et al. 2008).

In addition, there has been one terrestrial food-web study looking at the biomagnification of PFCAs and PFSAs (Muller et al. 2011). Reviews from Houde et al. (2006c, 2011), and Butt et al. (2010) provide insight into the food web studies. The general conclusion from these studies is for PFOS and the long-chain PFCAs there can be biomagnification in a given food-web. Unfortunately most of these studies combine samples from different time points and locations in order to understand biomagnification (Butt et al. 2010). These studies also use different matrices when talking about biomagnification, which can be problematic (Houde et al. 2006c). While these studies are significant, they may not give a complete picture about the biomagnification of PFAAs.

5.9 Future Research and Conclusions

Until recently, the data coming out of different laboratories has not been comparable and getting consistent data among laboratories was difficult. With the recent availability of Certified Reference Materials (CRMs), certified for PFAAs, there is a now resource to aid in the measurements of PFAAs (Reiner et al. 2012). The National Institute of Standards and Technology has a variety of biological Standard Reference Materials (SRMs) available, including fish tissue and bovine liver. The use of widely available reference materials, like SRMs, improves the comparability of measurements between individual laboratories.

There have been many Arctic studies from North America and Europe (Butt et al. 2010). Although recently there have been some studies, there is limited information from the Russian Arctic (Miljeteig et al. 2009). Since Russia encompasses nearly two-thirds of the polar Arctic, it is essential to understand the distribution of PFAAs in wildlife from this region. In addition to limited studies from the Russian Arctic, the distribution of PFAAs in wildlife populations from other regions around the world, especially in the Southern Hemisphere, including Africa, Australia, and South America, are still lacking. Measurements of PFAAs in wildlife from these locations would increase our understanding of the persistence of PFAAs in the Southern Hemisphere. There is also poor coverage of PFAAs from tropical areas and the open ocean food webs. Additionally, terrestrial wildlife studies have been limited. There is a need to understand transport of PFAAs to remote locations; especially the terrestrial locations that are only influenced by atmospheric transport.

Since there are many areas around the world which have not been examined for PFAAs, it is important to leverage already banked samples from these geographic locations. There are 22 environmental specimen banks around the world (www.inter-esb.org) which make up the International Environmental Specimen Bank community. These specimen banks have wildlife and environmental samples, collected in a systematic way, archived for long-term storage and future projects. They are an underutilized resource that can provided samples to answer research questions about environmental contamination, temporal trends, and spatial trends of emerging compounds of concern, like PFAAs.

A repository of measurement data, such as PFAA concentrations in wildlife, would be a useful tool for the collection of findings by individual laboratories. This could be in the form of a web-based database, possibly hosted by a consortium of world's environmental specimen banks. A web-based database which can provide easy access to sample information (type of sample, species, etc.) and concentration information from wildlife species around the world would be very useful to understand the global distribution of PFAAs and their precursors. The world's specimen banks that are a part of the International Environmental Specimen Bank community could combine their efforts to develop a database system that could be utilized to better understand the global distribution of PFAAs and other contaminants.

It is apparent that wildlife from around the world are exposed to PFAAs. There is a tendency for animals living closer to industrialized regions to have higher concentrations of PFAAs compared to those living in more remote locations. The main compound found in most wildlife species is PFOS; however, especially in the more recent studies, the long-chain PFCAs are frequently being detected and measured. Although there has been exponential growth in the analysis of wildlife samples, there are still data gaps that need to be filled to help us understand environmental exposure.

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ED_002330_00119699-00161

Chapter 6
Metabolism and Pharmacokinetics

Naomi Kudo

Abstract Perfluoroalkyl acids (PFAAs) are highly persistent and widely spread in the environment. PFAAs were detected in various wildlife and human after 1960s and the levels gradually elevated to 2000. In addition to the production of perfluorocarboxylic acids (PFCAs) themselves, fluorotelomer-based compounds were potential source of PFCAs. Fluorotelomer-based compounds can degrade through atmospheric oxidation and biodegradation to form PFCAs. The biological half-lives ($t_{1/2}$) of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), major contaminants in the environment, were calculated to be 3.5 and 8.5 years in human, respectively. To elucidate the mechanisms by which PFAAs accumulate in human, pharmacokinetics have been studied in experimental animals, however, in rats, mice, monkeys and other animals, half-life ($T_{1/2}$) were hours to days, therefore, great species-difference exist in $t_{1/2}$ between experimental animals and human. Recent studies identified partially the biological molecules responsible for protein binding, transmembrane transport of PFCAs. In addition, transplacental and lactational transports are thought to be an important exposure routes of these chemicals, because developmental toxicity of PFAAs is thought to be one of primary toxic events of PFAAs. Physiologically-based pharmacokinetic (PBPK) models are proposed to understanding kinetics of PFAAs in biological systems.

Keywords Absorption • Distribution • Gestational transfer • Lactational transfer • Protein binding • Clearance • Renal transport • PBPK model

6.1 Absorption

PFOA and its salts are well absorbed following oral exposure irrespective of species and sex. After a single oral dose of [^{14}C]PFOA (11.2 mg/kg) in male rats, at least 93 % of dosed [^{14}C]PFOA was absorbed (Johnson et al. 1979). In mice fed a high-fat

N. Kudo (✉)
School of Pharmaceutical Sciences, Josai University,
Keyakidai1-1, Sakado, Saitama 350-0295, Japan
e-mail: naokudo@josai.ac.jp

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_6

ED_002330_00119699-00162

diet, initial absorption of [^{14}C]PFOA was 98.8 % within 48 h (Jandacek et al. 2010). Female rats were administered 2 mg PFOA by stomach intubation, and entire dosed nonionic fluorine was recovered in urine and feces within 96 h, suggesting that dosed PFOA was entirely absorbed (Ophaug and Singer 1980). Serum level of organic fluorine was highest 4.5 h after administration, and then gradually decreased indicating that PFOA was rapidly absorbed from gastrointestinal tract. Hundley et al. administered a single 10 mg/kg oral gavage of [^{14}C]PFOA ammonium salt to male and female rats, mice, hamsters and rabbits (Hundley et al. 2006). The recovery of radioactivity in urine, feces, tissues, cage wash and expiration products was ranged 71–105 % 120–160 h after dosing. The results suggest that almost dosed PFOA ammonium salt was absorbed by gastrointestinal tract. In castrated Angus cattle, orally dosed [^{14}C]PFOA (1 mg/kg) was rapidly absorbed and excreted (101 % recovery) in the urine within 9 days of dosing (Lupton et al. 2012). The serum level reached maximum 24 h after the dosing. The mechanistic aspects of intestinal absorption of PFOA remain to be studied. Non-absorbable dietary lipid olestra (sucrose polyester) reduced initial absorption of tetrachlorobiphenyls but not [^{14}C] PFOA, suggesting that partition of PFOA to olestra is very low in intestine (Jandacek et al. 2010).

Perfluorocarboxylic acids (PFCAs) other than PFOA also seem to be well absorbed from intestine. Perfluorohexanoic acid (PFHxA) is likely to be rapidly absorbed and eliminated in male and female Sprague-Dawley rats and CD-1 mice, because essentially the 100 % of dosed [^{14}C]PFHxA (100 mg/kg) was eliminated in urine within 24 h (Gannon et al. 2011). In male and female Wistar rats, over 90 % of dosed perfluoroheptanoic acid (PFHpA) was found in urine (Kudo et al. 2001a).

Perfluorooctanesulfonate (PFOS) is also well absorbed from gastrointestinal tract in male and female rats (Chang et al. 2012). Male and female rats were administered orally with 4.2 mg/kg of potassium [^{14}C]PFOS, and the recovered radioactivity in carcass, urine, feces, plasma and red blood cells was almost 95 % of the dose. Only 3.6 % of dosed radioactivity was recovered in digestive tract. Fate of PFOS was studied in steers after a single oral dose of 8 mg/kg PFOS (Lupton et al. 2014). The recovery of PFOS in plasma, feces, muscle, liber, bile, urine, lung, kidney, spleen and carcass reminder was 60 % through 28 days after a dose. It is unclear whether lower bioavailability is responsible for less recovery of PFOS or not.

Plasma PFOA concentration was determined after repeated inhalation in rats by Kennedy et al. (1986; Hinderliter et al. 2006a). Male and female rats were exposed nose-only to aerosol atmospheres of 0, 1, 10 or 25 mg/m^3 PFOA for 6 h (Hinderliter et al. 2006a). Plasma PFOA concentrations were proportional to airborne concentrations in both male and female rats, but plasma PFOA was disappeared more rapidly in female rats. Following repeated inhalation exposures, male rats demonstrated an accumulative pattern while little daily carryover was observed in the plasma in female rats over the 3 weeks period (Hinderliter et al. 2006a).

PFOA is dermally absorbed, but permeability of PFOA is not fully elucidated yet. O'Mally and Ebbins showed high mortality of male and female rabbits in which approximately 40 % of the shaved trunk of animals were applied to 1,000

and 2,000 mg/kg PFOA in a saline slurry 24 h/day, 5 days/week, for 2 weeks suggesting that PFOA is dermally absorbed (O'Mally and Ebbins 1981). Rabbits and rats were dermally treated with ten applications of ammonium salt of PFOA at doses of 0, 20, 200 or 2,000 mg/kg, resulting in dose-dependent increase in blood organofluorine amounts (Kennedy 1985). Penetration of PFOA ammonium salt was estimated *in vitro* using rat and human skin (Fasano et al. 2005). PFOA ammonium salt penetrated rat skin with permeability coefficient of 3.25×10^{-5} cm/h, and only 1.44 % of applied chemical penetrated through rat skin by the end of 48 h exposure period. For human skin, permeability coefficient was 9.5×10^{-7} cm/h, and only 0.048 % of applied chemical was penetrated through human skin. Franco et al. estimated dermal penetration of [^{14}C]PFOA in human and mouse skin *in vitro* and in mouse in vivo (Franco et al. 2012). Mice were exposed to 25 uL of 0.5–2.0 % PFOA solution in acetone on the dorsal surface of each ear for 4 days. Serum concentrations of PFOA in mice were 150–226 ug/mL. *In vitro* skin permeation study revealed that 39 %, 23.4 % and 24.7 % of applied PFOA (0.5 mg/50 uL acetone) penetrated across mouse skin, human full-thickness skin and human epidermis samples (0.64 cm^2), respectively, within 24 h. They also compared permeability coefficient of PFOA in heat-separated human epidermal membrane between different pH. Permeability coefficient was $5–6 \times 10^{-2}$ cm/h at pH 5–5.5, whereas 5.5×10^{-2} cm/h at pH 2.25, suggesting that non-ionized PFOA easily penetrate the membrane than ionized PFOA. However, permeability coefficient was 100 times greater in this study than the study by Fasano et al. (2005). The difference was not fully explained, but acetone may facilitate permeability of PFOA in the latter study.

6.2 Distribution

6.2.1 Distribution to Blood and Tissues

PFOA is distributed mainly to the serum, liver and kidney in male rats after single and repeated exposure (Hundley et al. 2006; Ylinen and Auriola 1990; Vanden Heuvel et al. 1991; Kudo et al. 2007; Cui et al. 2010). PFOA levels of lung and heart are relatively high, followed by skin, testis and spleen. Very low but significant amounts were detected in adipose tissue and brain (Hundley et al. 2006; Kudo et al. 2001a). In female rats, more PFOA distributed to kidney than male rats 2 h after dosing (Vanden Heuvel et al. 1991). Tissue distribution of PFOA depends on the dose (Kudo et al. 2007; Cui et al. 2010). The ratio of PFOA concentration between plasma and liver and was 1:0.82 at 40 umol/kg while the ratio was 1:2.22 at 0.1 umol/kg 2 h after iv dose (Kudo et al. 2007). One possible explanation for liver-addressed accumulation is that transport system such as transporters is responsible for uptake of PFOA into hepatocytes (Han et al. 2008). The ratio of serum to whole blood concentrations of PFOA was approximated 2:1 (Kudo et al. 2007). Considering volume displacement by red blood cells, almost PFOA is in plasma or serum but not

in blood cells. In mice, PFOA accumulated primarily in liver, followed by blood skin, kidney and other tissues after 168 h after an single oral administration (Hundley et al. 2006). In chicken, high concentrations of PFOA were detected in blood, liver and kidney after repeated oral administrations (Yeung et al. 2009). Tissue levels of PFOA were determined in lactating cow fed PFOA-contaminated glass silage and hay (approximately 200 and 1,900 ug/kg dry matter, respectively) for 28 days (Kowalczyk et al. 2013). At the end of slaughting period, PFOA was detected in plasma, liver and kidney at the concentrations approximately 10 ug/kg. The levels in milk and muscle were one tenth and one hundredth of the former tissues, respectively. Tissue distributions of PFCAs having nine or more carbon atoms were poorly estimated. Perfluorodecanoic acid (PFDA) seems distribute to mainly serum, liver, kidney, lung and lesser to brain in rats after single ip dose (Ylinen and Auriola 1990). Perfluorononanoic acid (PFNA), PFDA, perfluoroundecanoic acid (PHUA) and perfluorododecanoic acid (PDoA) were detected in the kidney, liver and bladder of harbor seals from Dutch Wadden Sea (Van de Vijver et al. 2005). [^{14}C]PFHxA was shown to be distributed mainly to plasma, liver and kidney in male and female rats (Gannon et al. 2011). Large amounts of [^{14}C]PFHxA was detected in bladder in both mice and rats, and [^{14}C]PFHxA in all tissues rapidly disappeared, suggesting rapid urinary elimination.

In the case of PFOS and other perfluoroalkylsulfonates (PFASs), high concentration was observed in liver, serum and kidney. Distribution of potassium salt of [^{14}C] PFOS was determined in rats after a single oral dose of 4.2 mg/kg (Chang et al. 2012). Hepatic PFOS concentration was 21 ug/g and approximately ten times higher than plasma concentration. Concentrations of other tissues were less than plasma concentrations and the order of kidney, lung and spleen. The levels in brain and abdominal fat were extremely low. In steers, PFOS concentrations in plasma, liver and kidney were approximately 53 ug/kg, 18 ug/kg and 3.7 ug/kg, respectively, 28 day after single oral administration at 8 mg/kg (Lupton et al. 2014). In this study, PFOS was concentrated in bile where PFOS concentration was 37 ug/mL, while concentrations of other tissues were approximately 5 ug/g or less. Dose-related difference in tissue distribution of PFOS is demonstrated by Cui et al. (2010). In this study rats were received oral dose of PFOS at high dose (5 and 20 mg/kg) for 28 days, and tissue-specific accumulation was not significant compared to other studies. Kowalczyk et al. determined tissue distribution of PFAA in the cows fed naturally PFAA (PFBS, PFHxS, PFOS and PFOA)-contaminated food for 28 days (Kowalczyk et al. 2013). PFOS concentrations were 2,464, 2,952, 1,074, 145 and 9.1 ug/kg (L) in plasma, liver, kidney, and milk, respectively. It is noteworthy that muscle concentration was less than one tenth of liver, over 40 % of dosed PFOS was reserved in this tissue. In this study, concentrations of perfluorohexanesulfonic acid (PFHxS) were 419, 61, 98, 19, 1.9 ug/kg (L), respectively. The levels of perfluorobutanesulfonic acid (PFBS) were less than 1 ug/kg (L), although contamination levels of PFOS, PFHxS and PFBS in the diet were comparable.

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6.2.2 Distribution to Fetus and Breast Milk

Distribution of PFAAs to fetus and milk is paid special attention because these chemicals exhibit developmental toxicity in experimental animals at low dose (Chap. 8). Indeed, PFAAs were detected in human breast milk (So et al. 2006; Kärrman et al. 2007; Tao et al. 2008; Kim et al. 2011), neonatal blood immediately after birth (Spliethoff et al. 2008), umbilical cord blood or plasma (Kim et al. 2011; Spliethoff et al. 2008; Inoue et al. 2004; Apelberg et al. 2007; Midasch et al. 2007; Zhang et al. 2013a) and amniotic fluid (Zhang et al. 2013a; Stein et al. 2012). Pharmacokinetic study was performed in rats which were orally administered with ammonium salt of PFOA once daily at doses of 3, 10 or 30 mg/kg/day starting on gestation day (GD) 4 (Hinderliter et al. 2005). PFOA was detected in placenta, amniotic fluid, embryo, fetus, and milk indicating gestational and lactational transfer of PFOA. Steady-state concentrations in milk were approximately 10 % of those in maternal plasma (Hinderliter et al. 2005). On GD21, PFOA concentrations in amniotic fluid and fetus were slightly higher than those in milk, and PFOA concentrations in placenta were approximately two times of those in milk. The concentration of PFOA in fetal plasma was approximately half of those in maternal plasma. In female rats, estimation of PFOA transfer to milk and fetus is difficult in single dose study because of rapid disappearance (half-life of approximately 2 h) (Ohmori et al. 2003; Han et al. 2012). By contrast, half-life of PFOA is estimated to be approximately 16 days, therefore, mice were used for many studies (Lau et al. 2006; Abbott et al. 2007; White et al. 2007; Wolf et al. 2007; Reiner et al. 2009; Fenton et al. 2009). Disposition of PFOA was determined in mice received single oral dose of PFOA at doses of 0.1–5 mg/kg on GD17 (Fenton et al. 2009). Serum concentrations of pups were highest at PND1 and gradually decreased by PND18, suggesting that transplacental transfer is responsible for substantial serum PFOA in fetus. When mice were received repeated oral dose of PFOA at 5 mg/kg during gestation, whole blood concentrations of pups were higher than those in dams on postnatal day (PND) 10 and decreased to the level of dams on PND 20 (White et al. 2007). The same trend was observed in the study of Fenton et al. where mice received a single oral single dose at a dose of 5 mg/kg on GD17 (Fenton et al. 2009). In addition to gestational transfer, pups are exposed to PFOA by lactation. Abbott et al. demonstrated that serum PFOA levels in dams nursing pups up to weaning were significantly lower than those in dams without pups at weaning when mice were received repeated oral dose on GD1-GD17 at dose ranging from 0.1 to 1 mg/kg (Abbott et al. 2007). Cross-Foster study was performed in mice where significant amounts of PFOA were detected in control pups nursed by dams that received repeated oral dose of PFOA at a dose of 5 mg/kg on GD1-17 (Wolf et al. 2007). Milk:serum distribution ratio in dams were estimated to be 1:0.11–1:0.56 (Fenton et al. 2009). PFOA concentration of serum, urine and mammary tissue and milk in dams exhibited U-shape from GD18 to PND18 while inverse U-shaped curve was observed in the amounts of PFOA in pups, suggesting milk-borne PFOA transfer from dams to pups (Fenton et al. 2009). PFOS also distributes to fetus and milk in rats and mice.

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When pregnant mice were received oral dose of PFOS at 1, 2, 3 or 5 mg/kg from GD2 through GD20, serum concentrations of PFOS in pups were close to those in dams on GD21 (Lau et al. 2003). Similar results were demonstrated in the study of Chang et al., where rats were given daily oral doses at 0.1, 0.3 and 1.0 mg/kg/day from GD0 to GD20. On GD 20, serum levels of PFOS in fetus were close to those in dams (Chang et al. 2012). In the study of Luebker et al. both male and female rats were received daily dose of PFOS at dose levels of 0.1, 0.4, 1.6 and 3.2 mg/kg for 6 weeks prior to mating, during mating, and for female rats, through gestation and lactation (Luebker et al. 2005). At the end of lactation, serum levels of pups were close to those of dams. Cross-foster study suggested that pups are exposed to PFOS during lactation (Luebker et al. 2005). When mice were received oral doses of PFOS at 4.5, 6.5, 8.5 or 10.5 mg/kg/day from GD15 to GD18, serum levels of pups were approximately two times higher than those in dams on PND15 (Abbot et al. 2009). Dams without pups after PND6 had significantly higher levels of PFOS in serum than the dams with pups (Abbot et al. 2009). Borg et al. estimated tissue distribution of [^{35}S]PFOS that was intraperitoneally administered on GD16 (Borg et al. 2010). The levels of [^{35}S]PFOS in liver and lungs of fetuses and pups were higher than maternal blood level. Brain levels of [^{35}S]PFOS in fetuses and pups were higher than brain of dams and comparable to maternal blood.

Transfer of PFAAs to breast milk, fetus and amniotic fluid was estimated in humans by comparing PFAA concentrations between maternal serum, breast milk, and amniotic fluid. In general, transfer efficiency from maternal blood to cord blood is high while those from maternal blood to amniotic fluid and milk are low. In addition, PFOA exhibits higher transfer efficiency compared to PFOS. Carbon chain length seems to influence transfer efficiency of PFCAs. Kärrman et al. detected PFHxS, PFOS, PFOA, PFNA, PFDA, perfloroundecanoic acid (PFUnDA) and perfluorooctanesulfonamide (PFOSA) in serum and PFHxS, PFOS, PFOA, PFNA, and PFOSA in milk in 12 primiparous women (Kärrman et al. 2007). The mean ratio between serum and milk concentration was 1:0.01 for PFOS, 1:0.02 for PFHxS and 1:0.07 for PFOSA. The ratios for PFOA and PFNA were uncertain because these were detected only a few milk samples. In the study of Kim et al. where PFAA concentrations were determined in maternal blood serum, cord blood serum and breast milk in 35 women, the concentration was highest for PFOS followed by PFOA, PFUnDA and PFHxs in maternal serum, cord serum and breast milk (Kim et al. 2011). The mean ratios between maternal serum and milk were 1:0.03 for PFOS and 1:0.04 for PFOA, and those between maternal serum and cord blood serum were 1:1.93, 1.02, 0.72 and 0.48 for perfluorotridecanoic acid, PFOA, PFHxS, PFOS, respectively. Midasch et al. estimated the ratios between maternal plasma and cord plasma in 11 women advanced in pregnancy to be 1:0.6 and 1:1.26 for PFOS and PFOA, respectively (Midasch et al. 2007). According to Inoue et al. the ratio between maternal serum and cord blood serum was 1:0.32 in 15 pregnant women at gestation weeks between 38 and 41 (Inoue et al. 2004). Zhang et al. estimated the levels of various PFAAs in 27 matching samples of maternal serum, cord blood, amniotic fluid and placenta in pregnant women (Zhang et al. 2013a). PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA and PFDoA were detected in all samples while

PFHxA and perfluoroheptanoic acid (PFHpA) were not detected in some samples of maternal blood, placenta and cord blood. Only PFOA was frequently detected, and PFOS, PFDA, PFUnDA and PFDoDA were not detected at all in amniotic fluid samples. Comparison of transfer efficiencies from maternal blood to cord blood between PFAAs revealed a U-shaped trend of C_7–C_{12} PFCAs with increasing carbon chain length that seems to be an integrated result of opposite trend of the ratio between maternal blood and placenta and between placenta and cord blood. Both PFOS and PFHxS showed lower transfer efficiency compared to PFCAs. Similar trend in the ratio between maternal blood and cord blood were observed between C8 and C13 PFCAs by Liu et al. who estimated the levels of PFAAs in 50 pairs of women and their newborns (Liu et al. 2011). This trend was also observed in the ratio between breast milk and maternal blood although the ratio between breast milk and maternal blood was one order of magnitude lower than the ratio between cord blood and maternal blood (Liu et al. 2011). The ratio between amniotic fluid and maternal serum was estimated to be 1:0.039 for PFOS and 1:0.078 for PFOA in the study by Stein et al. (2012).

6.3 Protein Binding

Interactions of PFAA with biological matrices in plasma and tissues likely contribute their tissue distribution and bioaccumulation patterns. PFCAs bind to serum proteins (Ylinen and Auriola 1990; Jones et al. 2003). It was reported that more than 99 % of PFDA was bound to protein in the serum after a single dose in rats (Ylinen and Auriola 1990). The primary PFOA-binding protein in plasma is serum albumin (Han et al. 2003). Many experimental methods have been employed to study PFAA binding to albumins, including BSA, HSA and rat serum albumin (RSA). These involve not only traditional techniques such as size exclusion column, equilibrium dialysis (Chen and Guo 2009) and ligand displacement measurement (Jones et al. 2003; Han et al. 2003; Chen and Guo 2009) but also novel methods including monitoring protein's native fluorescence (Chen and Guo 2009; Wu et al. 2009; Zhang et al. 2009; Hebert and MacManus-Spencer 2010; MacManus-Spencer et al. 2010; Qin et al. 2010), surface tension (MacManus-Spencer et al. 2010), circular dichroism (Wu et al. 2009; Zhang et al. 2009), ligand blotting assay (Han et al. 2003), isothermal titration calorimetry (Wu et al. 2009), ^{19}F-NMR (Han et al. 2003; MacManus-Spencer et al. 2010), heteronuclear single quantum coherence NMR. Binding affinity (association constant, *Ka*) obtained by monitoring native albumin fluorescence and those by other methods are summarized in Table 6.1. The values are variable when different methods are employed. Some studies suggest that there are two groups of binding sites on serum albumin, namely, high affinity site and low affinity site (Wu et al. 2009; MacManus-Spencer et al. 2010; Bischel et al. 2010). *Ka* values between 10^1 and 10^3 M^{-1} can be categorized as the low affinity site, whereas *Ka* values between 10^4 and 10^6 M^{-1} can be categorized as the low affinity site (Han et al. 2012). Molecular modeling study where structure and energies of

Table 6.1 Association constant (*Ka*) of PFAA-albumin binding

Fluorescent method	HSA	BSA	RSA
PFHpA	9.4×10^{3} [a]		
PFOA	2.5×10^{4}, 5.5×10^{2} [b]	1.5×10^{5}, 8.0×10^{1} [d]	
	2.2×10^{4} [a]	4.4×10^{4} [e]	
	2.7×10^{5} [c]		
PFNA	5.0×10^{4}, 0.1×10^{2} [b]	6×10^{5}, 9×10^{2} [d]	
	2.6×10^{4} [a]		
PFDA	4.8×10^{4} [a]	3×10^{5}, 5×10^{2} [d]	
		6.9×10^{5} [e]	
PFUnDA	4.3×10^{4} [a]	0.2×10^{5}, 0.1×10^{2} [d]	
PFDoDA			
PFHxS	1.2×10^{4} [a]		
PFOS	8.9×10^{3} [a]		
	2.2×10^{4} [c]		
	4.4×10^{4} [a]		
Other methods			
PFBA	1.1×10^{6} [g]		
PFOA	3.1×10^{4} [h]	1.4×10^{6} (1.4), 0.2×10^{6} (4.3) [j]	2.8×10^{3} (7.8) [j]
	2.6×10^{3} (7.2) [i]	1.3×10^{5} [k]	
PFNA	2.1×10^{6} [a]	3.3×10^{6} (2.9), 1.1×10^{6} (4.6) [j]	
		2.6×10^{5} [k]	
PFDoA	1.2×10^{6} [g]		
PFBS	2.2×10^{6}, 6.5×10^{6} [g]		
PFOS	7.6×10^{6} [g]		

Values represent *Ka* (M^{-1}) and values in parenthesis represent number of binding sites
References for fluorescent method: [a]Hebert and MacManus-Spencer (2010), [b]Wu et al. (2009), [c]Chen and Guo (2009), [d]MacManus-Spencer et al. (2010), [e]Qin et al. (2010), [f]Zhang et al. (2009)
References for other method: [g]ligand-displacement measurement (Chen and Guo 2009), [h]equilibrium dialysis (Wu et al. 2009), [i]size-exclusion column (Han et al. 2003), [j]equilibrium dialysis (Bischel et al. 2010), [k]nano ESI-MS (Bischel et al. 2010)

the binding sites were determined, supported two groups of affinity sites for PFCAs (Chen and Guo 2009). Other studies suggest one affinity value between 10^4 and 10^6 M^{-1} (Chen and Guo 2009; Hebert and MacManus-Spencer 2010; Qin et al. 2010). No significant difference in *Ka* values is observed between PFHxA, PFOA, perfluorononanoic acid (PFNA) and PFDA for both HSA and BSA (MacManus-Spencer et al. 2010) while the binding of PFAAs to HSA or BSA exhibits a trend of increasing binding strength with increased chain length (Chen and Guo 2009; MacManus-Spencer et al. 2010; Qin et al. 2010; Bischel et al. 2010). Proposed number of binding site was ranged from 1 to 11 in these studies (Han et al. 2012). Some studies suggest PFOA binds to Sudlow's binding Site I and Site II. According to Salvalaglio et al. the binding sites are common either to fatty acid binding sites or Sudlow's binding Site I and Site II and the maximum number of binding for PFOA

and PFOS was 9 and 11, respectively (Salvalaglio et al. 2010). At low concentrations, however, binding stoichiometry of PFAA and albumins seem 1–2 (Salvalaglio et al. 2010). The physiological relevance of these binding sites remains unclear. To date, study that compare species difference in albumin binding is limited, significant difference in albumin binding is not shown between HSA and BSA in the above studies. Compared to PFCAs, information of the binding of PFSA is limited. To date, observed properties of protein binding cannot fully explain species- and sex-related difference in renal elimination of PFAA.

Binding of PFOA to proteins are studied in liver and testis (Kudo et al. 2007; Vanden Heuvel et al. 1992a). Covalent binding of PFDA to TCA-insoluble components was observed in liver and testis in vivo (Vanden Heuvel et al. 1992a). Kudo et al. demonstrated that PFOA distributed to membrane fraction of the liver compared to female rats after PFOA dosing (Kudo et al. 2007). PFOA binds to both liver form and kidney form of α2u-grobulins, male rat-specific proteins exist in liver, plasma and kidney, *in vitro* in physiological conditions (Han et al. 2004). PFOA seems to bind to α2u-grobulins on fatty acid-binding sites with *Kd* values in 10^{-3} M range, which is estimated by fluorescence competitive binding assay using dansyl undecanoic acid. Considering *Kd* values and concentrations of plasma and tissues of these proteins, α2u-grobulins cannot adequately explain sex difference in renal clearance of PFOA (Han et al. 2004). Binding of PFAAs to liver fatty acid-binding protein was estimated (Luebker et al. 2002; Woodcroft et al. 2010). In fluorescence competitive binding assay using dansyl undecanoic acid, IC_{50} values of PFOS and PFOA were calculated to be 0.01 uM and over 10 uM; these values are two or three order of magnitude higher that natural ligand oleic acid (Luebker et al. 2002). Binding affinity of PFOA, estimated fluorometrically, was shown to be an order of magnitude less than that of oleic acid (Woodcroft et al. 2010).

6.4 Metabolism

6.4.1 PFAA Metabolism

PFAAs are not metabolized in animals. Ophaug and Singer demonstrated that neither the ionic fluoride level in the serum nor the rate of ionic fluoride excretion in the urine was altered by PFOA administration (Ophaug and Singer 1980). Vanden-Heuvel et al. demonstrated that single radioactive peak corresponding to PFOA was detected in urine and bile samples after intraperitoneal administration of 9.4 umol/kg [^{14}C]PFOA (Vanden Heuvel et al. 1991). Thin layer chromatography was performed with liver extract and the chromatographic behavior of radioactivity was closely resembled that of PFOA. No evidence of phase II metabolite of PFOA was found in urine of rats received single intraperitoneal administration of 50 mg/kg PFOA (Ylinen et al. 1989). In *in vitro* experiments, no glucuronide conjugate of [^{14}C]PFOA was detected using microsomes prepared from rat and human liver, kidney and intestine *in vitro* (Kemper and Nabb 2005).

6.4.2 Metabolic Production of PFAAs

PFAAs are well known to be wide-spread contaminants although their chemical and physical properties are not consistent with those of that typically undergo atmospheric long-range transport. In addition, the production of PFCAs themselves was very small compared to the production of fluorotelomer-based polymer and phosphate (Buck et al. 2011). In addition to the direct release of PFAAs from industrial emissions and commercial products, these are produced by degradation of other precursor compounds including fluorotelomer alcohols (Butt et al. 2014). Precursor compounds degrade to form PFAAs through biotransformation by microorganisms and animals, and atmospheric oxidation (Young and Mubry 2010). Therefore, exposure to precursor chemicals of animals results in accumulation of PFAAs within their body. 1H, 1H, 2H, 2H-Perfluorodecanol (8:2FTOH), a fluorotelomer alcohol (FTOH), is used in most biotransformation studies *in vivo* and *in vitro*. Biotransformation of FTOH was reported by Hagen et al. in early 1980s (Hagen et al. 1981). They found PFOA, 2H, 2H-perfluorodecanoic acid (8:2FTCA), 2H-perfluorocecenoic acid (8:2FTUCA) and unidentified metabolite in plasma samples in male rats after single oral dose of 8:2FTOH. Plasma levels of 8:2FTCA and unidentified metabolite were transient whereas PFOA levels increased with time. Following studies in 2000s, additional metabolites PFNA (Martin et al. 2005; Kudo et al. 2005; Henderson and Smith 2007; Fasano et al. 2009; Himmelstein et al. 2012), PFHxA (Fasano et al. 2009; Himmelstein et al. 2012) 2H, 2H, 3H, 3H-perfluorodecanoic acid (7:3FTCA) (Fasano et al. 2009; Himmelstein et al. 2012), 2H, 3H-perfluorodecenoic acid (7:3FTUCA) (Nabb et al. 2007), and *O*-glucuronide and *O*-sulfate (Martin et al. 2005) were determined in 8:2FTOH treated animal samples. In *in vitro* studies using hepatocytes, liver microsome or cytosolic fractions, additional metabolites of 8:2FTOH were detected such as 2H, 3H-perfluorodecenoic acid (7:3FTUCA), 2H, 2H-perfluorodecanal (8:2FTAL), 2H-perfluorodecenal (8:2FTUAL), glutathione-conjugates of FTUAL (Martin et al. 2005; Fasano et al. 2009; Nabb et al. 2007), 7:2ketone, 7:3β-keto acid, 2H, 2H, 3H, 3H-perfluorodecanal (7:3FTAL), 2H, 3H-perfluorodecenal (7:3FTCA) (7:3FTUAL) and 7:3FTCA taurine conjugate (Nabb et al. 2007). Fasano et al. studied fate of [3-^{14}C]8:2FTOH after a single oral dose in both conditioned rats following 45 days of daily oral dose of unlabeled 8:2FTOH and non-conditioned rats (Fasano et al. 2009). Approximately 80–90 % of the total radioactivity was found in feces, and major analyte in feces was 8:2FTOH 168 h after [3-^{14}C]8:2FTOH dosing. In urine and tissues, approximately 5–10 % of radioactivity was found in both conditioned rats and non-conditioned rats. Inhalation study was performed by Himmelstein et al. (2012). Male and female rats were exposed by nose-only inhalation for 6 h at 8:2FTOH concentration of 3 mg/m^3 or 30 mg/m^3. During the exposure period, the levels of parent compound were low and it was not detected at 12 and 24 h, suggesting rapid clearance (elimination and/or metabolism). The most abundant metabolites were 7:3FTCA, PFOA and 7:2FTCA. One-compartment model analysis revealed that yields of 7:3FTCA and PFOA were approximately 2 % and 1 % or less,

respectively. As for humans, elevated levels of PFCAs in ski wax technicians compared to general population (Nilsson et al. 2010a). Exposed levels of 8:2FTOH were determined to be up to 92,000 ng/m^3 via air (Nilsson et al. 2010b). Precise analysis of blood samples of male professional ski wax technicians reveled that not only PFCAs of C_4–C_{18} and some PFAS, but 6:2FTUCA, 8:2FTUCA, 10:2FTUCA, 3:3FTCA, 5:3FTCA and 7:3FTCA were detected. The result indicates that FTOHs are metabolized to PFCAs in humans (Nilsson et al. 2013).

Mono- and di-fluoroalkyl phosphate esters (monoPAP and diPAP), fluorinated surfactants with high production volume, are candidates of PFCA source. DiPAP were orally or intravenously administered to rats, and kinetics of these chemicals were estimated (D'Eon and Mabury 2011). A mixture of 4:2, 6:2, 8:2 and 10:2 monoPAP and diPAP were intravenously or orally administered to male rats. Bioavailability of diPAPs decreased as chain length increased. In addition to parent chemicals, PFCAs of C_4–C_{11} were detected in plasma, suggesting that diPAPs undergo hydrolysis to produce FTOH which are metabolized to form PFCAs. By contrast, monoPAP were not absorbed from gut.

6.5 Clearance

6.5.1 Biological Half-Life

Early studies on PFAA paid attention to their toxicity. PFOA shows sex-related difference in LD_{50}, effects on lipid metabolism in rats (Kawashima et al. 1989; Uy-Yu et al. 1990; Griffith and Long 1980) whereas no significant sex-related difference was observed for PFDA (Olson and Andersen 1983; George and Andersen 1986). Following studies revealed that such differences in the magnitude of biological responses observed in PFOA are mainly due to the difference in $T_{1/2}$ (Ylinen et al. 1989, 1990; Vanden Heuvel et al. 1992b; Olsen et al. 2007; Kudo et al. 2000; Kudo and Kawashima 2003). Chain length dependent trend of $T_{1/2}$ of PFCAs in rats was demonstrated by Ohmori et al. (2003). Namely, PFCA with longer carbon chain length exhibits longer $T_{1/2}$. The study on retired fluorochemical production workers revealed that $T_{1/2}$ for PFOS, PHHxS and PFOA were calculated to be 4.8 years, 7.3 years and 3.5 years, respectively (Olsen et al. 2007), which is a magnitude of difference from other experimental animals. It is concluded that PFAAs exhibit species-, sex- and carbon chain length-dependent $T_{1/2}$. Species difference is the most important issue because it is an important factor in evaluating safety of these chemicals to human based on animal data. Studies on the difference in sex, carbon chain length and species have been performed mainly for PFOA (summarized in Table 6.2). Very short half-lives (approximately 2 h) of PFOA are observed in male and female rabbits and female rats (Ohmori et al. 2003; Kudo and Kawashima 2003). All animals studied exhibit shorter half-lives of days or weeks. Even in primates (Rhesus monkeys and Japanese macaques), half-lives are estimated to be a

Table 6.2 Half-life ($T_{1/2}$) in plasma or serum and renal clearance of PFAAs

	Rat		Mouse		Monkey		Rabbit		Dog		Cow	Human	
	M	F	M	F	M	F	M	F	M	F		M	F
PFCA													
PFBA	9.2 h[d]	1.8 h[d]	13 h[d]	2.9 h[d]	40 h[d]	41 h[d]						72 h[d]	87 h[d]
PFHxA	1 h[e]	0.4 h[e]			5.3 h[e]	2.4 h[e]							
	1.7 h	0.5 h											
PFHpA	2.4 h[f] (977)	1.2 h[f] (1462)											
PFOA	5.6 days[f] (31)	1.9 h[f] (988)	22 days[i]	16 days[i]	21 days[j]	33 days[j]	5.5 h[l] (640)	7 h[l] (670)	21 days[l] (43)	11 days[l] (50.8)	19 h[m, c]	2.3 years[n, b]	
					5.6 days[k, a] (15)	2.7 days[k, a] (32)						3.5 years[o, b]	
	12 days[g] (18.2)	3.4 h[g] (666)										3.3 years[p, b]	
												2.8 years[q]	2.3 years[p]
	13 days[h]												
PFNA	47 days[r]	2 days[r]	34–69 days[s]	26–68 days[s]									
	31 days[s]	1.4 days[s]											
	30 days[f] (0.72)	2.4 days[f] (64)											

PFDA	40 days[f] (0.96)	59 days[f] (0.96)										12 years[q]	4.5 years[q]
PFAS													
PFBS	2.1 h[e]	0.6 h[e]			15 h[e]	8.1 h[e]						1 month	
PFHxS	29 days[t]	1.8 h[t]	28–31 days[t]	25–27 days[t]	87 days[v]	141 days[v]						8.5 years[o, b] 35 years[q]	7.7 years[q]
PFOS	38 days[w]	62 days[w]	43 days[w]	38 days[w]	132 days[w]	110 days[w]					114 days[u, c]	5.4 years[o, b] 34 y[q]	6.7 years[q]

Values in parenthesis represent CLR (mL/day/kg). [a]Japanese macaque; [b]sex is not distinguished; [c]castrated cattles

References: [d]Chang et al. (2008), [e]Chengelis et al. (2009), [f]Ohmori et al. (2003), [g]Kemper (2003), [h]Benskin et al. (2009), [i]Lou et al. (2009), [j]Butenhoff et al. (2004), [k]Harada et al. (2005), [l]Kudo and Kawashima (2003), [m]Lupton et al. (2012), [n]Bartell et al. (2010), [o]Olsen et al. (2007), [p]Brede et al. (2010), [q]Zhang et al. (2013b), [r]De Silva et al. (2009), [s]Tatum-Gibbs et al. (2011), [t]Sundstrom et al. (2012), [u]Lupton et al. (2014), [v]Lau et al. (2007), [w]Chang et al. (2012), [x]bodyburden half-life

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month or less (Harada et al. 2005; Kudo and Kawashima 2003; Butenhoff et al. 2004). The most reliable human half-live of PFOA is shown to be 3.5 years (geometric mean) for retired fluorochemical production workers (Olsen et al. 2007) and 2.3 years for participants of C8 Health Project from mid-Ohio valley of West Virginia where environmental PFOA exposure level is high (Bartell et al. 2010). Half-life of PFOA was estimated to be 3.26 years in two-tear follow-up study of resident drinking water contaminated with PFOA (Brede et al. 2010). Species difference is shown in other PFCAs although available data are limited (Table 6.2). Half-life of PFOS also exhibits species difference (Han et al. 2012). Similar to PFCAs, Species difference in half-lives of PFOS and other perfluoroalkylsulfonic acids (PFAS) is observed. Longer half-lives are observed in humans compared to other animals; human $T_{1/2}$ of PFOS is 4.8 years while those in other animals are from several days to several months (Han et al. 2012). Recently, sex-difference was demonstrated in human, namely, $T_{1/2}$ of PFHxS and PFOS are 7.7 and 6.7 years in young females, respectively, whereas these are estimated to be 35 and 34 years, respectively, in males and old females (Zhang et al. 2013b).

6.5.2 Fecal and Urinary Elimination

PFAAs are nonvolatile and metabolically inert, total body clearance depends on elimination into urine and feces. PFAAs excreted in bile undergo fecal elimination or enterohepatic circulation. Fecal elimination of PFCAs was less than 5 % in both male and female rats 120 h after dosing (Kudo et al. 2001b). Biliary excretion of PFOA was studied in rats (Vanden Heuvel et al. 1991; Kudo et al. 2001b; Johnson et al. 1984) and humans (Harada et al. 2007). Estimated biliary clearance rates are 3.30 and 3.52 mL/kg/day in male and female rats, respectively, and 1.06 mL/day/kg in humans (Harada et al. 2007). PFCAs undergo enterohepatic circulation in rats and humans because cholestyramine treatment significantly increases fecal elimination of PFOA or PFOS in rats (Johnson et al. 1984) and that of PFOA in humans (Genuis et al. 2010). Fecal elimination rate in rats seems slow compared to urinary elimination rate due to slow biliary clearance and entero-hepatic circulation.

Renal elimination is the most critical process in determining total body clearance of PFAAs. Renal clearance (CL_R) is determined in various animals for PFOA and other PFCAs (Summarized in Table 6.2). Chain length difference in CL_R was demonstrated between PFHA, PFOA, PFNA and PFDA in rats (Ohmori et al. 2003). Pharmacokinetics of PFBS was determined as well (Chang et al. 2012). Between four and ten carbon chain length PFCAs, the trend of a shorter chain length leading to a shorter $T_{1/2}$ is observed except for PFBA (four carbons), suggesting that elimination machinery of PFBA may differ from the longer chain PFCAs (Han et al. 2012). In addition to carbon chain length difference, sex difference in CL_R is also interesting. Early studies demonstrated rapid urinary elimination of [^{14}C]PFOA in female rats compared to male rats (Vanden Heuvel et al. 1992b). Sex difference is prominent in rats (Vanden Heuvel et al. 1991; Ohmori et al. 2003; Kemper and

Nabb 2005) and Hamster (Hundley et al. 2006), but not observed in rabbits (Kudo and Kawashima 2003) and mice (Kudo and Kawashima 2003; Lou et al. 2009). In rats, sex hormones were identified as major factors in regulating CL_R. Castration of male rats greatly increased CL_R of PFOA, while testosterone treatment of castrated male rats or female rats reduced to the normal level of male rats (Vanden Heuvel et al. 1991; Kudo et al. 2002). By contrast, 17β-estradiol treatment of castrated male rats increased urinary PFOA elimination to the level of females (Ylinen et al. 1989). Urinary elimination increased as growing after weanling in female rats while male rats remain low (Hinderliter et al. 2006b). Recently, sex difference in CL_R was demonstrated in human for some PFAAs (Zhang et al. 2013b). Half-lives of PFOA and other PFCAs in males and older females were longer than those of females. The values of PFOA CL_R are greatly different between species. These trends of CL_R seem to explain difference in total clearance or $T_{1/2}$ between various species, sexes, and PFCAs having different carbon atoms. Indeed, linear relationship between total clearance (CL_{Tot}) and renal clearance (CL_R) is observed in male and female rats treated with PFHA, PFOA, PFNA or PFDA (Ohmori et al. 2003). For PFOA, negative correlation is observed between $T_{1/2}$ and CL_R in male and female animals (rats, mice, rabbits, dogs, Cynomolgus monkeys and Japanese macaques) Han et al. 2012). Therefore, it is concluded that slow renal elimination rate is responsible for prolonged $T_{1/2}$ in various animals. Estimated human CL_R of PFOA is 0.03 mL/day/kg (Harada et al. 2005) which is greatly lower than other animals including rats, mice, dogs, rabbits, hamsters, and even primates. In the study of Zhang et al. CL_R was estimated for PFOA and other PFAAs in healthy volunteers in China, and PFOA CL_R was estimated to be 0.29 mL/kg/day and 0.79 mL/kg/day for young female group and male and old female group (Zhang et al. 2013b). Though available information is limited for human, and exposure conditions to human are quite different from those in experimental animals, human CL_R of PFOA seems very low compared to other animals.

6.6 Renal Transport

One of the most important aspects of PFAA toxicity is long half-lives of these chemicals due to slow urinary elimination in human. Mechanistic studies have been performed to elucidate slow urinary elimination of PFOA in humans. Renal elimination is a combined process involving glomerular filtration, tubular secretion and tubular reabsorption. PFCAs, unbound (*fu*) or bound to small molecules in plasma, undergo glomerular filtration at glomerular filtration rate (GFR). Transporter proteins residing on basolateral membrane and brush-border membrane in proximal tubular cells are responsible for uptake into cells or efflux from cells of substrates. Renal secretion and reabsorption is the net result of these transport systems. Therefore, secretion and reabsorption are thought to be directional transcellular transport system across proximal tubular cells; the former involves basolateral uptake and apical efflux and the latter involves apical uptake and basolateral efflux.

Table 6.3 Net renal tubular secretion or reabsorption of PFOA in different species

Species	Gender	GFR (L/day/kg)	*CLR* (mL/day/kg)	Net secretion (mL/day/kg)	Net reabsorption (mL/day/kg)	Reabsorption (%)
Rabbit	Female	4	670	590		Not applicable
	Male	4	640	560		Not applicable
Rat	Female	14.4	666	378		Not applicable
Dog	Female	5.3	50.8		55	52
	Male	5.3	43		63	59
Japanese macaque	Female	8.5	32		138	81.2
	Male	8.5	15		155	91.2
Rat	Male	14.4	18.2		270	93.7
Mouse	Female	16.7	16		318	95.2
	Male	16.7	10		324	97.0
Human		2.57	0.03		51	99.94

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GFR Glomerular filtration rate
Net tubular secretion = *CLR* – *fu*·GFR, where *fu*, the unbound fraction, is assumed to be 0.02. Net tubular reabsorption = *fu*·GFR – *CLR*. Reabsorption (%) = (Net tubular reabsorption)/(*fu*·GFR) × 100

CL_R is expressed as following equation: $CL_R = GFR \times f_u +$ secretion – reabsorption . Therefore, (secretion – reabsorption) is obtained by subtracting GFR × *fu* from CL_R. Han et al. summarized net secretion or reabsorption for PFOA in rabbits, rats, dogs, mice, Japanese macaque and humans based on GFR and CL_R assuming that *fu* value is 0.02 in all species (Table 6.3) (Han et al. 2012). As mentioned above, PFCAs are highly bound to albumin in plasma with high affinity, however, available study is limited on differences in binding affinity between species, carbon chain length. Compared to calculated net absorption value with GFR, 99.94 % of PFOA is reabsorbed in human. In male and female mice, male rats and male Japanese macaques, over 90 % of PFOA is reabsorbed. By contrast, PFOA seems undergo active secretion in male and female rabbits and female rats. It is interesting that reabsorption rate in humans are slower than that in dogs, mice, male rats and Japanese macaques despite that the highest percentage of reabsorption is predicted in human. Low GFR in human may be one of the reasons for low CL_R of PFOA.

Early study demonstrated that probenecid inhibit renal elimination of PFOA, suggesting that some transporters are responsible for renal PFOA transport (Vanden Heuvel et al. 1992b). To explain sex hormone-regulated CL_R of PFOA, regulation of gene expression of transporters by sex hormone status was studied, and candidate transportes were proposed (Kudo et al. 2002). Candidate of PFOA transporters are organic anion transporting proteins of SLC22 family, SLCO family and ABC family, expressed on brush border membrane or basolateral membrane of proximal tubular cells. In humans, OAT1, OAT2, OAT3 and OATP4C1 are uptake transporters whereas MRP6 and OSTα/β are efflux transporters on basolateral membrane, and OAT4, URAT1, OATP1A2 and NPT1 act as uptake transporters whereas MRP2, MRP4 and BCRP act as efflux transporters (Han et al. 2012). In rats, uptake transporters are Oat1, Oat3 and Oatp4c1 whereas efflux transporters are Mrp6 and

Ostα/β on basolatetal membrane, and Oat2, Oat5, Urat1, Oatp1a1, Oatp1a3v1, Oatp1a3v2, Oatp1a6 and Npt1 act as uptake transporters whereas Mrp2 and Mrp4 act as efflux transporters (Han et al. 2012). PFOA transporter was first reported by Katakura et al. (2007). Rat organic anion transporter (Oat) 3 and rat organic anion transporting polypeptide (Oatp) 1 were shown to facilitate [^{14}C]PFOA uptake in xenopus oocytes. Expression of human OAT1, human OAT3, rat Oat1 or rat Oat3 facilitated PFOA uptake in HEK293 cells (Nakagawa et al. 2008). Following studies revealed that hOAT4 (Nakagawa et al. 2009; Yang et al. 2010), rOatp1a1 (Yang et al. 2009; Weaver et al. 2010), rat Oat1 (Weaver et al. 2010), rat Oat3 (Weaver et al. 2010), rat Urat1 (Weaver et al. 2010) and human URAT1 (Yang et al. 2010) facilitate PFOA transport when transfected in HEK293 cells or CHO cells. Human OAT2 (Nakagawa et al. 2008), rat Oat2 (Nakagawa et al. 2009; Weaver et al. 2010), human OATP1A2 (Yang et al. 2010) and rat Urat1 (Weaver et al. 2010) were shown not to facilitate PFOA transport. Indirect evidence that CL_R of PFOA in Eisai Hyperbilirubinemic rats, defect of Mrp2, was not different from that in normal Sprague-Dawley rats suggests Mrp2 is not responsible for renal PFOA transport (Katakura et al. 2007). It cannot be excluded the possibility that undefined transporters are responsible for renal PFOA transport. For quantitative analysis of renal transport, affinity between medium-chain PFCAs and transporters were determined; estimated *Km* value of rOat1 is approximately 50 uM for PFHA and PFOA (Nakagawa et al. 2008; Weaver et al. 2010), those of rOat3 are 80 uM for PFOA (Nakagawa et al. 2008) or 66 uM for PFOA for PFOA (Weaver et al. 2010) and 175 uM for PFNA (Weaver et al. 2010), and, those of rOatp1a1 are 162 uM (Yang et al. 2009) or 127 uM (Yang et al. 2009) for PFOA, 21 uM for PFNA (Weaver et al. 2010) and 29 uM for PFDA (Weaver et al. 2010). As for human transporters, *Km* value of hOAT1 for PFOA is 48 uM (Nakagawa et al. 2008), that of hOAT3 is 49 uM (Nakagawa et al. 2008), that of hOAT4 is 172–310 uM and that of hURAT1 for PFOA is 64 uM (Yang et al. 2010). Among PFOA transporters, apical uptake transporters are rOatp1a1, hOAT4 and hURAT1 and basolateral uptake transporters are rOat1, rOat3, hOAT1 and hOAT3 (Han et al. 2012). No efflux transporter is determined to date. Slow renal elimination of PFNA and PFDA may be due to their higher affinity to rOatp1a1 that is responsible for reabsorption. However, slow urinary elimination rate of PFDA cannot be explained by rOatp1a1 alone because of faint expression of rOatp1a1 in female rats. rOatp1a1 is highly expressed in rat liver, but physiological significance in the liver is not clear. For systematic understanding of renal clearance of PFAAs, more information is required on transporters, transport systems both in in vivo and *in silico*.

6.7 Pharmacokinetic Model

Pharmacokinetic properties of PFAAs have been studied in both experimental animal and humans. The most notable aspect of pharmacokinetics for PFAAs is that they exhibit significant differences in plasma half-lives and renal clearance between species, sex, and carbon chain length. To assess human risk of PFAAs, framework

is required to understand and estimate human pharmacokinetics based on animal study and available human data. Currently proposed physiologically-based pharmacokinetic (PBPK) models incorporated a saturable renal tubular reabsorption process (Lou et al. 2009; Andersen et al. 2006; Tan et al. 2008; Loccisano et al. 2011, 2012a). These models contain compartments for plasma, liver, kidney, filtrate, skin, and a lumped compartment for remaining body tissues, therefore, tissue:plasma partition coefficients for these tissues, which are based on the data obtained by experiments, are used as parameters. Renal reabsorption maximum (*Tmc*) and affinity constant (*Kt*) for reabsorption process are applicable to renal reabsorption model. PBPK models also involve parameters for biliary excretion, urinary elimination, fecal elimination, unsorbed dose, liver protein binding, intestinal absorption, plasma protein binding as chemical parameters, and for body weight, cardiac output, blood flow, fractional tissue volumes, fractional blood flows and hematocrit as physiological parameters. Parameters obtained by fitting are different between the models applied; *Tmc* values for PFOA in male and female rats were estimated to be 0.516 mg/h/kg and 0.00935 mg/kg/h, respectively, in the study of Tan et al. (2008), while these were 270 mg/h/kg and 3 mg/h/kg, respectively, in the study of Loccisano et al. (2012a). The former study doesn't involve liver protein binding and the latter study applied *Km* value of rat Oatp1a1 for PFOA as *Tmc*. In the monkey model, *Kt* value for PFOA was estimated by fitting to be 0.055 mg/L (0.13 uM) (Loccisano et al. 2011), which is two or three order magnitude lower than that of human transporters, estimated *in vitro* (Han et al. 2012). Recently, it was reported that probenecid, a inhibitor for SLC22 family transporters such as Oat1 and Oat3, effectively inhibited urinary excretion of PFOA in female rats but rather enhanced in male rats and in isolated perfused rat kidney (Han et al. 2012). Possible explanation is that PFOA renal tubular secretion is responsible for PFOA elimination in urine in female rats or that basolateral efflux to circulation is inhibited in male rats. As for PFOS, PBPK models are proposed by Andersen et al. (2006), Tan et al. (2008), and Loccisano et al. 2011, 2012a), although mechanistic analysis in vitro is limited compared to PFCAs. Recently, a new PBPK model was developed based on a previously reported model (Loccisano et al. 2011) where lung and brain are added to body compartment but skin was removed, and compared simulated data with experimental data in the study of Perez et al. (2013). Considering transplacental transfer during gestation and transfer to milk during lactation, PBPK models were developed in mouse for PFOA (Rodriguez et al. 2009) and in rat for PFOA and PFOS (Loccisano et al. 2012b, 2013).

6.8 Conclusion

Renal elimination is a key determinant of biological half-life of PFAAs. Renal PFAA elimination mechanisms are studied in the aspect of membrane transport by transporters across both basolateral membrane and brushborder membrane of renal tubular cells. These data greatly improve our understanding of PFAA renal

transport. However, it remains to be solved how renal tubular secretion system contribute net transfer across renal tubular cells. Transport system for PFAS is not studied yet except for inhibition study. In addition to transporter study, studies on the properties of albumin binding are increasing. More information on mechanistic representation of renal tubular transfer, estimation of albumin binding properties, and human data is required to improve PBPK model to predict human kinetics.

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ED_002330_00119699-00186

Chapter 7
Metabolic Effects PFAS

Qixiao Jiang, Hui Gao, and Lei Zhang

Abstract Among the various biological effects PFAS perfluoroalkyl and polyfluoroalkyl substances (PFAS) exert on living organisms, metabolic effects are important and have attracted considerable attention in PFAS studies *in vitro* and *in vivo*. Although PFAS are metabolically inert themselves, they can interfere with endogenous metabolic processes and thus do have the ability to exert effects on metabolism. The alteration on metabolism could induce a wide range of biochemical and physiological changes. Metabolic effects have various connections with other systemic toxicities induced by PFAS and potentially serve as the fundamental basis for other observed toxicities. Conversely, other systemic toxicities could potentially affect the metabolic balance of an organism, and thus induce secondary metabolic effects as well. This chapter discusses the molecular basis of PFAS-induced metabolic effects including experimental animal and human data regarding metabolic effects. While the major focus of this chapter is on metabolic effects, some systemic and organ-specific toxicities are also discussed, as it is necessary for a comprehensive discussion. A good understanding of PFAS-induced metabolic effects could help us to better handle the potential health risks associated with PFAS exposure.

Keywords Metabolic effects • Structure-activity relationship • Peroxisome proliferator-activated receptors • Estrogen receptor • Thyroid hormone receptor • Leptin receptor • Carbohydrates • Fatty acids • Uric acid • Thyroid function

This chapter covers the following topics on the metabolic effects of PFAS:

- **Structure-activity relationship**
  Introduce the structural basis of the metabolic effects of PFAS
- **The molecular targets i.nvolved in the metabolic effects**
  Discuss the molecular targets of PFAS-induced metabolic effects

Q. Jiang (✉) • H. Gao • L. Zhang
Department of Pharmacology, Qingdao University Medical College,
422 Boya building, 308 Ningxia Road, Qingdao 266071, Shandong, China
e-mail: jiangq08@126.com

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_7

ED_002330_00119699-00187

- **Laboratory data on the metabolic effects of PFAS**
  Summarize experimental animal data on the metabolic effects of PFAS
- **Human data on the metabolic effects of PFAS**
  Summarize human data on the metabolic effects of PFAS
- **Organ specific metabolic effects**
  Discuss the metabolic effects on main affected organ systems separately

7.1 Relationship Between the Structure of PFAS and the Effects on Metabolism

PFAS are a class of organofluorine compounds that have all hydrophobic hydrogens replaced with fluorine. Most PFAS share the common structure of a long fatty chain. Therefore, they have similar chemical properties based on their structural similarity. The PFAS most commonly found in human blood are PFOS ($C_8F_{17}SO_3$), PFOA ($C_8F_{15}O_2H$) and PFHxS ($C_6F_{13}SO_3$) (Fig. 7.1 and 7.2). The images were represented by the ChemBioDraw software (http://scistore.cambridgesoft.com/) and Discovery Studio Viewer 2.5 (Accelrys Software Inc.).

The Log P values of PFOS, PFOA and PFHxS were predicted by ChemBioDraw software. Theses Log P values suggest that the representative PFASs are hydrophobic and they can easily penetrate through the cell membrane. This lipophilic property also makes it difficult for the excretion of these PFASs.

The long fatty chain structures of the representative PFAS indicate that hydrophobic interactions play an important role in the binding of these molecules to their receptors. The sulfonic acid and carboxylic acid groups contribute to the polar interactions or hydrogen binding interactions of PFAS-receptor binding.

Peroxisome proliferator-activated receptor alpha (PPARα), a major regulator of lipid metabolism in the liver, was a main target for PFAS (Rosen et al. 2008). Molecular docking was performed to predict the binding patterns of PFAS in the structure of PPARα. The Glide 5.5 software (Schrö dinger Inc, supported by Shanghai Institute of Materia Medica Chinese Academy of Sciences) was used in the docking process. The A chain of PPARα (PDB Entry: 4BCR, www.pdb.org) was used as the receptor. A cubic box (with length of 36 Å) containing the whole receptor

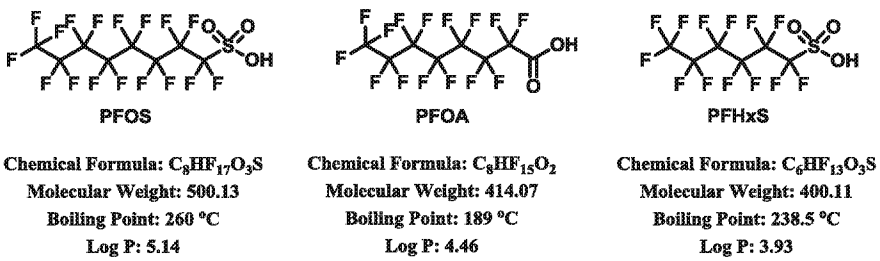

Fig. 7.1 The structures and chemical properties of representative PFAS

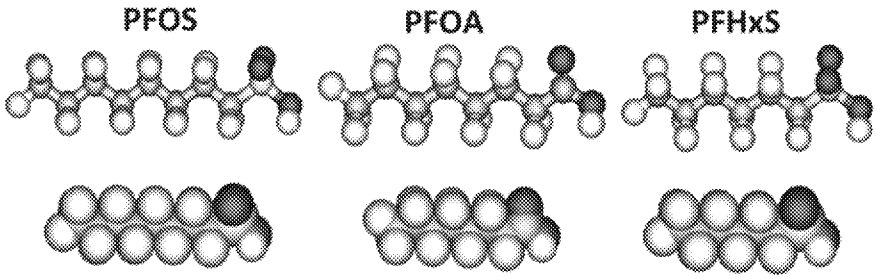

Fig. 7.2 3D structures of representative PFASs, the molecules were displayed by ball-stick and CPK models, respectively

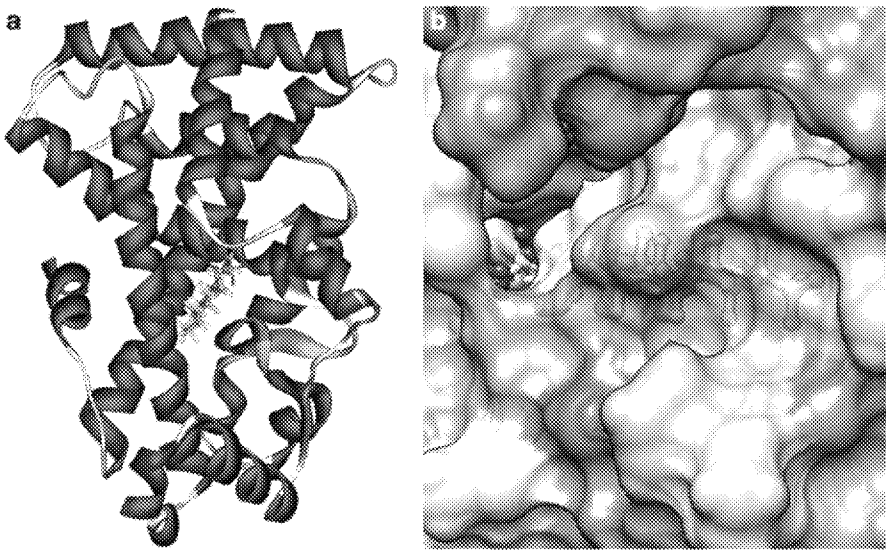

Fig. 7.3 Ribbon (**a**) and surface (**b**) representation of PFASs in the structure of PPARα

(chain A) was defined as the active site. Standard precision was selected to make the docked the ligands (PFOS, PFOA and PFHxS) search their binding site from the whole protein. Discovery Studio Viewer 2.5 and UCSF Chimera 1.8 software were used for the result representation (Pettersen et al. 2004).

As shown in Fig. 7.3, the representative PFASs (PFOS, PFOA and PFHxS) binds to the same site in the structure of PPARα. The PFAS ligands also share similar binding modes in their binding sites. The fatty chains of PFASs penetrate into an internal pocket of the PPARα structure, and the acid groups located in the opening of the pocket.

The binding site of PFAS was lined by residue Met220, Cys276, Thr279, Ser280, Thr283, Ile317, Phe318, Met320, Leu321, Val324 and Met355. The ligand (WY11468) in the structure of PPARα also locates in the hydrophobic pocket

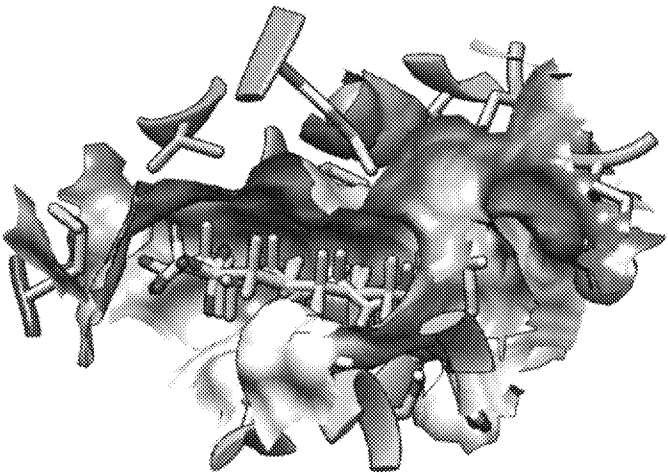

Fig. 7.4 3D representation of PFOS, PFOA and PFHxS in the binding site of PPARα

formed by the above residues. In Fig. 7.4, the surface was colored from blue to orange with the decrement of polarity. Therefore, hydrophobic interactions play a key role in the binding of PFAS in the interaction site of PPARα.

Peroxisome proliferator-activated receptor gamma (PPARγ), which regulates fatty acid storage and glucose metabolism, is also the target of PFAS. Molecular docking studies were also performed to investigate the binding of PFAS in the structure of PPARγ. The structure of PPARγ with a decanoic acid in its structure (3U9Q) was used as the receptor in the docking study. Other parameters were set as the same with data in the PPARα study (Fig. 7.5).

The PFASs (PFOS, PFOA and PFHxS) also bind to the same region in the structure of PPARγ. There is a long and narrow opening at the binding site of PFAS. The whole structure of PFAS locate into the pocket close to the surface of the structure (Fig. 7.6).

The PFAS bind to the same site with the ligand decanoic acid, which was made by Cys285, Arg288, Ser289, Ala292, Ile326, Tyr327, Leu330, Leu333, Phe363, Met364 and Hie449. Hydrophobic interactions, formed by the fatty chain of PFAS and surrounding amino acids, make important contributions to the ligand-receptor binding. Hydrogen bond interactions also facilitate binding of PFAS to PPARγ. PFOS and PFOA share similar binding patterns; their acid groups form hydrogen bond interactions with Arg288. While PFHxS has a different binding mode (the acid group extends to the other side of the pocket), the sulfonic group binds to the NH of Hie449 by hydrogen bond interactions.

Estrogen receptor alpha (ERα) was also demonstrated to be a receptor for the binding of PFAS in human bodies. The molecular docking process was also performed to predict the binding mode of PFASs in the structure of ERα. The A chain of ERα (2QZO) was selected as the receptor in the docking studies. Other parameters were also set as the same with data in the PPARα study (Fig. 7.7).

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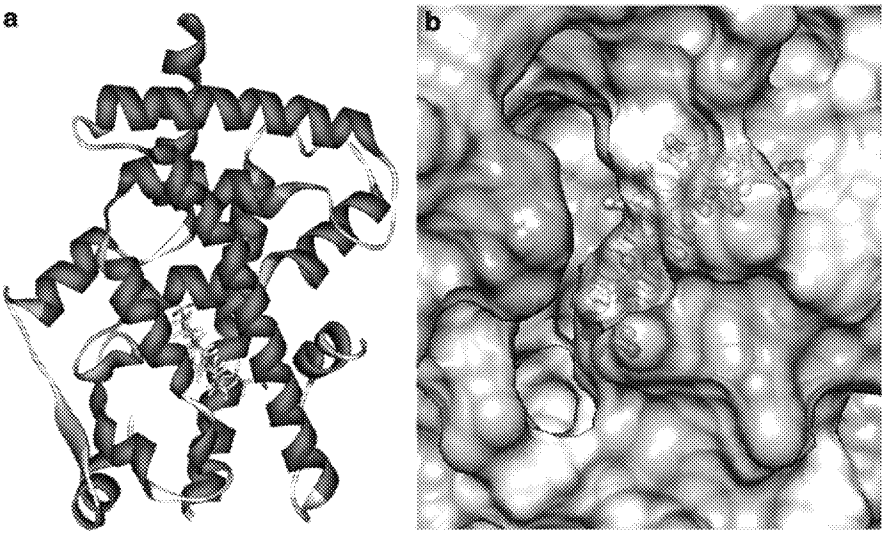

Fig. 7.5 Ribbon (**a**) and surface (**b**) representation of PFASPFASs in the structure of PPARγ

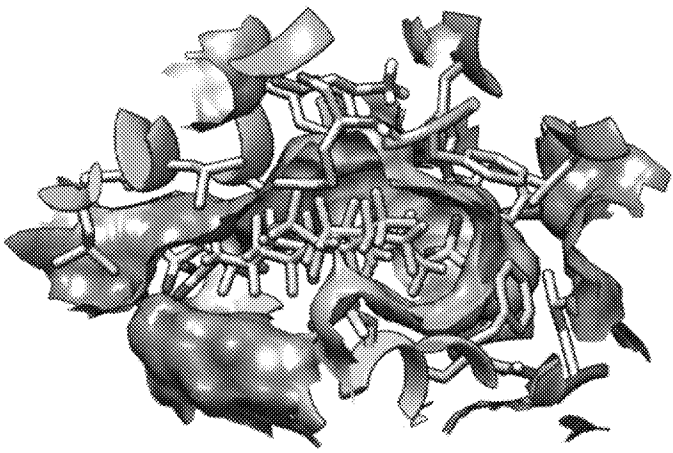

Fig. 7.6 3D representation of PFOS, PFOA and PFHxS in the binding site of PPARγ

Unlike the binding patterns of PFAS in PPARα and PPARγ, the representative PFAS bind to the surface domain of ERα. The long fatty chains of PFAS extend into a narrow channel, and the acid groups locate in the opening of the channel. There are good spatial fittings between the structures of the ligands and the surface of the binding site (Fig. 7.8).

The binding site of PFAS in the structure of ERα was lined by residue Glu323, Pro324, Ile326, Glu353, Met357, Ile386, Leu387, Gly390, Trp393, Arg394, Phe445

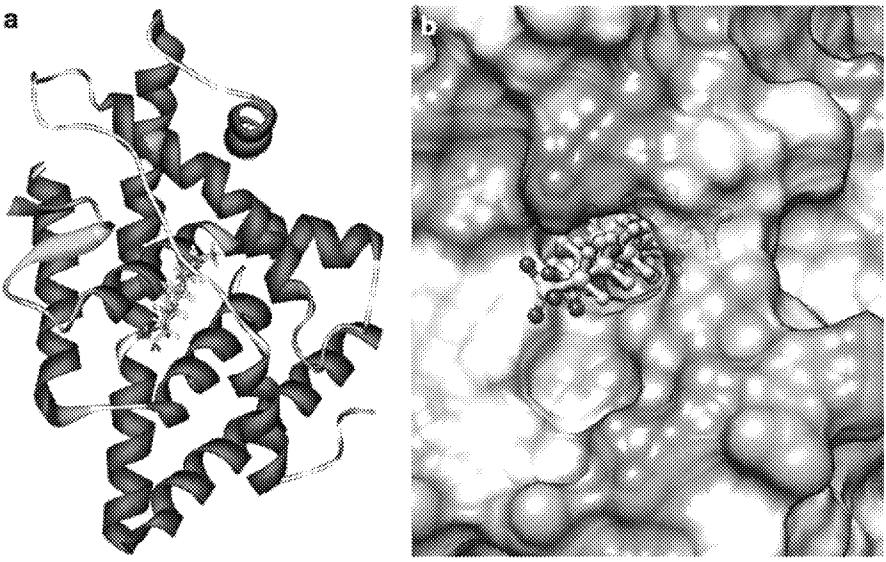

Fig. 7.7 Ribbon (**a**) and surface (**b**) representation of PFASs in the structure of ERα

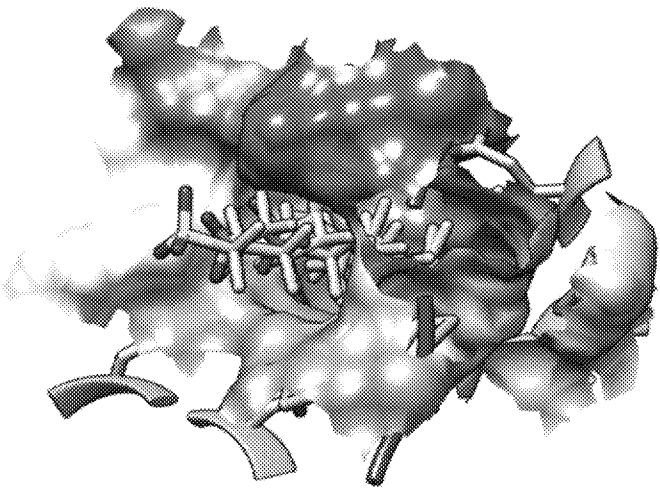

Fig. 7.8 3D representation of PFOS, PFOA and PFHxS in the binding site of ERα

and Lys449. Hydrophobic interactions formed between representative PFAS and surrounding amino acids make major contributions to the ligand-receptor binding.

The docking results show that the representative PFAS could bind to the active site of PPARα and PPARγ, or bind to the region close to the active site of ERα. It is

difficult for endogenous ligands to bind to the catalytic sites, if the PFAS have occupied those regions. Thus, PFAS disturb the function of these receptors by interfering with the ligand-receptor interactions.

7.2 The Molecular Targets Involved in the Metabolic Effects of PFAS

PFAS induce metabolic effects by interacting with various molecular targets, including specific and non-specific ones. This section gives a comprehensive review of the known specific molecular targets involved in the metabolic effects of PFAS. The non-specific effects are also mentioned.

7.2.1 Receptor Specific Metabolic Effects of PFAS

PFAS can induce metabolic effects by affecting specific receptors. These effects are relatively well-studied and characterized. Receptor-specific metabolic effects can be induced by primary or secondary actions. The primary action is due to the ability of PFAS to directly mimic endogenous ligands of the target receptors, thus agonizing or antagonizing the target receptors. This type of effects could be attributed mainly to the chemical structure of the PFAS, as detailed in Sect. 4.1. On the other hand, the secondary action of PFAS on receptors may not be attributed to their ligand-mimicking, but to their effects on signaling pathways by interference with the levels of ligands or receptors. While the primary actions, especially impacts on the PPARs, are well characterized, the second actions are less studied and harder to elucidate. Nevertheless, this section covers both primary and secondary actions of PFAS on the receptors that could induce metabolic effects.

7.2.1.1 PPARα

As detailed in Sect. 4.1, the main molecular targets of PFAS are the peroxisome proliferator-activated receptors (PPARs), of which PPARα and PPARγ are the major ones. PPARα is a nuclear receptor mainly known for its role in regulation of lipid metabolism. High level of PPARα expression is found in the liver. Other tissues with high levels of expression include kidney, heart, muscle and adipose tissue. The endogenous ligand for PPARα is fatty acid. Upon activation, PPARα dimerizes with Retinoid X receptor (RXR), binds to peroxisome proliferator hormone response elements (PPREs), and regulates various genes, including those responsible for fatty acid metabolism such as fatty acid binding proteins (FABPs) and acyl CoA oxidase (ACOX) (Latruffe et al. 2000).

Most PFAS have affinity for PPARα, and serve as agonists. Upon exposure, PFAS could induce the expression of downstream genes and hence a series of biological and/or pathological alterations in the exposed organism. PFOA is one of the most prominent PFAS in the environment and is known for its potency to act on the PPARα receptor. Many studies have confirmed that PFOA could activate PPARα, as reported by Rosen et al. (2008), Minata et al. (2010), and Strömqvist et al. (2012). However, the effect of PFOA on PPARα is not always agonism. For example, when PFOA and the PPARα agonist WY14,643 were compared for their transcriptional effects, PFOA induced PPARα independent effects, mainly xenobiotic metabolism gene inductions (Rosen et al. 2008). Furthermore, in PPARα-null mice, PFOA is still capable of inducing a series of gene expression changes, confirming the PPARα independent role of PFOA (Rosen et al. 2008). In another study on developing chicken embryos, it was also observed that WY 14,643 could induce similar but not exactly identical effects to PFOA, suggesting that the effect of PFOA on PPARα is not a purely agonism (Jiang et al. 2013).

Other PFAS, such as PFOS and PFHXS, also possess the characteristics of PPARα agonism, but the degree of agonist effect varies depending on the specific compound and target species. Due to this variability, one should take caution when evaluating the effects of PFAS on metabolism based solely on PPARα agonism.

7.2.1.2 PPARγ

Aside from PPARα, PFAS could also affect PPARγ. Like PPARα, PPARγ belongs to the PPARs family, but the endogenous ligands are mainly eicosanoids. PPAR γ mainly regulates fatty acid storage and glucose metabolism. PFAS, such as PFOA and PFOS, are demonstrated to be capable of activating PPAR γ as well as PPARα (Takacs and Abbott 2007). Thus PPAR γ is also an important molecular target for the ability of PFAS to induce metabolic effects.

7.2.1.3 Estrogen Receptor

PFAS are known as endocrine disruptors, and have been shown to be capable of directly interfering with estrogen receptor (Liu et al. 2007; Kjeldsen and Bonefeld-Jørgensen 2013). Since estrogen receptor has the ability to regulate metabolism, the interference with estrogen receptor could indeed result in profound metabolic effects. More detailed information about the effects of PFAS on estrogen receptors, however, will be discussed in other chapters.

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7.2.1.4 Thyroid Hormone Receptor

The thyroid hormone signaling pathway is important for development, cariogenic metabolism, and the cardiovascular system. Impacts on thyroid hormone receptors could lead to disrupted metabolism. Thyroid hormone receptors have two subtypes: TRα and TRβ. Both TRα and TRβ can attach to thyroid hormone response elements (TRE) in the DNA, and become activated when thyroid hormones, T3 or T4, bind to the receptors, initiating various gene transcriptions.

In laboratory models, PFAS were associated with decreased circulating thyroid hormone levels. Wei et al. (2008) reported that PFOA exposure inhibited genes responsible for thyroid hormone biosynthesis in rare minnows. However, contradictory results were reported in human studies. Jain (2013) summarized NHANES 2007–2008 data that indicated an association between elevated circulating total T3 levels and PFOA exposure levels. Total T4 levels were also found to be increased when circulating PFHxS levels were elevated. Because of the important role of TH signaling in development and the cardiovascular system, researchers started to observe an association between PFAS-induced developmental toxicity and the interruption of thyroid hormone levels. Ongoing research will likely shed more light to better delineate this characteristic of PFAS.

7.2.1.5 Leptin Receptor

Leptin is a hormone that regulates appetite, thus is involved in the etiology of obesity and potentially other metabolic-originated diseases. Leptin receptor is located in the hypothalamus. When leptin binds the receptor, the receptor activation induces physiological effects, mainly regulating energy intake and expenditure. Thus leptin signaling is important to metabolism.

PFAS, when developmentally exposed to rodents, are known to induce increased body weight later in life. Hines et al. (2009) reported that in their developmental mouse model, a low dose of PFOA (0.01–0.3 mg/kg) significantly increased body weight and leptin levels in the mice when they reached mid-life. This suggests that a disrupted leptin signaling pathway could contribute to PFOA-induced body weight increases. However, PFAS-induced alterations in leptin levels are not completely understood at this point. Leptin is a hormone associated with the maintenance of a lean body weight, but PFAS both increased leptin levels and body weight. When additional data, such as leptin receptor alterations following developmental PFOA exposure become available, this effect might be better elucidated.

7.2.2 Non-specific Metabolic Effects of PFAS

Although many specific molecular targets have been identified for PFAS, there are conditions where non-specific metabolic effects can occur. When systemic exposures to PFAS occur at high doses, there could be non-specific effects that occur throughout the living organism, including metabolic effects. These effects have been observed both in human and in experimental animal models, but have been relatively less studied.

Change in body weight is one major endpoint that has been reported to be affected by PFAS exposure. Epidemiological data from 2005 to 2010, in a Mid-Ohio Valley community, revealed a negative association between PFOS exposure and birth weight among full-term infants (Darrow et al. 2013). In animal studies, high doses of PFAS significantly decreased body weight (Hines et al. 2009; Wolf et al. 2007). No exact mechanism has been identified for this type of general toxicity. Interference with fatty acid metabolism might partially contribute to the body weight loss, but is not sufficient to account for the reported changes alone.

Other non-specific effects also exist, such as development retardation. Such effects are usually difficult to quantify, and can be widely assigned to metabolic effects. With additional studies, the precise molecular targets and mechanisms behind these effects might be identified.

7.3 Laboratory Data on the Metabolic Effects of PFAS

This section covers existing laboratory data on metabolic effects of PFAS. For clarification, direct metabolic effects (alterations in metabolic substrate levels or metabolic activity levels) and indirect metabolic effects at genetic level (alterations in metabolism-related gene expression) are discussed separately.

7.3.1 Direct Metabolic Effects

7.3.1.1 Effects on Fatty Acids

The impact on fatty acid metabolism is the most prominent effect observed in laboratory animals or cell cultures following PFAS exposure. As mentioned in previous sections, PFAS can bind to and activate PPARα. Since PPARα is the major component in regulating fatty acid metabolism, it could induce a series of metabolic effects. This is one of the major mechanisms for the metabolic effects of PFAS. Numerous studies have reported impacts on fatty acid metabolism following PPARα activation (Abbott et al. 2012; Arukwe and Mortensen 2011; Yang 2010).

However, PPARα may have independent effects on other biological activities, as demonstrated by Rosen et al. (2008).

PFOA has been shown to interfere with fatty acid metabolism and cholesterol synthesis in the liver (Haughom and Spydevold 1992). Cholesterol metabolism is especially interesting, since a significant decrease in Hmgcr, acyl CoA cholesterol acyltransferase (ACAT) activity is observed that is consistent with a decreased serum cholesterol level after 24 h when PFOA was fed to rats. Similar results were reported by Guruge et al. (2006), in which Hmgcr activity was significantly down-regulated at doses of 5, 10 and 15 mg/kg PFOA in female rats.

In addition to the changes of these cholesterol metabolism-related genes, alterations in cholesterol levels were also directly observed. PFOA and PFOS exposure induced a decrease of serum cholesterol levels in male Sprague-Dawley (SD) rats (Martin et al. 2007). An earlier study also revealed that relatively high doses of PFOA exposure in Wistar rats could lead to cholesterol accumulation in the liver (Kawashima et al. 1995). The decrease in serum cholesterol levels and the increase in liver cholesterol levels can be potentially explained by the fact that PPARα agonism enhanced the cholesterol reverse transport to liver (Bighetti et al. 2009). Guruge et al. (2006) proposed an interesting hypothesis with respect to PFOS. Since PFOS could be incorporated into cell membranes and affect cell membrane fluidity and membrane potential (Hu et al. 2003), the role of PFOS seems to be similar to that of cholesterol, thus the reduction of cholesterol could be the result of functional substitution by PFOS. While the interference with PPARα is generally thought to be the main cause of the cholesterol reduction, this functional substitution hypothesis could indeed explain the cholesterol-lowering effect in lab models of PFAS. Nonetheless, more work is needed before solid conclusions can be reached.

7.3.1.2 Effects on Carbohydrates

Little is known about PFAS exposure-induced effects on carbohydrate levels. However, the effect of PFAS exposure on glycogen is better studied and known. In one study, exposure to PFOA depleted glycogen in zebrafish livers, indicating that the metabolic effects of PFAS include impacts on glycogen, especially on glycogen deposition (Hagenaars et al. 2013). This gives interesting mechanistic evidence to the decreased hatchability of chickens following PFAS exposure (O'Brien et al. 2009). Since the chicken hatching process largely depends on the deposited glycogen in the hatching muscle, the interrupted glycogen deposition could potentially contribute to the decrease in hatchability following PFOA exposure. Further investigation may provide more evidence if and how PFAS exposure affects carbohydrate levels.

7.3.1.3 Effects on Other Metabolic Substances

In addition to the effects of PFAS exposure on the major metabolic substrates such as fatty acids, cholesterol and carbohydrates, other compounds related to metabolism may also be affected by PFAS exposure. Carnitine is an important endogenous compound that facilitates fatty acid beta oxidation. Peng et al. (2013) demonstrated that carnitine metabolism is disturbed by PFOA exposure in L-02 cells. Carnitine concentration was decreased with PFOA exposure, while the other metabolic carnitine congeners, including acetyl carnitine, propionyl carnitine, butyrylcarnitine and valerylcarnitine, were all increased. The enzymes responsible for carnitine metabolism, including CPT1A, CPT2, CACT and CRAT, were all increased as well. Considering the important role of carnitine in fatty acid metabolism, the contribution of carnitine interference could be important. However, the dose used in this study was significantly higher than levels associated with human exposures.

Another target of PFAS is thyroid hormone, a major regulatory hormone of metabolic function. PFAS are known to affect thyroid hormone signaling pathways, which potentially contributes to the metabolic effects induced by PFAS exposure. PFOS exposure (15 mg potassium PFOS/kg) in rats transiently increased tissue availability of the thyroid hormones and the turnover of T4, thus reducing serum TT4. However, PFOS did not induce a classical hypothyroid status (Chang et al. 2008, 2009).

Other than the direct change of metabolic substrate levels following PFAS exposure, metabolic processes could also be affected by PFAS exposure. Bjork et al. (2011) reported that in primary rodent hepatocytes, both PFOA and PFOS exposure induced a substantial shift from carbohydrate metabolism to fatty acid oxidation. Hepatic triglyceride accumulation was also observed. The effect was more pronounced for PFOA than PFOS. The metabolic shift induced by PFAS exposure has multiple indications. It could directly impair energy production or affect oxygen consumption, thus contributing to systemic toxicity; it might also induce generation of reactive oxygen species and downstream cellular and tissue damage.

7.3.2 Indirect Metabolic Effects at Genetic Level

Aside from directly changing the levels of fatty acid, cholesterol and thyroid hormones, PFAS can affect the expression levels of various genes associated with metabolism. These effects are primarily mediated through PPARα agonism, but PPARα independent effects may also exist.

Following exposure of 0.5 mg/L PFOS in zebrafish larva, various proteins responsible for metabolism were affected. NDPK-Z2, UMP-CMP kinase, AK2, Ckmb protein, CS, phosphoglycerate mutase 1 and OSBP1A were all decreased (Shi et al. 2009). These proteins are responsible for nucleic acid, carbohydrate, alcohol, and cholesterol metabolism. Guruge et al. (2006) also reported that PFOA exposure induced expression level changes in multiple genes responsible for

peroxisomal and mitochondrial fatty acid beta oxidation. The prominent genes include acyl-CoA oxidase, enoyl CoA hydratase, carnitine palmitoyltransferases and acetylCoA dehydrogenase. The increase in the level of expression of these genes indicated that PFOA promotes fatty acid metabolism. Similar results were obtained by other researchers, including Krøvel et al. (2008), whose study showed that PFOS induced an elevation in PPARα and acyl CoA oxidase expression.

Peng et al. (2013) carried out an *in vitro* study with L-02 cells exposed to PFOA, and found that multiple genes for metabolic pathways were affected, including genes for lipid metabolism, carbohydrate metabolism, amino acid metabolism and xenobiotic metabolism. In a zebrafish study by Hagenaars et al. (2013), PFOA was found to interfere with mitochondrial membrane permeability and subsequent impairment of aerobic ATP production. Depletion of liver glycogen and elevated anaerobic metabolism gene expression were detected, potentially the result of compensation for decreased aerobic metabolism. This study also confirmed that the mitochondrial electron transport activity was decreased in PFOA-exposed zebrafish livers. Lipid metabolism related genes were also found altered by PFOA exposure. Common carp with PFOS exposure at 0.1–1 mg/L had alterations in gene expression, including energy metabolism genes such as apoE, chymotrypsinogen B1, ATPase2, protein phosphatase 1, glucokinase, cytochrome C oxidase, and NADH dehydrogenase 1 beta subcomplex 1; all of these are involved in lipid transport, protein metabolism, glycogen and glucose metabolism and electron transport chain (Hagenaars et al. 2008).

In summary, PFAS exposure-induced alterations in metabolic gene expression are important parts of PFAS-induced metabolic effects. These alterations in gene expression could be the cause or result of changes at the levels of metabolic substrates (fatty acids, carbohydrates, etc.).

7.3.3 Body Weight

In adult animals, body weight generally is decreased when high dose of PFAS are applied. For example, Lefebvre et al. (2008) reported significant body weight decreases following dietary PFOS exposure in adult SD rats. This body weight reduction was considered as a mark of general toxicity, and only was observed at doses higher than human exposure levels. Nonetheless, PFAS can affect body weight via interference of signaling pathways at much lower doses. As mentioned in Sect. 7.4.2.1.5, leptin levels are affected by PFOA exposure in animal models. This effect seems to be more prominent in developing organisms than in adults, thus will be discussed in details in the following subsection.

7.3.4 Metabolic Effects in Development

Metabolic effects of PFASs are not limited to their effects on adult humans and animals. Developmental toxicity is a point of concern for PFAS. Because metabolic effects are one of the primary effects that PFAS induce, many studies actually suggest that PFAS-induced developmental toxicity could partially contribute to metabolic effects.

Generally, in utero exposure to PFAS will induce changes in gene expression similar to that of adult exposures. Bjork et al. (2008) showed that in utero exposure of PFOS in SD rats led to significant increases in hepatic peroxisomal proliferation genes, along with fatty acid transport, oxidation, biosynthesis genes, and increase of bile acid synthesis genes. LV et al. (2013) found that gestational and lactational exposure to PFOS induced elevated fasting serum insulin and leptin levels, impaired glucose tolerance and hepatic steatosis and increased gonadal fat pad weight in Wistar rats. Similar results were obtained after developmental exposure of PFOS to CD1 mice. When F1 pups reached adulthood, insulin resistance and glucose intolerance were observed (Wan et al. 2014). These types of metabolic effects were not evident after adult exposures, but were detectable following developmental exposures. Thus, the developmental metabolic effects of PFAS should receive greater attention.

In summary, many investigations confirm PFAS-induced metabolic effects. Metabolic substrates, including fatty acids, cholesterol and glycogen; other metabolic-related compounds, including leptin, carnitine and thyroid hormone; and genes related to metabolism have all been found to be affected by PFAS exposure. Body weight and specific developmental metabolic effects were also found to be significantly affected by PFAS exposure *in vivo*.

7.4 Human Data on the Metabolic Effects of PFAS

This section covers existing human data on the metabolic effects of PFAS. The effects of PFAS on serum levels of lipids, uric acid and thyroid hormone are discussed.

7.4.1 Effects on Serum Lipid Level

As mentioned in the previous section, decreases in PFAS levels are associated with increased levels of lipids in animal studies (Lau et al. 2007). However, considerable epidemiologic evidence suggests a positive association of PFAS, particularly PFOA and PFOS, with lipid levels in humans, which contradicts what would be deduced from results of animal studies.

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As early as in 2000, Olsen et al. (2000) reported that PFOA exposure correlated with increased cholesterol levels in an occupational cohort. However, the association was not statistically significant. Similar results were obtained in another occupational study (Olsen et al. 2007) and a community study (Emmett et al. 2006b). In contrast, nine studies including four occupational studies (Olsen et al. 2003; Sakr et al. 2007a, b; Costa et al. 2009), two studies of a highly exposed community (Frisbee et al. 2010; Steenland et al. 2009) and three general population studies in adults, children and adolescents (Nelson et al. 2010; Geiger et al. 2014; Eriksen et al. 2013), reported statistically significant correlations between plasma PFAS and serum cholesterol levels.

Of the 12 studies, 10 studies in adults did not provide uniform findings on whether PFAS exposure was involved in the development of elevated cholesterol level whereas both cross-sectional studies that examined the association between PFOA and PFOS exposure in relation to dyslipidemia in children and adolescents reported statistically significant results. The community-based study including 12,476 children in a Mid-Ohio river valley area conducted by Frisbee et al. (2010) reported that total cholesterol demonstrated a consistent increase for each increase in PFOA or PFOS quintile: a 4.6 mg/dL and 8.5 mg/dL increase in the covariable-adjusted EMM of total cholesterol between first and fifth quintile of PFOA and PFOS, respectively. A recent study (Geiger et al. 2014), which evaluated 877 adolescents between 12 and 18 years old, revealed exposure to PFOA and PFOS was independently correlated with elevated serum total cholesterol levels at "background" exposure levels lower than seen in the average American population.

Nine out of the 12 studies were cross-sectional whereas 3 studies were longitudinal. Sakr et al. (2007b) documented multiple measurements of PFOA and cholesterol in 454 workers over an average of 10 years. Costa et al. (2009) reported the health outcomes of 30 years (1978–2007) of medical surveillance of 53 male workers (20–63 years) engaged in a PFOA production plant. Olsen et al. (2003) reported the results of two measurements in 174 workers. All three of these studies suggested a significant correlation between serum cholesterol and PFOA exposure.

Other than total cholesterol levels, the relationship of PFAS exposure with other lipids, including high density lipoprotein (HDL), low density lipoprotein (LDL), and triglyceride has also been investigated. Mounting evidence showed a positive association of PFOA exposure with LDL (only the occupational study published in 2009 reported that increased PFOA levels had no association with LDL levels (Costa et al. 2009)). In addition, a similar magnitude of association was found between PFOS and LDL (Steenland et al. 2009; Frisbee et al. 2010; Nelson et al. 2010), which leads to the postulation that PFAS are associated with increased LDL levels via a common mechanism. The positive associations between PFOA and PFOS in serum and LDL cholesterol was further demonstrated in a recent published longitudinal study that reported within-individual changes in serum PFOA and PFOS and changes in serum lipid LDL over a 4.4-year period (Fitz-Simon et al. 2013). Compared to the relatively consistent positive relationship of PFOA and PFOS with LDL, changes in the serum HDL level and triglycerides were not consistently associated with PFOA and PFOS exposure. In occupational studies,

different associations between PFOA exposure and HDl or triglyceride have been reported. PFOA is associated with increased total and LDL but not HDL (Sakr et al. 2007a), increased total cholesterol but not triglycerides or HDL (Costa et al. 2009), and increased triglycerides but not LDL (Olsen et al. 2007). The recent cross sectional study in a general population of adolescents also reported non-significant associations of PFOA and PFOS with abnormal HDL and triglycerides (Geiger et al. 2014).

The mixed results from these studies make it difficult to draw conclusions on the effect of PFAS exposure on serum lipids. In addition, the markedly varied strength of the association of PFOA or PFOS and lipids makes it even more problematic to interpret these results. Furthermore, it is still unknown whether peroxisome proliferation, the mechanism of action identified in animal studies, plays a role in changes of lipid levels in humans, given the discrepancy between human and animal studies on the associations between PFAS and serum lipid levels. Therefore, additional studies are needed to get the full picture on the effects of PFAS on serum lipids level in humans.

7.4.2 Effect on Uric Acid Levels

Uric acid is a natural byproduct of purine metabolism. Elevated serum uric acid levels have an underlying role in the pathophysiology of gout, and emerging studies have showed that higher serum levels of uric acid are associated with dyslipidemia (Lin et al. 2006), increased makers of inflammation (Mijatovic et al. 2011), and insulin resistance (Facchini et al. 1991). Considerable epidemiologic evidence suggests that increased uric acid is a risk factor for hypertension (Shankar et al. 2007), diabetes mellitus (Bandaru and Shankar 2011), chronic kidney disease (Cain et al. 2010) and cardiovascular disease (Fang and Alderman 2000).

The positive associations between PFAS exposure and elevated serum uric acid levels have been reported in four studies in adult populations highly exposed to PFAS (Costa et al. 2009; Sakr et al. 2007a, b; Steenland et al. 2010a, b) and two studies in the general population at lower "background" exposure levels of PFAS (Geiger et al. 2013; Shankar et al. 2011). The highly exposed populations included occupational cohorts of employees from PFAS-handling plants (Sakr et al. 2007a, b) and residents from the Ohio River Valley who were highly exposed to PFOA in contaminated drinking water by a nearby chemical plant (Steenland et al. 2010a, b). The general population studies have included both adult and child subjects (Geiger et al. 2013; Shankar et al. 2011). All of these studies are cross-sectional except for the one conducted by Costa et al. (2009). Serum PFOA and uric acid levels were repeatedly measured over a 7-year period for longitudinal analysis in 56 workers (Costa et al. 2009), which suggested a significant association between uric acid and PFOA.

Positive associations were consistently found in all cross-sectional studies, although the overall strength of the association between PFAS and uric acid varies.

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In the study by Costa's group (2009), the uric acid levels were 6.29 μg/ml for 34 currently PFOA exposed workers versus 5.73 μg/ml for 34 matched non-exposed workers (P = 0.04). In the cross sectional study by Steenland et al. (2010b), significant association of both PFOA and PFOS with serum uric acid were found, although PFOS showed a less pronounced trend. Recent studies (Shankar et al. 2011) in the general population additionally demonstrated that the association of PFOA and PFOS with increased serum uric acid was independent of age, sex, race and ethnicity, body mass index, diabetes, hypertension, and serum cholesterol level (Geiger et al. 2013).

7.4.3 Effect on Thyroid Function

Experimental evidence has shown that PFAS exposure impaired thyroid hormone homeostasis by reducing T3 and T4 in rats and monkeys (Lau et al. 2007; Butenhoff et al. 2002). In addition, *in vitro* modeling of human thyroid function showed PFAS may be able to decrease thyroid hormone levels by competing with T4 for binding to the human thyroid hormone transport protein transthyretin (TTR). Hence, a number of studies have investigated the effect of PFAS exposure on clinical markers of thyroid function. These studies include occupational studies (Olsen et al. 2003), highly exposed community studies (Emmett et al. 2006a, b; Bloom et al. 2010) and general population studies (Knox et al. 2011).

In an early occupational study (Olsen et al. 2003), PFOA or PFOS exposure was found to have no association with T3, T4 or TSH. The longitudinal analysis of three thyroid hormone measurements in 174 workers in the same occupational cohort also suggested no association between thyroid hormone and PFOA. A later occupational study of 552 employees in three plants reported modest associations of PFOA with T3 whereas no association with T4 or TSH (Olsen et al. 2007). Both community studies (Bloom et al. 2010; Emmett et al. 2006b) found no significant association between PFOA exposure and levels of TSH. The smaller one of these two studies also found that the level of free thyroxine was not significantly associated with PFAS (Bloom et al. 2010).

The correlations between thyroid disease and PFAS exposure have also been explored. Pirali et al. (2009) reported no significant association of intrathyroidal concentration of PFOA or PFOS with underlying thyroid disease when compared with controls. However, contradictory results were reported in a study from National Health and Nutrition Examination Survey (NHANES) data (Melzer et al. 2010), which found higher concentrations of serum PFOA and PFOS were associated with current thyroid disease in the U.S. general population.

Sex steroids modulate the homeostasis of thyroid hormone by affecting the clearance of thyroid-binding globulin (TBG) synthesized in the liver (Tahboub and Arafah 2009). Given that PFOA levels showed moderate gender differences with a longer half-life in men (Steenland et al. 2010a, b) and PFOS had a significantly inverse association with serum estradiol (Knox et al. 2011), it is an important issue

to address the gender difference in thyroid function in response to PFAS exposures. In the cross-sectional report, which was stratified by gender, it was found that both PFOA and PFOS levels had a significant association with thyroid disease in females whereas much less precise association was only found between PFOS and thyroid disease in males (Melzer et al. 2010).

Overall, a conclusion could not be drawn from the current available evidence due to the small sample size and self-reported system of data collection. More evidence is needed to clarify the mechanisms involved and provide solid interpretation regarding the role of PFAS in modulating thyroid hormones in humans.

7.5 Organ-Specific Metabolic Effects

In previous sections of this chapter, PFAS-induced metabolic effects were discussed at the systemic level. This subsection will specifically discuss organ-specific metabolic effects. The major organ systems to be covered include cardiovascular, skeletal muscle, liver, central nervous system and reproductive system.

7.5.1 Cardiovascular System

The cardiovascular system is one of the most important organ systems in a living organism and it is vulnerable to the metabolic effects of PFASs, mainly due to several characteristics: it is dependent on fatty acid metabolism; circulating fatty acids and cholesterol may have detrimental effects on cardiovascular tissue; and it is influenced by thyroid hormone.

Mature myocardium depends on fatty acid metabolism as the main energy source. To be more specific, fatty acid beta-oxidation is the major energy-production pathway in the heart; PPARα is expressed in the heart and regulates fatty acid metabolism (Barger and Kelly 2000). As described in other chapters, and in previous sections of this chapter, PFAS have the ability to interfere with the PPARα signaling pathway. As a result, the cardiovascular system could be affected by PFAS exposures due to metabolic disturbances of fatty acid metabolism. As PFOA has been associated with cardiovascular disease and peripheral arterial disease (Shankar et al. 2012), PFAS exposures could induce cardiovascular disturbances.

Thyroid hormone is important in metabolic regulation, energy expenditure and blood pressure regulation. The cardiovascular system is regulated by various neurohormonal factors including thyroid hormone. Since PFAS are known to interfere with thyroid hormone, it is possible that the cardiovascular system is affected secondary to the interference of the thyroid hormone signaling pathway. In a study by Curran et al. (2008), serum thyroid hormone levels were decreased in PFOS-treated rats, however, no apparent cardiovascular changes were observed. The lack

of significant cardiovascular changes suggests lack of direct cardiovascular effects associated with PFAS exposure.

Both experimental animal and human data indicate that PFAS exposures are associated with cardiotoxicity. In a study with chicken hatchlings, Jiang et al. (2012) demonstrated that developing chicken hearts were affected, with altered morphology and function, following developmental exposure to PFOA starting at a dose of 0.5 mg/kg, An additional study associated these changes with PPARα and BMP2 signaling pathways (Jiang et al. 2013). For human studies, it is more difficult to draw clear conclusions as several studies report opposite findings. As discussed previously, Shankar et al. (2012) demonstrated increased cardiovascular risk associated with PFAS exposure. However, Steenland et al. (2010a) indicated no clear association was established between PFAS exposure and cardiovascular risk. Therefore, additional investigations are needed to establish if PFAS exposure increases cardiotoxicity and the risk of cardiovascular disease.

7.5.2 Skeletal Muscle

Skeletal muscle guides most voluntary mechanic movements throughout the body, which expends a large amount of energy. The energy resources include glycogen and fatty acid. Thus, skeletal muscle, as a result of its dependence on energy resources, is a potential target of PFAS-induced metabolic effects. Moreover, skeletal muscle also plays important roles in metabolic regulation. For example, skeletal muscle dysfunction has significant impacts on insulin resistance.

Glycogen, a main energy resource for skeletal muscle function, is generated from metabolism of carbohydrates. PFAS are known to induce detrimental effects on glycogen metabolism, mainly glycogen deposition, hence affecting muscle function and metabolic homeostasis (Hagenaars et al. 2013; Ørtenblad et al. 2013). In certain circumstances, this effect could have severe outcomes. For example, in the late stage of chicken embryo development, the deposition of glycogen in hatching muscle is critical for successful hatching (Pulikanti et al. 2010). Exposure to PFAS has been reported to affect the homeostasis of glycogen deposition process (Hagenaars et al. 2013), thus the developmental toxicity of PFAS in egg laying organisms are potentially attributed to disrupted glycogen deposition.

Fatty acid metabolism is important especially in prolonged skeletal muscle work. PPARα expression levels are high in skeletal muscle, thus the disruption of PPARα signaling in skeletal muscle and downstream fatty acid beta-oxidation interruption are potential targets for PFAS. However, the evidence of the effect of PFAS exposure on fatty acids was largely limited to liver fatty acid metabolism. More studies are needed to examine the localized effect of PFAS on skeletal muscle fatty acid metabolism.

7.5.3 Liver

Liver is the primary organ of endogenous and exogenous substance metabolism. Carbohydrates, fatty acids and protein are all processed mainly in the liver, making it the major organ for metabolism of these substances. Due to the high level of PPARα expression in the liver, PFAS exposure may lead to profound hepatotoxicity.

PFAS are widely known to induce hepatomegaly and overexpression of fatty acid beta-oxidation-related genes both *in vitro* and in experimental animal models. For example, hepatomegaly has been observed following PFOA and PFOS exposure in SD rats (Cui et al. 2009). Kudo et al. (2006) also reported induction of hepatomegaly and peroxisomal beta-oxidation in rats. Carcinogenesis generally occurs following long-term and relatively large doses of PFAS, and is generally considered not applicable to humans (Post et al. 2012). However, the potential for carcinogenicity in liver should not be under-looked when evaluating potential health risks to humans.

Fatty acid metabolism is the major metabolic pathway being affected by PFAS exposure, raising the concern of fat deposition in the liver (fatty liver), as observed in Kudo and Kawashima (1997) and Tan et al. (2013). However, considering the doses of PFAS used in these studies (up to 10 mg/kg in Std:ddY mice in Kudo and Kawashima and up to 5 mg/kg in C57BL/6N mice in Tan et al.), it is highly unlikely that PFAS is a major risk associated with fatty acid metabolic effects in the liver of humans at current exposure levels.

In humans, hepatotoxicity is generally less prominent, mainly being detected as elevated cholesterol levels (discussed in previous sections) and an increase in biomarkers of liver damage. For example, Gallo et al. (2012) reported elevation of ALT following PFOS and PFOA exposure in an exposed population in West-Virginia and Ohio (the C8 project population). Frisbee et al. (2010) also reported that an increase in cholesterol levels were associated with higher circulating PFAS levels. This study suggested that metabolic effects of PFAS are not simply activation of PPARα and increased beta oxidation, as the latter should decrease cholesterol levels instead of increase, as demonstrated by these epidemiologic studies.

7.5.4 Reproductive System

To our knowledge, reproductive effects of PFAS exposure mainly impacts sex hormones. Experimental animal studies have demonstrated the association between PFOA exposure with increased estradiol levels and decreased testosterone levels (Lau et al. 2007). Three cross sectional occupational studies also investigated the relationship between hormones and serum levels of PFOA (Sakr et al. 2007a; Costa et al. 2009; Olsen et al. 1998). An early study by Olsen et al. (1998) reported results in male workers only. In this study, estradiol, 17-hydroxyprogesterone, prolactin and bound testosterone were measured and no significant association was found

between PFOA exposure and these hormones. However, the power of the study had been compromised by the fact that most of the population was in the two lowest exposure groups (<1 and <10 ng/ml) with only five subjects in the highest exposure group (>30 ng/ml). Sakr et al. (2007a) reported a sex-related association between PFOA exposure and sex hormones. In male subjects, a significant association of serum PFOA levels with both estradiol and testosterone was found. In contrast, no significant correlation was found for hormones in the 243 female subjects. Costa et al. (2009) reported the data of 56 workers in routine occupational surveillance, in which no association between serum PFOA and sex hormones (estradiol and testosterone) was observed. As far as PFOS is concerned, a significant inverse association between PFOS and serum estradiol was found in women age from 42 to 65 years old (Knox et al. 2011). Given the moderate sex difference in median PFOA levels (Steenland et al. 2009) and the significantly longer half life of PFOA in male rats, more studies with consideration of sex difference is needed to address the effects of PFAS exposure on sex hormones.

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Chapter 8
Developmental Toxicity

Barbara D. Abbott

Abstract This chapter provides an overview of the developmental toxicity resulting from exposure to perfluorinated alkyl acids (PFAAs). The majority of studies of PFAA-induced developmental toxicity have examined effects of perfluorooctane sulfonate (PFOS) or perfluorooctanoic acid (PFOA) and there is only limited information available for other members of this family of chemicals. In this chapter, there are separate overviews of the developmental toxicity of PFOS and PFOA, along with a summary of studies available for perfluorobutyrate (PFBA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA). In general, among the PFAAs that do produce developmental toxicity in one or more laboratory species, prenatal PFAA exposure in teratology studies typically does not result in major malformations and significant findings are often limited to the higher exposure levels. The postnatal effects in rats or mice exposed to PFAAs are typically increased mortality in the first hours or week after birth, effects on weight which may persist beyond weaning, delayed eye opening, abnormal mammary gland development, and liver hypertrophy. The role of peroxisome proliferator activated receptor-alpha (PPARα) in mediating developmental effects is discussed, including insights from genetically modified mice, PPARα knockout mice, and mice expressing the human PPARα gene. Pharmacokinetic issues are relevant to selecting an appropriate animal model for developmental studies and regarding the influence of rapid clearance on manifestation of developmental toxicity. Whether or not a particular PFAA will cause developmental toxicity depends on levels and timing of fetal exposure and is influenced by species and

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B.D. Abbott (✉)
Toxicity Assessment Division (B105-04), National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, 109 T.W. Alexander Dr, Research Triangle Park, NC 27711, USA
e-mail: Abbott.barbara@epa.gov

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_8

ED_002330_00119699-00212

gender specific pharmacokinetic characteristics that impact exposure of the conceptus throughout gestation and during the lactational period. Factors influencing the pharmacokinetics and developmental outcomes include chemical characteristics of a particular PFAA (carbon chain length, functional moiety – carboxylate or sulfonate), species specific characteristics (sex and species specific expression of particular transporters in the kidney that influence clearance), timing and level of exposure to the developing fetus, and ability of the PFAA to activate PPARα (human, mouse, and rat PPARα differ in responses to PFAA, carboxylates are more effective than sulfonates, and longer carbon chain PFAA are more potent than short chain PFAA). The expression and activation of PPARα is necessary for mediating developmental effects of PFOA and PFNA, but the early postnatal deaths caused by exposure to PFOS were not dependent on expression of PPARα.

Keywords Developmental toxicity • Teratology • Peroxisome proliferator activated receptor (PPAR) • Postnatal toxicity

8.1 Introduction

The developmental toxicity of perfluorinated alkyl acids (PFAAs) has been evaluated in several species, including fish, rats, mice, monkeys, and humans. The majority of the studies that evaluated the developmental effects of PFAAs focused on perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), although there are studies for a few of the other perfluorinated compounds, such as perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluorobutyrate (PFBA), perfluorobutane sulfonate (PFBS), and perfluorohexane sulfonate (PFHxS). A comprehensive review of the developmental toxicity of the PFAAs was published by Lau et al. (2004), and subsequently updated (Lau et al. 2007). Subsequent to the Society of Toxicology Contemporary Concepts in Toxicology Symposium "Perfluoroalkyl Acids and Related Chemistries: Toxicokinetics and Modes-of-Action Workshop", held in 2007, a report was published summarizing new information from presentations at that meeting, including developmental toxicity and potential roles of nuclear receptors in producing PFAA toxicity (Andersen et al. 2008). These reviews (Andersen et al. 2008; Lau et al. 2004, 2007), provide a thorough overview of what was known at that time regarding developmental toxicity of the perfluorinated compounds. This chapter integrates that information and provides a current overview of the developmental toxicity of PFOS and PFOA, followed by a summary of the available reports of developmental toxicity for other PFAAs. The role of the peroxisome proliferator activated receptor-alpha, (PPARα) in mediating PFAA-induced developmental toxicity is discussed, including the insights provided by use of genetically modified mice, both PPARα-null mice and mice expressing the human PPARα gene.

8.2 PFOS and N-Ethylperfluoroocatnesulfonamido) Ethyl Alcohol (N-EtFOSE)

Teratology studies, two-generation reproductive studies, and cross-foster studies have been conducted using PFOS and N-EtFOSE (Case et al. 2001; Christian et al. 1999a, b; Lau et al. 2003; Thibodeaux et al. 2003). In these studies, using rat, rabbit and mouse, there were typically few teratological findings, and these were generally only seen at higher doses. N-EtFOSE is metabolized and degrades in the environment to PFOS and generally gives results in these studies that are similar to PFOS. Case et al. (2001) conducted developmental toxicity studies of both PFOS and N-EtFOSE in rat and rabbit, using doses ranging from 1 to 20 mg/kg/day (rat, N-EtFOSE) and 0.1–3.75 mg/kg/day (rabbit, both compounds). At the highest doses, maternal toxicity (reduced feed consumption and body weight) was associated with increased abortions in rabbits (both compounds), increased late resorptions in rabbits (N-EtFOSE), and reduced fetal weights in rats (N-EtFOSE) and rabbits (PFOS). External, soft tissue, and skeletal fetal examinations did not reveal any malformations in either species. These findings were generally supported by a study of PFOS in mice and rats (Thibodeaux et al. 2003), in which malformations were reported only at the highest doses in both species. In that study, Sprague-Dawley rats were dosed orally at 1–10 mg/kg/day from gestation day (GD) 2-20 and CD-1 mice were dosed at 1–20 mg/kg/day from GD1-17, and serum and liver levels of PFOS increased with dose. Maternal weight gains were reduced in both rat and mouse, and small effects on fetal weight were noted in the rat. This study also reported increased liver weight in mouse dams, and effects on thyroid in the rat and mouse dams with reduced thyroxine (T_4) and triiodothyronine (T_3) with no change in thyroid-stimulating hormone (TSH). At the highest doses in rat and mouse, delayed ossification and edema were reported, along with several malformations including cleft palate and cardiac abnormalities (ventricular septal defect, and enlarged right atrium). The potential for PFOS to cause cleft palate was examined further using ICR mice dosed from GD1-17 and using palatal organ culture (Era et al. 2009). The incidence of cleft palate correlated with modest increases in PFOS in fetal serum, as doses to the dam increased from 13 to 20 mg/kg/day. Cleft palate induction was attributed to effects of PFOS that prevented elevation of palatal shelves above the tongue during palatogenesis.

Thibodeaux et al.'s (2003) paper was accompanied by a companion paper in which the postnatal effects of PFOS exposure were reported (Lau et al. 2003). Dams were exposed to PFOS using the same dosing regimen (described in the companion paper) and allowed to give birth. Pups were born alive, but those from dams in the highest dose groups (rats and mice) became pale, inactive, moribund, and died within hours of birth. Pups from the dams dosed at lower levels of PFOS (rats dosed at 5 mg/kg/day and mice dosed at 15 mg/kg/day), survived longer but over 95 % of those pups died within the first 24 h, and about half of offspring died from dams dosed at 3 mg/kg/day (rat) and 10 mg/kg/day (mouse). Cross-fostering the pups

exposed in utero to control dams at birth failed to change the outcomes. Among surviving pups, delayed eye opening, persistent lags in growth, and increased liver weight were noted. Serum T_3 and T_4 were decreased in the surviving rat pups, although TSH was not affected. An earlier two-generation reproductive study of N-EtFOSE in rats also reported reduced pup survival in the first 3 days of life and weight gain deficits among survivors (Christian et al. 1999a). The dramatic effects on postnatal survival were further supported by a reproductive study in rats (Luebker et al. 2005a), in which male and female rats were dosed orally with PFOS (0.1–3.2 mg/kg/day) across two generations (both sexes prior to mating, during mating, and females through gestation and lactation). PFOS exposure reduced body weight and feed consumption in both sexes of the parental generation (F_0) at 0.4 mg/kg/day and higher, and at the two higher doses there was substantial neonatal toxicity. There were no effects on reproductive performance, and no effects of PFOS were found on embryos examined on GD10. At the high dose, the length of gestation was decreased, the number of implantation sites was decreased, and there were increased numbers of stillborn pups and pups dying postnatally. The increased F_1 pup lethality, developmental delays, and decreased body weight gain were noted after a maternal dose of 1.6 mg/kg/day. A cross-foster study indicated that in utero exposure contributed to the postnatal pup mortality and that the combination of both pre- and postnatal exposure produced greater responses than either exposure period alone. Luebker et al. (2005b) followed up on these observations with a study designed to define the dose-response and evaluate pharmacokinetic and biochemical parameters, including measurement of serum lipids, glucose, mevalonic acid, thyroid hormones, milk cholesterol, liver lipids, pharmacokinetic parameters (serum and liver levels). Dams were dosed 6 weeks prior to mating through postnatal day (PND) 4 with doses ranging from 0.4 to 2.0 mg PFOS/kg/day. Gestation length and viability were decreased at 0.8 mg/kg/day and higher, but this was not correlated with effects on the biochemical measures. Transfer of PFOS from the dam to the fetus was confirmed and levels in the fetus correlated with postnatal lethality.

The reports of postnatal lethality after in utero exposure to PFOS were interesting, but an explanation for how this could be happening required studies that would focus on the symptoms exhibited soon after birth in those pups exposed to the higher doses of PFOS. Lau et al. (2003) and Thibodeaux et al. (2003) reported that pups became pale and inactive, and in a subsequent study, Grasty et al. (2003) further defined the prenatal window of susceptibility, reporting that the pup mortality could be produced solely by an exposure late in gestation. In that study, Sprague-Dawley rats were dosed orally with 25 mg/kg/day for four consecutive days across different gestational ranges of days, or at 25 or 50 mg/kg/day only on GD19-20. After 4 days of dosing, reduced maternal weight gain, food and water consumption, and pup weights were observed. Pup survival was reduced in all PFOS-exposed groups, and late gestational exposure (GD19-20 with exposure to 25 or 50 mg PFOS/kg/day) was sufficient to reduce survival to only 3 % of pups by PND5. Lung maturation is a key event during the late stages of gestation, and interference with lung development could impact functionality and subsequent survival. Grasty et al. 2003 study

revealed effects of prenatal PFOS exposure on the morphology of the fetal and neonatal lung. Based on histological differences and reduced expansion of the lungs (at necropsy, fetal or neonatal lungs were perfused via the trachea with formalin and the degree of expansion was noted to be less in PFOS-exposed offspring), it was proposed that effects on lung maturation or function of surfactant were involved in the neonatal deaths.

The hypothesis that effects on the lung were involved in postnatal mortality is consistent with observations of the distribution of PFOS in dams, fetuses, and pups in a study using whole-body autoradiography and liquid scintillation counting to localize and quantify the distribution of PFOS (Borg et al. 2010). C57Bl/6 mouse dams were injected on GD16 with ^{35}S-PFOS, 12.5 mg/kg, and distribution of PFOS was determined on GD18, GD20 and PND1. At GD18, PFOS levels were two to three times higher in fetal lungs, liver, and kidneys than in maternal blood, and by PND1 levels in neonatal lungs were much higher than in the GD18 fetuses. This evidence of distribution in substantial levels to fetal and neonatal lung, supports a proposed role for effects on that organ in the early mortality observed after birth. Further studies of effects of PFOS on lung maturation in the rat (Grasty et al. 2005), described increased thickness of the alveolar walls in the PFOS-exposed neonates (Sprague-Dawley rats exposed to 25 or 50 mg/kg/day on GD19-20) and the ratio of tissue to airway space increased. This was interpreted to indicate immaturity of the exposed lungs, with potential failure of alveoli to inflate properly with the onset of respiration at birth, potentially indicating interference with surfactant, which is essential for proper dilation of the alveoli. However, the study did not detect any effects of PFOS on surfactant phospholipid concentrations or molecular speciation, and analysis of gene expression related to alveolar differentiation revealed no differences from controls. It was further speculated that the surfactant properties of PFOS itself were interfering with surfactant function and this is supported by other evidence that PFOS and PFOA have the potential to interact with surfactant, based on interactions with dipalmitoylphosphatidylcholine (DPPC), one of the major components in surfactant, and the abilities of PFOS and PFOA at low concentrations to migrate from water into DPPC monolayers and bilayers changing fluidity and phase transitions (Gordon et al. 2007; Lehmler et al. 2006; Matyszewska et al. 2007; Xie et al. 2007a). The potential for PFOS to affect lung development was supported by a later study in ICR mice, dosed orally at 0.1–2 mg/kg/day from GD0-18, in which pups died within 24 h and abnormal lung histology was described as "atelectasis-like" (collapsed lung), (Yahia et al. 2008). This study also reported dilated intracranial blood vessels. Similarly, in Sprague-Dawley rats dosed orally with PFOS at 0.1 or 2 mg/kg/day from GD1-21, the lungs of the offspring at the high dose were reported to have severe histolopathological changes, oxidative injuries, and cell apoptosis, accompanied by altered expression of genes related to oxidative stress and cell death (Chen et al. 2012). Although additional studies are needed to clarify the mode of action for PFOS-induced developmental toxicity, the evidence supports a mechanism affecting lung function to explain the early deaths among newborn rodents exposed to PFOS during gestation (The potential role of PPARα is discussed in a separate section below).

8.3 PFOA

The developmental toxicity of PFOA has been extensively studied in multiple species, including monkeys, mice, fish, rats, rabbits, and humans (via epidemiological studies), and more information regarding these studies can be found in the reviews mentioned earlier (Andersen et al. 2008; Lau et al. 2004, 2007). The study of the developmental toxicity of PFOA has been complicated by the finding of sex and species differences in pharmacokinetics which results in major differences in time required to eliminate PFOA. Sex and species differences in elimination of PFOA are covered in detail in Chap. 6 (the chapter of this book dealing with pharmacokinetics), however, in order to discuss the developmental toxicity of PFOA and the most appropriate animal model for such studies, it is necessary to mention these issues. Briefly, the handling of PFOA by transporters in the kidney is of particular importance for explaining sex and species differences in elimination. The organic anion transporters (Oat) in the kidney move the perfluorinated carboxylates across membranes and are key players in renal elimination. There are multiple transporters involved and the specific sex and species dependent expression and the timing of acquisition of expression in the young animal are relevant to explaining differences in PFOA elimination (Buist et al. 2002; Buist and Klaassen 2004; Weaver et al. 2010). The half-life of PFOA elimination is estimated to be 3.8 years in humans with little difference between genders; in the rat, the half-life for elimination is 4–6 days in the male, but only 2–4 h in the female (Lau 2012). Of particular relevance to studies of developmental toxicity, the female rat eliminates PFOA very rapidly and the exposure of the conceptus is likely to be limited, making it less likely that developmental toxicity will be observed. The rabbit also exhibits a short half-life of elimination for PFOA, 7 h in the female and 5.5 h in the male, (Hundley et al. 2006). Mice present an appropriate model for studies of developmental effects of PFOA, as, similar to the case in humans, there is little difference between sexes in half-life of elimination. The half-life of PFOA in mice is 17 days in the female and 19 days in the male. With a gestational period in mice of approximately 20–21 days, it is likely that daily dosing during gestation will achieve continuous exposures throughout development and into the lactational period. Thus the mouse presents a gestational exposure model that would be similar to that expected in the human where the half-life of elimination is in years.

Considering the short half-life of PFOA in female rats and rabbits, it is not surprising that the early teratology studies in those species did not report any significant findings (Gortner 1982; Staples et al. 1984). A two-generation reproductive study of PFOA in the Sprague-Dawley rat with oral exposures of 1, 3, 10, or 30 mg/kg/day and beginning dosing of the parental generation at about 6 weeks prior to mating, showed few significant effects. There was decreased body weight and increased liver and kidney weight in males of the parental and F_1 generation at all exposures. High dose F_1 pups had decreased body weight at birth and preputial separation and vaginal opening were delayed, but there were no effects on later reproductive performance. No adverse effects for F_1 (mortality, weight, sexual

maturation) were observed at 10 mg/kg or below. At the 30 mg/kg level, mortality was observed in the F_1 pups after weaning and there was delay in achieving sexual maturity (males and females). The findings in the F_2 generation were unremarkable.

Study of developmental toxicity of PFOA in the mouse model gave a profile of effects that was reminiscent of outcomes observed following PFOS exposure in rats or mice. CD-1 mice dosed orally with PFOA at 1, 3, 5, 10, 20, or 40 mg/kg/day on GD1-17 revealed full-litter resorption at the high dose, effects on dam weight gain during pregnancy at the 20 mg/kg dose, and enlarged liver in the dams at all doses (Lau et al. 2006). Some dams were necropsied at GD18 and the remainder were allowed to give birth for postnatal observations. Among the GD18 dams, the percent of live fetuses and fetal weights was lower in the 20 mg/kg group, some structural abnormalities were seen in the fetuses, but no increase in malformations was found in any of the dose groups lower than 20 mg/kg. In the postnatal study, the incidence of live births and pup survival were lower in both the 10 and 20 mg/kg groups and survival was also decreased in the 5 mg/kg group. Growth deficits occurred in all but the 1 mg/kg group and delays in eye opening at 5 mg/kg or higher were observed. In a follow-up study from this laboratory, cross-foster experiments exposed the timed-pregnant CD-1 mouse to PFOA on GD1-17 at 0, 3, or 5 mg/kg/day (Wolf et al. 2007). At birth, pups were cross-fostered to create sets of pups that were exposed in utero only, lactationally only, both in utero and lactationally, or not exposed in either developmental period. Among the PFOA-treated dams, relative liver weight increased, but weight gain in pregnancy and litter size were unaffected. In utero exposure alone at 5 mg/kg group (no lactational exposure) was sufficient to produce the postnatal body weight deficit and delay in eye opening, but only the pups exposed to 5 mg/kg/day both in utero and lactationally had significantly reduced pup survival from birth to weaning. The study also included restricted windows of exposure from GD7-17, 10–17, or 13–17 at 5 mg/kg/day, or from GD15-17 at 20 mg/kg/day. Similar to the findings from the PFOS window-of-exposure studies, PFOA exposure late in gestation at a high level (GD15-17, 20 mg/kg) was sufficient to produce reduced survival at birth and reduced birth weight. The GD7-17 and 10–17 exposure at 5 mg/kg/day also reduced pup weight gain, and delayed eye opening and hair growth. The cross-foster and window of exposure studies indicated that, in a dose-related manner, exposure in utero was sufficient to produce developmental toxicity, that gestational and lactational exposure both contribute to the adverse effects, and that late gestational exposure was sufficient to affect survival.

Cross-foster and window-of-exposure studies in CD-1 mice, similar in design to those described for the developmental toxicity studies above, were conducted to evaluate effects of PFOA on mammary gland development. White et al. (2007) dosed CD-1 mice on GD1-17, 8-17, 12-17, at 0 or 5 mg/kg/day and examined mammary glands of dams and female pups at PND10 and 20. PFOA reduced body weights at birth that persisted until weaning. Effects on differentiation of the dam's mammary glands in the GD1-17 and 8-17 exposure groups were found on PND10, and on PND20 delays in epithelial involution and altered milk protein gene

expression were noted. All of the female pups exposed to PFOA in utero showed significantly delayed mammary epithelial branching and delayed mammary growth at both PND10 and 20. In a cross-foster study in which dams were dosed at 5 mg/kg/day on GD1-17 (White et al. 2009), both in utero only and lactational only exposures produced delays in mammary gland development detectable as early as PND1 and morphological effects that persisted beyond PND63. Exposures late in gestation on GD15-17 produced mammary gland effects similar to those observed after exposures occurring throughout gestation. As these studies did not identify a no-effect level for mammary gland outcomes, further studies examined exposures to lower levels of PFOA (Macon et al. 2011; White et al. 2011). CD-1 mice dosed orally from GD1-17 or 10-17 were treated with 0, 0.3, 1.0, 3.0 mg/kg/day or 0, 0.01, 0.1, or 1.0 mg/kg/day, respectively. Relative liver weights increased in all groups in offspring of the GD1-17 exposure and at 1.0 mg/kg in the GD10-17 exposure group. Mammary epithelial stunting was observed in all of the groups from both exposure paradigms. The results in the GD10-17 exposure group showed effects on mammary development at doses which did not affect liver weight. The mammary gland effects in the GD1-17 group persisted to 12 weeks of age, and again it was not possible to identify a no-effect level. In a transgenerational study (White et al. 2011), pregnant CD-1 mice were dosed during gestation (0 or 1.0 mg/kg/day on GD1-17) and their F_1 and F_2 offspring were exposed chronically via drinking water at 5 ppb. The F_1 offspring were bred to produce F_2 which were observed from PND1-63. Mammary glands of parental, F_1 and F_2 females were examined throughout the study. Gestational exposure induced delayed mammary gland development and chronic, low-dose exposure in drinking water also altered mammary gland morphology. It should be noted, that in this animal model, the morphological effects on the F1 female's mammary glands did not impair nutritional support of offspring as there was no effect on growth of the pups of these females, suggesting that lactational nutrition was adequate. These studies identified mammary gland as a developing system that is highly sensitive to PFOA exposure, demonstrating responsiveness at exposures below those required to affect liver weight changes, and further showed that there was not substantive recovery as these morphological effects persisted to later life stages.

8.4 Peroxisome Proliferator-Activated Receptor Alpha (PPARα)

The perfluorinated compounds are known activators of peroxisome proliferation and this effect is well characterized in the liver. The induction of peroxisomes in response to PFAA exposures is a direct response to activation of the PPARα receptor. Activation of PPARα by PFOA is considered a key event in the mode-of-action of PFOA-induced liver tumors in the rat (Kennedy et al. 2004; Klaunig et al. 2003). The PPARα pathway is also an important regulator of lipid and glucose homeostasis, regulating inflammation, cell proliferation and differentiation (Escher and Wahli

2000). Since PFOA was known to act through PPARα to produce effects in the liver, it was relevant to determine the role of the pathway in PFAA-induced developmental toxicity. PPARα knockout (KO) mice (transgenic mice on the 129S4/SvJae background were genetically altered to no longer express PPARα) and wild type (WT) mice (on the genetically similar 129S1/SvlmJ background) were used to test the involvement of the PPARα pathway after exposure to PFOA, PFOS, and PFNA (Abbott et al. 2007, 2009; Wolf et al. 2010).

Pregnant WT and KO mice were dosed orally with PFOA on GD1-17 at 0, 0.1, 0.3, 0.6, 1, 3, 5, 10, or 20 mg/kg/day. In WT and KO mice, PFOA at 5 mg/kg and above resulted in full-litter resorptions in all dams, suggesting that these strains of mice were more sensitive than the CD-1 mice (which did not have full-litter resorptions at 5 mg/kg/day), and that a PPARα-independent mechanism was responsible for the early loss of the embryos at high doses. However, PPARα was required for PFOA to produce postnatal lethality (Abbott et al. 2007), as WT litters exposed to 0.6 or 1 mg/kg/day had only 43 % of pups survived to weaning, whereas survival in KO litters was unaffected at up to 3 mg/kg/day. Pups heterozygous for PPARα expression that were born to either WT or KO dams experienced increased incidences of postnatal mortality after PFOA exposure in utero. Thus, maternal factors, such as background genetics or potential effects of PPARα KO on the dam, were not involved and expression of only one copy of the PPARα gene was sufficient to make pups susceptible to PFOA-induced postnatal mortality. Delays in eye opening and reduced postnatal pup weight also occurred in WT PFOA-exposed litters and these effects also appeared to depend on PPARα expression. However, it is possible that other factors contribute to these outcomes, as there was a slight effect on these endpoints in the KO at 3 mg/kg.

Developmental toxicity after exposure to PFNA was also examined in the WT and PPARα KO mice using the approach described above for PFOA (Wolf et al. 2010). WT and KO mice were dosed orally with PFNA on GD1-18 at 0.83, 1.1, 1.5, or 2 mg/kg/day. In WT litters, PFNA reduced the number of live pups at birth and decreased survival to weaning in the 1.1 and 2 mg/kg groups. Delayed eye opening and decreased pup weights were also seen at 2 mg/kg. KO litters did not have reduced survival or effects on pup weight or developmental delay. It was clear that, as with PFOA, the developmental toxicity of PFNA was mediated by activation of PPARα and in the absence of expression of that receptor there was no effect on survival, pup weight, or eye opening.

This was not the case for the developmental toxicity induced by PFOS. Exposure to PFOS in utero resulted in neonatal deaths in both the WT and KO litters. The protocol for testing PFOS in the WT and KO differed from that described for PFOA and PFNA, as the fetuses were only exposed later in gestation and postnatal survival was monitored from PND1-15. As it was previously shown that PFOS could induce excessive postnatal mortality with exposures only in the latter stages of development in rats, the WT and KO mice were dosed orally with PFOS from GD15-18 at 0, 4.5, 6.5, 8.5, or 10.5 mg/kg/day (WT) or 0, 8.5, or 10.5 mg/kg/day (PPARα KO). Survival was decreased in both WT (4.5, 8.5, and 10.5 mg/kg/day) and KO (8.5 and 10.5 mg/kg/day) litters and eye opening was delayed in the 8.5 mg/kg WT group

and the 10.5 mg/kg KO group. The effects in the WT may have been more pronounced compared to KO; among WT litters exposed to 10.5 mg/kg/day only 26 % of the pups were alive on PND15, but 62 % survived to PND15 in the KO litters exposed at this dose. The effects of PFOS on survival in the KO litters indicated that expression of PPARα was not required to mediate this response. This strongly suggests that the mechanism leading to early postnatal deaths after exposure to PFOS differs from that occurring after exposure to either PFOA or PFNA and may indeed involve effects of PFOS on the function of surfactant in the lung of the neonate. Although both PFOA and PFOS are surfactants with the ability to partition into DPPC layers (as mentioned in the PFOS discussion), experimental evidence suggests that PFOS is more likely to be disruptive, with a partition coefficient about four times that of PFOA (Matyszewska et al. 2007; Xie et al. 2007b). The linkage between distribution of PFOS to the fetal/neonatal lung and the potential for PFOS to interfere with surfactant function are the current explanations for the induction of lethality that is independent of PPARα expression.

The studies in the PPARα KO mice are clear in demonstrating that for PFOA and PFNA the postnatal developmental toxicity is mediated via activation of the PPARα pathway. This is further supported by studies showing that PPAR receptors are expressed in developing embryos and embryonic/fetal tissues are capable of responding to perfluorinated chemical exposures with altered gene expression (Abbott 2009; Abbott et al. 2012; Rosen et al. 2007, 2009). All of the three PPAR receptor isoforms (alpha, beta, and gamma) are expressed in specific patterns in developing organ systems from early prenatal stages in the mouse, human, and rat (Abbott 2009). In a study of gene expression, PFOA exposure altered expression of PPARα-regulated genes in multiple developing organs of the CD-1 mouse fetus (Abbott et al. 2012). CD-1 mouse embryos were exposed to PFOA during gestation (pregnant mice dosed orally with PFOA at 5 mg/kg/day from GD1-17) and profiles across time for expression of the PPAR receptors and genes regulated by nuclear receptors were described for multiple organs at prenatal (GD14, GD17) and postnatal (PND1, 7, 14, 21, 28) time points. PFOA exposure altered gene expression as early as GD14 in liver and heart and effects on genes regulated by PPARα were found in kidney, intestine, stomach, lung, adrenal, spleen, and thymus across the pre- and postnatal ages. Clearly, the PPAR pathway is present in developing organ systems and capable of responding to perfluorinated chemicals that activate PPARα.

8.5 Other PFAAs: Developmental Toxicity Studies

The interactions of PFAAs with nuclear receptors is covered in Chap. 13 and will be dealt with in much more detail in that chapter. However, in order to discuss the developmental toxicity of PFAAs, other than PFOS and PFOA, it is helpful to briefly comment on the ability of members of the PFAA family of compounds to activate PPARα. A number of in vitro studies have established that PFAAs are able to activate PPARα and that the responsiveness of both human and mouse PPARα

depends on the carbon chain length (shorter are less active that longer, e.g., PFBA is less active than PFOA), the functional moiety (carboxylates are more active than sulfonates), and the species (human PPARα being less active than mouse PPARα), (Wolf et al. 2008, 2012). Similar profiles of potency were shown in mouse and human hepatocytes, based on effects of PFAAs on gene expression (Rosen et al. 2013). The ranking of the compounds' ability to activate PPARα seems to parallel the potential for the various members of the PFAA family to cause developmental toxicity. The two shorter carbon chain compounds, PFBA and PFBS, have very short half-lives of elimination, show little activation of PPARα in the in vitro assays (requiring high concentrations), and appear to have few developmental effects and those were noted mainly at high doses. The study of PFBA in CD-1 mice administered oral doses from GD1-17 at 0, 35, 175, or 350 mg/kg/day (Das et al. 2008). The highest dose resulted in significant increases in full-litter resorption and increased maternal liver weights, but neonatal survival and postnatal growth were unaffected. Eye opening was delayed in all PFBA dose groups and onset of puberty was delayed in the two highest dose groups. The general lack of developmental toxicity except at the highest doses was attributed to the rapid elimination of the chemical in the dams. A two-generation study of PFBS in the rat using doses from 30 to 1,000 mg/kg did not find any significant effects on fertility or reproduction in the parental and F_1 generation (Lieder et al. 2009). Postnatal survival, development, and growth of the pups was unaffected in the F_1 and F_2 generations, with the exception of a slight delay in onset of puberty and weight gain for F_1 males in the highest dose group. As with PFBA, this may be related to rapid elimination of the compound in the dam.

The developmental toxicity of perfluorohexanoic acid (PFHxA) was recently studied in Crl:CD1 (ICR) mice (Iwai and Hoberman 2014). After exposure from GD6-18 to doses ranging from 100 to 500 mg/kg/day, maternal mortality was noted at 350 and 500 mg/kg/day and pup weights at birth were reduced in this dose group. Stillbirths, reduced viability of newborn, delayed development, and reduced pup weights were noted at 175, 300, and 500 mg/kg/day. In a developmental study in rats, oral gavage of PFHxA on GD6-20 at 20, 100, or 500 mg/kg/day, produced maternal toxicity (reduction in body weight and weight gain from GD6-21) at 500 mg/kg and the only developmental toxicity was a reduction in fetal weights at that dose (Loveless et al. 2009). The relative lack of developmental toxicity at non-maternally toxic doses, correlates with a rapid elimination of PFHxA in rats and mice (Gannon et al. 2011). In fact the half-life for elimination in rats (approximately 2 h in male and female rat) appeared to be the same or even shorter than that found for PFBS (3.1 h in males and 2.4 h in female rats) (Chengelis et al. 2009).

In vitro assays for activation of PPARα, predicted lower potency of the sulfonates relative to the carboxylates and lower activity for shorter chain compounds, such as PFHxS, relative to the C8 compounds PFOA and PFOS. The rapid elimination of PFHxS in female rats would also be a major factor influencing whether or not PFHxS produced developmental effects in that species. The female rat has a serum half-life of elimination of about 2 days, compared to a month in male rats and mice of both sexes (Sundstrom et al. 2012). In a one-generation reproductive and developmental toxicity study in rats with oral doses up to 10 mg/kg, there was a

general lack of developmental toxicity (York 2003). In that study no effect on fertility or reproductive outcomes was found and no effects were found for viability or growth of the pups.

A small amount of information is available regarding the developmental toxicity of two of the longer chain carboxylate compounds, PFNA and PFDA. In a teratology study of PFDA, Harris and Birnbaum (1989) dosed mice orally with PFDA in corn oil from GD6-15 (0.03–12.8 mg/kg/day) or 10-13 (0.25–32 mg/kg/day) and reported no malformations in the fetuses on GD18, but found increased fetal deaths and decreased live fetal weight at the high doses that were maternally toxic. As this study did not include postnatal observations, it is not known if PFDA would have affected postnatal survival or growth. PFNA has pharmacokinetic features similar to those described for PFOA, with a sex difference in the elimination of the compound in the rat where there is a serum half-life of 30.6 days in male rats and more rapid elimination (1.4 days) in the female rat (Tatum-Gibbs et al. 2011). This difference is less evident in mice, where males and females have a similar half-life of elimination and Tatum-Gibbs also noted that PFNA is more persistent in the mouse compared to the rat. PFNA is also a potent activator of PPARα in the in vitro reporter assays. These attributes suggest that PFNA would cause developmental toxicity in the mouse. In the previously described WT and PPAR KO study this was the case, with increased neonatal deaths and other effects in the WT, but an absence of effects in the PPARα KO. Also, in a study with CD-1 mice, oral dosing with PFNA from GD1-17 at 1, 3, or 5 mg/kg/day resulted in effects on postnatal survival with 80 % of the neonates in the 5 mg/kg group dead within 10 days of birth, delays in eye opening, and delayed onset of puberty (Lau et al. 2009; Das et al. 2015).

Although there is a lack of information regarding the developmental toxicity of most of the perfluorinated compounds, with PFOS and PFOA being the most studied, the information for PFBA, PFBS, PFHxS, and PFNA suggest that pharmacokinetics and potency for activation of PPARα are important factors in determining the developmental toxicity of a perfluorinated compound. Those with fast elimination and relatively low potency for activating PPARα showed lesser capacity for developmental effects. Also the in vitro assays predicted that these PFAAs were not as capable of activating the human PPARα reporter. This prediction is supported by studies using humanized mice (hPPARα), that express the human (and not the mouse) PPARα gene (Albrecht et al. 2013). Wild type (WT), PPARα KO, and hPPARα mice were dosed orally with PFOA from GD1-17 at 0 or 3 mg/kg/day. Postnatal survival was affected in WT, but not in PPARα KO or hPPARα mice. At the 3 mg/kg/day dose no effects were found in weight gain, eye opening, or mammary gland development in any of the genotypes. However, at weaning relative liver weight was increased in WT, but not in PPARα KO or hPPARα mice. Although inconsistent with other reports for effects in mice at this dose (likely due to differences in mouse strain), the endpoints with effects suggest that human and mouse PPARα respond differently to PFOA such that at the same level of exposure the pups expressing mouse PPARα experience developmental toxicity and those with a human PPARα do not have adverse effects. It is possible that these humanized mice

could respond at higher doses or that the model does not provide all of the components of the pathway required for a human PPARα response, but the lack of a response in hPPARα mice is in alignment with lesser responses of human PPARα in the in vitro reporter assays (Wolf et al. 2008, 2012).

8.6 Summation

The ability of PFAAs to induce developmental toxicity depends on levels and timing of fetal exposure and is influenced by species and sex specific pharmacokinetic characteristics that impact exposure of the conceptus throughout gestation and during the lactational period. Factors influencing the pharmacokinetics and developmental outcomes include chemical characteristics of a particular PFAA (carbon chain length, functional moiety – carboxylate or sulfonate), species specific characteristics (sex and species specific expression of particular transporters in the kidney that influence clearance), timing and level of exposure to the developing fetus, and ability of the PFAA to activate PPARα. In vitro studies show that human, mouse, and rat PPARα are not equivalent in their responses to PFAAs, carboxylates being more effective that sulfonates, and longer carbon chain PFAAs more potent that short chain PFAAs. The expression and activation of PPARα were shown to be necessary for mediating developmental effects of PFOA and PFNA, but the early postnatal deaths caused by exposure to PFOS were not dependent on expression of PPARα. In general, among the PFAAs that do produce developmental toxicity in one or more laboratory species, prenatal PFAA exposure in teratology studies typically does not result in major malformations and significant findings are often limited to higher exposure levels that result in maternal toxicity as well. The postnatal effects in rats or mice exposed to PFAAs that cause developmental toxicity are typically increased mortality in the first hours or week after birth, effects on weight which may persist beyond weaning, delayed eye opening, potential for delayed puberty, abnormal mammary gland development, and liver hypertrophy.

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Chapter 9
Neurotoxicity

Henrik Viberg and Espen Mariussen

Abstract The developing central nervous system is susceptible to exposure to many different classes of chemicals and environmental pollutants and this is also true for the PFCs. In epidemiological studies it has been seen that kids from mothers with high PFOS and PFOA concentrations show delayed motor and cognitive development and the prevalence of ADHD is higher in these children. The epidemiological findings are supported by several studies in laboratory animals, where it has been seen that PFOS, PFOA and PFHxS exposures during the gestational period increased the locomotor activity and caused an inability to habituate to new environments. These chemicals also affects molecular targets in the brain of test animals after gestational exposure and in the newborn period and the cholinergic system may be a possible target for the PFCs. Also in cell culture studies PFCs have been shown to be neurotoxic and affect different subtypes of PKC, strengthening the animal studies. All these possible effects of PFCs are similar to what earlier have been seen for PCBs and PBDEs and there may be possible problems with co-exposures from these different groups of chemicals.

Keywords Central nervous system • Brain development • Behavioral toxicity • Neuromotor maturation • Calcium-dependent signaling

H. Viberg (✉)
Department of Organismal Biology, Division of Environmental Toxicology, Uppsala University, Norbyvägen 18A, 75236 Uppsala, Sweden
e-mail: henrik.viberg@ebc.uu.se

E. Mariussen
Protection and Societal Security Division, Norwegian Defence Research Establishment (FFI), P.O. Box 25, NO-2027 Kjeller, Norway
e-mail: espen.mariussen@ffi.no

© Springer International Publishing Switzerland 2015

J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_9

ED_002330_00119699-00228

Abbreviations

FTOH	Fluorotelomer alcohol
PFBA	Perfluorobutyric acid
PFBS	Perfluorobutane sulfonic acid
PFC	Perfluoroalkylated compounds
PFDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHSor PFHxS	Perfluorohexanesulfonic acid
PFHxA	Perfluorohexanoic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOC	1H-perfluorooctane
PFOS	Perfluorooctanesulfonic acid
PFOSA	Perfluorooctanesulfonamide
PFPA	Perfluoropropionic acid
PFTA	Perfluorotetradecanoic acid
TFAA	Trifluoroacetic acid

9.1 Introduction and Background

From the fertilization of the egg, through gestation and during the first years after birth, the brain is subjected to a continuously development and disturbances during susceptible periods can induce many different types of negative alterations. Transfer of xenobiotics occurs from the mother to the fetus through the umbilical cord, via mother's milk to the newborn and via direct inhalation and ingestion to the newborn and toddler. Knowledge about the exposure situation in fetuses, newborns, toddlers in addition to adults is therefore important in order to predict toxic effects. Exposure to environmental contaminants have been suspected as agents for an increased prevalence of attention deficit hyperactivity disorder (ADHD) and susceptibility of dementia disorders, such as Parkinson's disease (Barkley 1998; Brown et al. 2005; Hardell et al. 2002; Hoffman et al. 2010; Lai et al. 2002; Rice 2000; Schettler 2001). It has been hypothesized that environmental contaminants can affect cognitive functions, such as learning and behavior, and motor skills (Grandjean and Landrigan 2006; Grandjean et al. 2014; Mariussen and Fonnum 2006). Human exposures to PFCs are reviewed in Part I of this book, but it is worth repeating that certain tissues and fluids are of extra interest concerning the possible developmental neurotoxic effects of PFCs. Generally it can be said that the concentrations of PFCs are significantly higher in maternal serum than in umbilical cord serum (Apelberg et al. 2007; Inoue et al. 2004; Midasch et al. 2007; Monroy et al. 2008). Unlike PFOS, PFOA appears to cross the placental barrier unhindered (Midasch et al. 2007). Only a few studies have analyzed the levels of PFCs in brain tissue. The PFC found in the

highest concentration in brain tissue is PFOS, followed by PFOA, which probably reflects their historical use, persistency and accumulation potential, and rate of elimination (Mariussen 2012). In general the levels of the PFCs are lower in the brain than in other organs, such as the liver and kidney, and even in developmentally exposed laboratory animals (Kawamoto et al. 2011; Mariussen 2012; Sato et al. 2009). Both the adult and developing brain are protected by the so-called blood brain barrier (BBB) reducing the access of both exogenous and endogenous compounds into the brain (Ek et al. 2012). The properties of PFCs as surfactants may, however, modulate the membrane fluidity of the cells (Levitt and Liss 1986) and there are *in vitro* studies implicating that PFOS can increase the permeability of the blood brain barrier. Wang and co-workers showed that PFOS induced disassembly of endothelial tight junctions in brain endothelial cells (Wang et al. 2011) and later it was also observed that PFOS reduced mRNA expression of cellular adhesion markers in neuronal cells (Choi et al. 2013). Newborns, toddlers and children are the most exposed part of the population, on a body weight basis, since they tend to inhale and ingest more than the adult population (Trudel et al. 2008). Furthermore, it is a well-known fact that many environmental pollutants end up in the mother's milk, exposing the nursing neonate to a cocktail of chemicals, including several of the PFCs, such as PFOS, PFOA, PFHxS, PFBA, PFHxA, PFHpA and PFNA (Antignac et al. 2013; Mondal et al. 2014; Sundstrom et al. 2011). The concentrations of the PFCs in breast milk tend to be highest in mothers who is nursing for the first time compared to mothers who have previously nursed (Tao et al. 2008). One study have compared concentrations of PFOS between the adult and juvenile brain showing a higher relative concentration in brain of the rat fetuses compared with the brains from their mothers (Chang et al. 2009), indicating that the BBB of the fetus has increased permeability of PFOS. Bearing in mind that the PFCs are only one group of toxicants reaching potential targets in the brain it is of importance to both screen the extent of exposure and to evaluate their hazardous potential.

9.2 Epidemiological Studies

It is always hard to study toxicological effects in humans especially when it comes to reproductive and developmental effects. Despite that, effort has been put into epidemiological studies to investigate if there are connections or correlations between levels/concentrations of PFCs in maternal serum, umbilical cord serum and birth weight, size and other markers of development in humans. This is summarized in Chap. 14, but some of the effects seen in these epidemiological studies indicate that PFCs can contribute to developmental neurotoxic effects in human. In 2007, reports came from Maryland, U.S., that both PFOS and PFOA concentrations in umbilical cord serum were negatively associated with birth weight and head circumference. When looking at maternal concentrations of PFOS and PFOA in relation to motor and mental developmental in children, it can be seen that children from mothers with high PFOS concentration are slightly delayed in time of sitting

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without support (Fei et al. 2008) and also self-reported birth defects were associated with high PFOA exposures (Stein et al. 2009). The same research group used the same cohort to look into the correlation between the serum levels of PFCs in children and teenagers and the prevalence of ADHD, and found indications that high levels of PFCs, especially PFOA and possibly also PFHxS could be factors behind the induction of ADHD (Stein and Savitz 2011). Higher risk of ADHD in relation to PFC levels has also been proposed by other researchers and in that study it was not only the usual suspects mentioned, but also PFNA (Hoffman et al. 2010).

9.3 Animal Studies (*In Vivo* and *Ex Vivo* Studies)

Despite few studies it is plausible to believe that PFCs have toxic effects in humans, even though there are limited methods of measuring both exposure and effects. Instead animal studies are used to investigate neurotoxicity and there is a number of different experimental methods that can measure a variety of endpoints from several different exposure paradigms, with different doses, in several different species. Results generated from animal studies can be used to extrapolate and predict human toxicity and are therefore of great importance. Generally, it can be said that PFC neurotoxicity (mainly PFOS and PFOA) have been studied in all types of animals including fishes, birds and mammals, but the vast majority of studies have been done on rodents (Table 9.1). Usually, in order to exert an effect a compound has to be present in the target organ. In the section about the toxicokinetics of PFCs it was described that these compounds can reach the brain, both during development and in adults, which indicates that neurotoxic effects may arise. There are several known neurotoxic effects of PFCs and here we will look into some of them, starting with neuropsychiatric and neuromotoric effects. Effects that can be linked to the above mentioned epidemiological findings. An overview of studies on neurobehavioral effects of different PFCs are, in addition, presented in Table 9.1.

PFOS exposure in mice during the gestational period (6 mg/kg bw/day) delayed a couple of landmarks of neuromotor maturation, such as decreased resistance to backward pull on postnatal days 10 and 11 and decreased climb ability and forelimb strength on postnatal day 11. These effects were transient and not seen later during the postnatal period (Fuentes et al. 2007b). In a more recent study dams were exposed to different doses (0.1, 0.3, and 1.0 mg/kg bw/day) of PFOS from gestational day 0 through postnatal day 20. PFOS treatments had no effect during the postnatal period when looking at the auditory startle response and learning and memory in a swim maze. However, locomotor activity increased in PFOS treated animals (0.3 and 1.0 mg/kg bw/day) on postnatal day 17, which ultimately leads to the inability of the animals to habituate to the novel test environment (Butenhoff et al. 2009a).

The behavioral effects of PFC exposure, such as negative impact on memory, learning, and motor functions, may involve effects on several neurochemical targets. A major challenge is to link the behavioral effects to changes in the nervous tissue,

Table 9.1 Summary of neurobehavioral studies of PFCs* on rodents, birds and fish

Animal	Exposure and chemical	Behavioral effects	References
Neonatal rats	Dams exposed orally to 3 mg/kg PFOS*, from GD 2 to GD 21	No effects	Lau et al. (2003)
Neonatal rats	Dams exposed orally to 0.1, 0.4, 1.6 and 2 mg/kg/day PFOS 42 days prior to mating until lactation day 20	Delay in surface and air righting among offspring in the 1.6 mg/kg group	Luebker et al. (2005)
Neonatal rats	Dams exposed orally to 0.1, 0.3 and 1.0 mg/kg PFOS from GD 0 to PND 20	Male displayed increased motor activity and reduced habituation in high dose group	Butenhoff et al. (2009a)
Adult rats	Oral exposure to 0.3, 1, 3 and 10 mg/kg PFHxS for 40–50 days	No effects in the functional observational battery or motor activity	Butenhoff et al. (2009b)
Adult rats	Oral exposure to 0, 6, 30 and 150 mg/kg/day PFBA for 28 days	No effects in hearing, static rightning, grip strength or motor activity. Delayed pupillary reflex in the high dose group	Butenhoff et al. (2012)
Adult rats	Oral exposure to 0, 1.2, 6 and 30 mg/kg/day PFBA for 90 days	No effects in hearing, static rightning, grip strength or motor activity. Delay in pupillary reflex in the high dose group	Butenhoff et al. (2012)
Adult rat	Oral exposure to 30 mg/kg/day PFOA for 28 days	Small decrease in motor activity	Butenhoff et al. (2012)
Adult male mice	Adult exposure by gavage to 3 and 6 mg/kg/day PFOS for 4 weeks	Small effect on activity in open-field and on retention tests	Fuentes et al. (2007a)
Neonatal mice	Dams exposed orally to 6 mg/kg/day PFOS from GD 12 to 18	Delayed neuromotor maturation	Fuentes et al. (2007b)
Neonatal mice	Dams exposed orally to 6 mg/kg/day PFOS from GD 12 to 18	Combination of PFOS and restraint stress reduced mobility in the open-field test	Fuentes et al. (2007c)
Neonatal male mice	Mice exposed to a single oral dose of 0.75 and 11.3 mg/kg PFOS at PND 10	Effects on spontaneous behavior and habituation in 2 – and 4 month old mice in the high dose group	Johansson et al. (2008)
Neonatal male mice	Mice exposed to a single oral dose of 0.58 and 8.7 mg/kg PFOA at PND 10	Effects on spontaneous behavior and habituation in 2- and 4 month old mice in all the groups	Johansson et al. (2008)
Neonatal male mice	Mice exposed to a single oral dose of 0.72 and 10.8 mg/kg PFDA at PND 10	No effects	Johansson et al. (2008)

(continued)

Table 9.1 (continued)

Animal	Exposure and chemical	Behavioral effects	References
Neonatal mice	Dams exposed orally to 6 mg/kg/day PFOS from GD 12 to 18. Half of the exposed animals were attributed to restraint stress	Prenatally exposed to mice spent more time in the center of the open-field device. Stress counteracted the effect	Ribes et al. (2010)
Neonatal male and female mice	Mice exposed to a single oral dose of 0.61, 6.1 and 9.2 mg/kg PFHxS at PND 10	Effects on spontaneous behavior in high dose group in 2 and 4 month mice	Viberg et al. (2013)
Chicken	Fertilized chicken eggs injected on embryonic day 0–5 mg/kg or 10 mg/kg PFOS	Reduced imprinting performance at hatching day 1	Pinkas et al. (2010)
Chicken	Fertilized chicken eggs injected on embryonic day 0–5 mg/kg or 10 mg/kg PFOS	Reduced imprinting performance at hatching day 1	Pinkas et al. (2010)
Zebrafish	0.5 μM PFOS in water	Effects on swimming speed after stimulus	Chen et al. (2013)
Zebrafish larvae	0.03–3,000 mg/L of TFAA, PFBA, PFOA, PFNA, PFDA, PFBS, PFOS	Effects on locomotor behavior. Longer carbon chain PFCs were more potent than shorter chain PFCs. PFOS most potent	Ulhaq et al. (2013)
Zebrafish larvae	0.1 and 1 mg/L PFOS	Persistent hyperactivity in the high dose group	Spulber et al. (2014)

**PFBA* perfluorobutyric acid, *PFOA* perfluorooctanoic acid, *PFNA* Perfluorononanoic acid, *PFDA* perfluorododecanoic acid, *PFBS* perfluorobutane sulfonic acid, *PFHxS* perfluorohexanesulfonic acid, *PFOS* perfluorooctanesulfonic acid, *TFAA* Trifluoroacetic acid

which often probably result from effects on several neurochemical targets. In some of the studies of which neurobehavioral effects of PFCs have been elucidated, efforts have been performed to reveal neurochemical effects *ex vivo* (Table 9.2). In a series of studies it has been shown that different PFCs may affect spontaneous behavior and cognitive functions after administration of a single dose at specific time points (Johansson et al. 2008, 2009; Viberg et al. 2013). Here the spontaneous behavior, locomotion (horizontal movement), rearing (vertical movement) and total activity, was measured for an hour. In the beginning of the 60-min test period the activity was decreased in animals exposed to PFOS, PFOA, and PFHxS, but in the end these animals had not habituated to the novel environment and the activity was higher than in the control animals. This type of behavior was observed both in 2 and 4 months old animals and these behavioral effects were persistent and actually worsened with age. A fourth perfluorinated compound, PFDA (perfluorodecanoic acid) had no effects on adult behavior. So not all PFC have the potency to induce behavioral and cognitive disturbances. When looking at the neurochemical targets it was showed that mice exposed to single doses of PFOS (11.3 mg/kg), PFOA

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Table 9.2 Summary of *ex vivo* studies of PFCs*

Animal	Chemical and concentrations	Effects	Ref
Mice and rats	Peroral adult exposure to one single dose to 125, 250 and 500 mg/kg PFOS*	No effects on brain neurotransmitter levels of norepinephrine, dopamine, serotonin, glutamate, GABA or glycine, 24 and 48 h after exposure of 250 mg/kg. No brain histopathological changes detected	Sato et al. (2009)
Rats	Maternal peroral exposure to 3.2 mg/kg PFOS in food from GD 1 to PND 35. Pups exposed after weaning (PND21) to PND 35 by cross-fostering model	Effects on mRNA expression of calcium related signalling molecules (NR2B, CaM, CaMKIIα, CREB). At PND 1 an increase in NR2B, CaM, CaMKIIα. At PND 7 increase in CREB. At PND 35 a decrease in NR2B	Liu et al. (2010a)
Rats	Maternal peroral exposure to 3.2 mg/kg PFOS in food from GD 1 to PND 21. Pups exposed to 3.2 mg/kg in food to PND35	At PND 1 and 7 micro-arrays study showed effects on genes involved in neurodevelopment and synaptic plasticity (ligand receptor interaction, calcium signalling, cell communication, long term potentiation/depression). Less effects on PND 35	Wang et al. (2010)
Rats	0, 2, 8 and 32 and 128 ppm PFOS in the diet for 13 weeks (approximately 0.12, 0.5, 2.1 and 8.5 mg/kg/day)	No brain histopathological changes detected	Kawamoto et al. (2011)
Rats	Maternal peroral exposure to 0.1, 0.6 and 2.0 mg/kg/day PFOS from gestational day (GD) 0–20	Dose dependent decrease in mRNA expression of synaptophysin and synapsin (Syn1 and 2) in hippocampus in pups at postnatal day 0 (PND 0) and 21. Ultrastructural changes in hippocampus at PND 21	Zeng et al. (2011a)
Rats	Maternal peroral exposure to 0.1, 0.6 and 2.0 mg/kg/day PFOS from gestational day (GD) 0–20	At PND 0 and 21 an increase in glial brain fibrillary acidic protein and S100 calcium binding protein B. An increased mRNA expression of TNF-α, IL-1β, AP-1, CREB, NF-kappa-B. A reduction in brain synapsin and synaptophysin	Zeng et al. (2011b)
Rats	Maternal peroral exposure to 3.2 mg/kg PFOS in food from GD 1 to PND 7	Reduction in expression of miRNA involved in neurodevelopment and synaptic transmission. Reduction in synapse-associated proteins, vGlut, NGRF and TrKC	Wang et al. (2011)

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Table 9.2 (continued)

Animal	Chemical and concentrations	Effects	Ref
Rats	Adult rats administered 1.7, 5.0, and 15 mg/L PFOS in drinking water for 91 days.	Increase in expression of CaMKII and pCREB in cortex and hippocampus. Upregulation of transcription factors c-jun in hippocampus and and cortex, and c-fos in hippocampus	Liu et al. (2010b)
Mice	One subcutaneous administration of 50 mg/kg PFOS at (PND 7, 14, 21, 28 and 35	24 h after exposure a reduction in brain superoxide dismutase (SOD) activity in male rats exposed at PND 7 and 21. A reduction in brain antioxidant capability in male rats exposed at PND 21	Liu et al. (2009)
Mice	Peroral exposure, administered once, of 22 μmol/kg (11.3 and 8.7 mg/kg PFOS and PFOA) to 10 days old mice	24 h after exposure, both compounds increased the concentrations of CaMKII, GAP-43 and synaptophysin in hippocampus. PFOA increased concentration of Tau in hippocampus. Both compounds increased the concentration of synaptophysin and Tau in cerebral cortex	Johansson et al. (2009)
Mice	Peroral exposure, administered once, of 14 or 21 μmol/kg (6.1 or 9.2 mg/kg) PFHxS to 10 days old mice	24 h after exposure a reduction in levels of BDNF and GAP-43 in cerebral cortex in (9.2 mg/kg). An increase in CAMKII and Tau in hippocampus in both groups. An increase in synaptophysin in hippocampus (9.2 mg/kg)	Lee and Viberg (2013)
Chicken	Administration of one dose (5 mg and 10 mg/kg) PFOA and PFOS in egg at incubation day 0	At hatching day 1 an overall increase in brain cytosolic PKC (PKC αβγ) in animals exposed to PFOA, and an overall decrease in cytosolic PKC in animals exposed to PFOS	Pinkas et al. (2010)
Chicken	Administration of one dose (8.9, 94, 890, and 9,300 ng/egg PFHxS and 9.7, 94, 1,000, and 9,700 ng/egg PFHxA) in egg at incubation day 0	Upregulation of neurogranin mRNA in chicks exposed to 890 and 38,000 ng/egg PFHxS	Cassone et al. (2012)

* *PFHxA* perfluorohexanoic acid, *PFOA* perfluorooctanoic acid, *PFHxS* perfluorohexanesulfonic acid, *PFOS* perfluorooctanesulfonic acid

(8.7 mg/kg) and PFHxS (6.1 mg/kg) 10 days after birth, had increased levels of the proteins CaMKII, synaptophysin and tau in hippocampus 1 day after the exposure (Johansson et al. 2009; Lee and Viberg 2013). It was, in addition, shown that PFOA and PFOS induced increased levels of synaptophysin in the mice cerebral cortex (Johansson et al. 2009). The effects on CaMKII by PFOS, PFOA and PFHxS are supported by changes in the gene expression of calcium-dependent signaling molecules in rat hippocampus after perinatal PFOS exposure. The expression of calcium-related signaling molecules, which are critical to the function of the central

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nervous system, such as N-methyl-D-aspartate receptors, calmodulin, $Ca^{(2+)}$/calmodulin-dependent kinase II alpha and cAMP-response element-binding, were increased in the PFOS exposure group on postnatal day 1 (PND 1). In some cases these changes lasted for only a short period in postnatal life, but calmodulin and the N-methyl-D-aspartate receptor subtype-2B were still reduced on postnatal day 35 (Liu et al. 2010a). Furthermore these proteins are involved in neuronal growth, synaptogenesis and mediation of neurotransmitter release and indicate that the exposure of PFCs may influence the development of the juvenile mouse brain related to cognitive functions. Synaptophysin, which is a synaptic vesicle associated protein, has for example also been shown to be involved in modulation of cognitive functions such as learning and memory, and novelty exploration (Schmitt et al. 2009). A similar study was performed by (Zeng et al. 2011a, b) who exposed pregnant rats daily from GD2 to GD21 for 0.1, 0.6 and 2.0 mg PFOS/kg/day. The levels of synaptophysin and synapsin in hippocampus were analyzed at PND 0 and 21 showing a reduction in the levels in hippocampus, and an increase in the levels of synaptophysin and a decrease in the levels of synapsin in the cerebral cortex. Synapsin are synaptic vesicle associated proteins involved in the regulation of neurotransmitter release, and in the study by Zeng et al. (2011a) it was also claimed that PFOS induced morphological changes in the synaptic structure and reduced numbers of synaptic vesicles. The discrepancy from the findings by Johansson et al. (2009), who observed an increase in the levels of synaptophysin in the mice brain, may be due to the different administration procedure. The juvenile rats in the study by Zeng et al. (2011a, b) were exposed chronically during pregnancy and the juvenile mice in the study by Johansson et al. (2009) were administered a single oral acute dose 10 days after birth. These studies, therefore, indicate that the PFCs might influence synaptic plasticity, which may have consequences for neuronal development.

When the neonatally animals, which showed effects on cognitive function after exposure to PFOS, PFOA, and PFHxS, were challenged with nicotine in adulthood, their response was changed compared to normal animals.

Control animals became significantly hyperactive by the adult nicotine injection, while the neonatally exposed animals reacted totally opposite with very little activity, displaying a clear hypoactivity. This indicates that PFC could affect the cholinergic transmitter system during the neonatal brain development, because the cholinergic system is involved in many physiological functions, including cognitive capacity (Johansson et al. 2008; Viberg et al. 2013). Other studies support that the cholinergic system could be a target for developmental PFC exposure. For example choline acetyltransferase, a very important enzyme in the cholinergic system of mammals, is involved in the recycling of the neurotransmitter acetylcholine by joining of Acetyl-CoA and choline to reform acetylcholine. In utero exposure to 3 mg PFOS/kg bw/day, during the gestational period, in rats resulted in decreased activity of choline acetyltransferase in prefrontal cortex at different postnatal ages (Lau et al. 2003). Interestingly these effects on cognitive function, behavior and motor activity, are similar to developmental neurotoxicological effects seen after gestational or neonatal exposure to other persistent organic pollutants, such as PCBs and PBDEs (Eriksson 1998; Eriksson et al. 2001; Viberg et al. 2003a, b). In addition, the

mechanistic background to these disturbances are also similar, meaning that they seem to affect the same types of proteins and the same transmitter systems (Eriksson 1998; Viberg et al. 2002, 2007).

Pinkas and co-workers exposed chicks prenatally to single doses of PFOS and PFOA at incubation day 0 for 5 and 10 mg/kg (Pinkas et al. 2010). The chicks were subjected to behavioral testing at hatching day 1 and showed impaired imprinting behavior. An *ex vivo* examination of the brains showed that the PFOS exposed birds had an overall reduction in the levels of different cytosolic PKC isoforms (PKC -α, -β, -γ), whereas PFOA induced an overall increase in the levels of cytosolic PKC. No effects on membrane bound PKC were found. According to the authors, translocation of cytosolic PKC to the membrane is required for imprinting and plays a role in the transfer of cholinergic input involved in learning and memory. Different PKC isoforms have previously been postulated as possible targets following both adult and developmental exposure to halogenated aromatic hydrocarbons, such as the PCBs (Kodavanti et al. 1994, 1998; Yang et al. 2003). The doses used in the experiment by Pinkas et al. (2010) lead, however to high mortality. Between 30 and 50 % of the exposed eggs did not develop embryos indicating that the doses used were detrimental to the chicks leading to other substantial non-neurotoxic effects. Additional studies have been done by Sean Kennedy's research group, who exposed chickens *in ovo* to high doses of PFHxS and PFHxA. They saw that PFHxS induced increases in mRNA levels of neurogranin in cerebral cortex (Cassone et al. 2012). Neurogranin is expressed solely in central nervous system, particularly in dendrites, and is a calmodulin-binding protein, participating in the protein kinase C signaling pathway. PFHxA on the other hand did not have an effect on the mRNA levels of neurogranin.

PFCs can affect the nervous system of mammals and birds, but other studies have also shown that fish are susceptible to PFC exposure during their development. PFOS and PFOA are the most studied (Shi et al. 2009; Spulber et al. 2014; Zhang et al. 2011), and there is one particular interesting study out showing that water exposure to several different PFCs, in zebrafish, can cause behavioral disturbances in locomotor activity. Among the PFCs inducing behavioral disturbances were TFAA, PFNA, PFBS and PFOS. When looking at the structure of the PFCs, PFCs with longer carbon chain length and with attached sulfonic groups showed larger potential to affect locomotor behavior in zebrafish larvae (Ulhaq et al. 2013).

9.4 Cell Cultures (*In Vitro* Studies)

In order to look into the developmental neurotoxicity of PFCs and to get a better understanding of the potential mechanisms behind the neurotoxic effects, *in vitro* experiments have been conducted and investigated such as on cell differentiation and synaptic plasticity (Table 9.3). It has been shown that PFOSA and PFOS (50–250 μM) promote differentiation of the PC12 cell into the cholinergic phenotype at the expense of the dopaminergic phenotype (Slotkin et al. 2008). At the highest

Table 9.3 Summary of *in vitro* studies of PFCs*

Preparation	Chemical and concentrations	Effects	Ref
PC 12 cells	10–250 μM PFOS*, PFOA, PFOSA, PFBS	PFOS promoted differentiation of ACh phenotype at the expense of DA phenotype. Induction of lipid peroxidation and ROS, reduction in cell viability	Slotkin et al. (2008)
PC12 cells	100–500 μM PFHxS	Reduced cell viability and caspase-3 activation; activation of ERK (pro-apoptotic), JNK (anti-apoptotic) and p38MAPK. Protection by NMDA receptor antagonist and ERK-antagonist	Lee et al. (2014a)
Rat cerebellar granule cells	3 and 30 μM PFOS	Reduced cell viability and caspase-3 activation; activation of ROS and PKC. PKC antagonists and antioxidant (N-acetylcysteine) were protective	Lee et al. (2012)
Rat cerebellar granule cells	12–100 μM PFOS, PFOSA, PFOA, FTOH 8:2	Reduced cell viability (EC50 PFOS, PFOA, PFOSA and FTOH: 61, >100, 13 and 15 μM respectively) and induction of ROS (EC50 PFOS, PFOA, PFOSA and FTOH: 27, 25, 57 and >100 μM respectively)	Reistad et al. (2013)
Rat cerebellar granule cells	10 and 30 μM PFOS	Reduced cell viability and caspase-3 activation; activation of ERK (pro-apoptotic). PKC antagonist was protective	Lee et al. (2013)
Rat cerebellar granule cells	100–500 μM PFHxS	Reduced cell viability and caspase-3; activation of ERK (pro-apoptotic) and JNK (anti-apoptotic). Activation of ROS	Lee et al. (2014b)
Rat cerebellar Purkinje cells	30 μM PFOS	PFOS decreased action potential frequency. Influenced Ca, Na and K-currents toward a hyperpolarized state	Harada et al. (2006)
Rat primary hippocampal neurons and slices	10–100 μM PFOS	Increased frequency of miniature postsynaptic currents (mPSCs) and the amplitude of field excitatory postsynaptic potentials. Increased inward Ca-currents and intracellular Ca, inhibited by L-type Ca-channel inhibitor. Suppression of synaptogenesis in cultured neurons	Liao et al. (2008)
Rat primary hippocampal neurons	50 and 100 μM PFPA, PFBA, PFOA, PFDA, PFTA, PFBS, PFHS, PFOS, PFOC	Increased frequency of mPSCs. The increase was proportional to carbon chain length, and the carboxylates were less potent than the sulfonates	Liao et al. (2009b)

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Table 9.3 (continued)

Preparation	Chemical and concentrations	Effects	Ref
Rat primary hippocampal neurons	10–100 μM PFOS	Increase in K currents and glutamate activated currents	Liao et al. (2009a)
Rat primary hippocampal neurons	30–300 μM PFOS 100–300 μM PFOS	Elevation of intracellular Ca from intracellular Ca stores and induction of ROS. A role of ryanodine and inositol triphosphate receptors	Liu et al. (2011)
Neural stem cells	25–100 nM PFOS	Decreased cell viability (100 nM). Lower number of proliferating cells (50 nM) and an increase in neuronal differentiation. Upregulation of PPARγ	Wan Ibrahim et al. (2013)
N2a neuronal cells	EC50 PFOS 196–471 μM EC50 PFOA 389–632 μM	Decreased cell viability. Decreased expression levels of mRNA of differentiation markers (NSE, GFAP, and CNP). Reduction in expression of mRNA of cell adhesion markers (E-cadherin and connexion)	Choi et al. (2013)
HBMEC brain endothelial cells	50–160 μM PFOS	Disassembly of endothelial tight junctions, via the phosphatidylinositol-3 kinase/ Akt-pathway, increasing the permeability of PFOS	Wang et al. (2011)

**PFPA* perfluoropropionic acid, *PFBA* perfluorobutyric acid, *PFOA* perfluorooctanoic acid, *PFDA* perfluorododecanoic acid, *PFTA* perfluorotetradecanoic acid, *PFBS* perfluorobutane sulfonic acid, *PFHS* or *PFHxS* perfluorohexanesulfonic acid, *PFOS* perfluorooctanesulfonic acid, *PFOC* 1H-perfluorooctane, *PFOSA* perfluorooctanesulfonamide, *FTOH* fluorotelomer alcohol

concentration, the effect of PFOSA (100 μM) switched and promoted differentiation into the dopaminergic phenotype. No mechanisms for the effects were postulated, but it was suggested that the induction of oxidative stress could be a factor. PFOSA was shown to induce lipid peroxidation and was also the most cytotoxic compound. Wan Ibrahim and co-workers examined the effects of low PFOS concentrations (12.5–100 nM) on differentiation of neural stem cell (Wan Ibrahim et al. 2013). It was shown that 100 nM PFOS reduced cell viability, whereas the lower concentrations increased neuronal differentiation, as shown as a lower numbers of proliferating cells and a higher number of neurite bearing cells. The effect was attributed to PPARγ activation.

One strategy to evaluate neurochemical targets of PFCs has been to exploit their effects on cell viability, which may indicate a neurotoxic potential of the compounds. Reduced cell viability may be a response of a range of cellular processes triggered by the toxic agents, such as oxidative stress, disruption of the calcium homeostasis, and effects on neurotransmission and signaling. These are cellular processes that are important for neuronal development and survival. Cerebellar

granule cells (CGCs) have been a convenient model to evaluate the neurotoxic potential and mechanisms of effect of a range of environmental toxicants, such as polychlorinated biphenyls, brominated flame retardants as well as PFCs. In two studies by Lee and co-workers, CGCs were exposed to PFOS (3 and 30 μM) and PFHxS (≥100 μM). It was observed induction of apoptosis as shown by increased caspase-3 activity and induced DNA-fragmentation (Lee et al. 2012, 2014b). The PFOS induced apoptosis was connected to activation of different subtypes of protein kinase C (PKC-α, PKC-βII and PKC-ε). The effect of PFOS and PFOA on PKC-translocation was also observed by Pinkas et al. (2010) on developmentally exposed chicks as described in the previous section. PKC is involved in a range of processes in the brain such as cognitive functions, learning and memory and several of studies have shown that other environmental toxicants, such as PCBs, dioxins and brominated flame retardants also influence PKC activity (Kodavanti et al. 1994). This may have implications on the risk of being exposed to mixtures of contaminants which have effects on similar targets. A later study by Lee and co-workers showed that the PFOS activation of PKC was followed by activation of the ERK-pathway (Lee et al. 2013), which is one of the mitogen-activated protein kinases (MAPKs). Also PFHxS was shown to induce ERK (Lee et al. 2014b). By inhibiting the ERK-pathway, the PFOS and PFHxS induced apoptosis was blocked. The ERK-pathway has also been shown activated in cerebellar granule cells by tetrabromobisphenol A (TBBPA), which is a brominated flame retardant and possibly by hydroxyl-PCBs, which are metabolites of PCBs (Dreiem et al. 2009), and recently by PFHxS in PC12 cells (Lee et al. 2014a).

Reactive oxygen species (ROS) is a collective term for short lived, highly reactive compounds, often including oxygen radicals and non-radical products of oxygen. The brain is especially vulnerable to oxidative damage, partly because of its high oxygen demand, corresponding to about 20 % of the basal oxygen consumption. The membrane lipids of the nerve cells are rich in polyunsaturated fatty acids which are sensitive to attack from ROS. Nerve cells have often a large surface area making them more exposed to attack from ROS. ROS are typically generated as by-products in cellular metabolism, from toxic agents, inflammations and diseases (Halliwell and Guttenridge 1999). Oxidative stress refers to the consequence of a mismatch between the production of ROS and the ability of the cell to defend itself against them. In the study by (Lee et al. 2012) it was shown that PFOS induced production of reactive oxygen species (ROS), and the detrimental effect of PFOS, both the PKC-activation and apoptosis, was blocked by pretreatment of N-acetylcysteine (NAC). NAC is used as a scavenger of ROS-products. Reistad and co-workers exposed CGCs to four different PFCs to evaluate their potential to affect cell viability and induce ROS-formation (Reistad et al. 2013). The effect of the PFCs varied of which PFOSA and FTOH 8:2 were considerably more cytotoxic than PFOS and PFOA. PFOSA and FTOH 8:2 had EC50-values of 13 and 15 μM respectively, whereas PFOS had an EC50-value of 61 μM. PFOA did not induce cell death at concentrations up to 100 μM. Similar to these studies Slotkin et al. (2008) showed that PFOSA was the most potent in reducing the cell viability of PC12 cells followed by PFOS, and PFOA did not induce loss of cell viability. An interesting

observation in the study by Reistad et al. (2013) was the lack of correlation between cytotoxicity and ROS formation. PFOS and PFOA were equally potent ROS inducers, with EC-50 concentrations of 27 μM and 25 μM respectively, but PFOA did not induce cell death. PFOSA induced ROS with an EC50-concentration of 57 μM whereas FTOH had no effect, but they were equally cytotoxic. For PFOS Lee et al. (2012) found a correlation between ROS formation and apoptosis in cerebellar granule cells, whereas this appeared not to be the case in PFHxS induced apoptosis (Lee et al. 2014b). Slotkin et al. (2008) found a correlation between cell viability and lipid peroxidation in PC12 cells exposed to PFOSA and PFOS. Similar correlation has also been found for PCBs, OH-PCBs and TBBPA (Dreiem et al. 2009; Mariussen et al. 2002; Reistad et al. 2007). Cellular damage should probably ultimately lead to increased ROS formation. A possible explanation for the lack of correlation between cell death and ROS formation may be the selectivity of the method used to identify ROS and the time after exposure when the endpoints were measured. In addition, the PFCs also differ with respect to their physical-chemical properties such as water solubility, which probably will reflect their ability to reach targets in cells.

A crucial factor for normal functioning cells is maintenance of the intracellular Ca^{2+}-homeostasis. Ca^{2+} is an important second messenger in the cells. However, a sustained increase of the intracellular level of Ca^{2+} may induce formation of reactive oxygen species (ROS) followed by cellular injury. Calcium is also crucial for neurotransmitter release. Upon stimulation of a neuron the transmitter molecules are released from the nerve terminal into the synapse by a Ca^{2+} dependent process. In a study by Harada and co-workers it was shown that 30 μM of PFOS had a modulating effect on ion currents in rat cerebellar Purkinje cells leading towards a hyperpolarized state (Harada et al. 2006). The effect involved voltage gated Ca^{2+}, Na^{+} and K^{+} channels. In a later study, Liao and co-workers also showed that PFOS increases K^{+} currents at doses over 10 μM towards a hyperpolarized direction in hippocampal neurons (Liao et al. 2009b) similar as observed by Harada et al. (2006) on cerebellar Purkinje cells. Effects of PFOS on nervous ion currents were also found by Liao, who showed increased Ca^{2+} currents recorded in the CA1 region of hippocampal slices and in cultured hippocampal neurons (Liao et al. 2008). It was also shown that PFOS inhibits neurite growth and synaptogenesis in cultured neurons. The effects could be blocked by the L-type voltage gated Ca^{2+} channel blocker nifedipine. These findings indicate that PFOS may facilitate influx of calcium leading to an increased susceptibility of calcium related effects, which ultimately may lead to reduced cell viability or impairment of cellular growth. A structure activity study showed that the effect on the calcium currents increased with the carbon chain length of the tail moiety of the PFCs, and that the effects of the carboxylated compounds were less pronounced than the sulfonates (Liao et al. 2009a). The effects of PFOS and PFOA on the Ca^{2+}-homeostasis in hippocampal neurons have been elucidated in more detail by Liu and co-workers, showing that the PFCs affect several calcium dependent processes (Liu et al. 2011). The sulfonated PFOS (30 μM) was more potent than the carboxylated PFOA (100 μM) to induce elevated intracellular concentrations of Ca^{2+}. The increase intracellular Ca^{2+} appeared to be of both

extracellular origin involving voltage gated Ca^{2+} channels, as shown by Liao et al. (2008), and intracellular origin such as activation of ryanodine receptors and inositol phosphate-3 (IP_3)-receptors. The disturbance of the Ca^{2+}-homeostasis was followed by an increase in oxidative stress, as measured with DCF, and an increased expression of calcineurin, which is a Ca^{2+} activated protein phosphatase. Another interesting finding by Liao et al. (2009a) was that a low concentration of PFOS (1 μM) increased inward glutamate currents whereas higher concentrations of PFOS (10 and 100 μM) dose-dependently reduced the inward glutamate currents. Glutamate is the quantitatively the most important excitatoric neurotransmitter in the brain. Glutamate is an excitotoxin so a prolonged stimulation of glutamatergic receptors in the brain may cause a sustained elevation of the intracellular Ca^{2+} level in the neuron, which can mobilize Ca^{2+}- dependent processes, leading to inflammation, ROS production, and ultimately cause cell death (Fonnum 1998). Hippocampus, which is mainly glutamatergic, is one of the major brain areas concerned with the acquirement of memory, and only minor damage to this area is sufficient to produce memory disturbances (Bliss and Collingridge 1993; Fonnum et al. 1995; Milner 1972; Victor et al. 1961).

Cytotoxicity and oxidative stress may also be induced as a consequence of inflammatory responses, such as immune responses. In prenatally PFOS exposed rats it was observed an increase inflammatory response in the juvenile rat brains as shown by increased levels of the astrocyte markers fibrillary acidic protein and S100 Ca^{2+}-binding protein B in hippocampus and cortex (Zeng et al. 2011b). It was also found an increase in the mRNA levels of proinflammatory cytokines, such as interleukin 1β, tumour necrosis factor α, AP-1, NF-kappa-B and CREB. Changes in the mRNA levels as a response on an exposure may not necessarily imply changes or harmful effects on a higher protein or cellular level. There is, however, previously been shown that PFOS and PFOA enhance inflammatory responses of macrophages to lipopolysaccharide in mice, indicating that PFCs may be implicated in stress responses related to the immune system (Qazi et al. 2009).

9.5 Summary and Conclusion

There is no doubt that PFCs can induce developmental neurotoxic effects, since research in humans, animals and cell cultures all point in the same direction. Functional effects in animals, such as impaired behavior and cognitive functions, have also been investigated to elucidate the mechanisms behind the effects. Disturbances in the processes of synaptogenesis, dendritic outgrowth and ontogeny of neurotransmitter systems all look as plausible mechanisms and apoptosis, specific proteins, signaling molecules, calcium homeostasis as well as oxidative stress can be the molecular reasons behind the disturbances of these processes. It is important to remember, though, that the real world is much more complicated than exposure to one single compound at the time. Therefore, effects of PFCs in combination and/or in combination with other environmental pollutants need to be investigated.

At the moment it is hard to find good examples of neurotoxic effects after combination exposure to PFCs or PFCs and other types of chemicals, but one study shows that combined exposure to low doses of PFOA and the polybrominated diphenyl ether PBDE 209, during the neonatal period, can interact and exacerbate adult functional neurobehavioral effects, compared to the single compounds alone (Johansson 2009).

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Chapter 10
Immunotoxicity of Perfluoroalkylated Compounds

Deborah E. Keil

Abstract The following chapter reports on the health impacts of perfluoroalkylated compounds in the context of *in vitro* and *in vivo* immunotoxicology studies as well as epidemiology studies. In general, elevated serum PFAA levels reported in adults and children correlate with observed changes in health to include decreases in serum vaccine titers and IgE levels, or increases in antinuclear antibodies, asthma, the common cold, and gastroenteritis. Laboratory studies demonstrate direct, *in vitro* effects of perfluorinated compounds modulating TNF-α, IL-6 and IFN-γ. These studies may be linked mechanistically to alterations reporting decreases in vaccine antibody titers in human reports and dose-responsive, decreases in IgM antibody production in animal models. To some extent, perfluoroalkylated compounds are thought to modulate gene regulation via peroxisome proliferator activated receptor alpha (PPARα) and to a lesser extent via peroxisome proliferator activated receptor gamma (PPARγ), yet species differences affecting the expression of this receptor complicates this interpretation as an underlying mechanism in humans. Mechanisms of action beyond PPAR-mediated effects represents new directions and are also discussed. As we learn more about the relationship between perfluoroalkylated compounds and emerging health issues, this may challenge current benchmark thresholds in drinking water to ensure adequate protection for human health.

Keywords PFOS • PFOA • PFAAs • Immunotoxicity • Immunosuppression

10.1 Immunotoxicity in Human Studies: *Epidemiology*

In an ongoing epidemiological study of humans living near a PFC production facility in West Virginia, levels of IgA, IgE (females only), and C-reactive protein decreased with increasing PFOA serum concentration in adults. Additionally,

D.E. Keil, Ph.D., M.T. (ASCP), D.A.B.T. (✉)
Department of Microbiology and Immunology, Montana State University,
109 Lewis Hall, Bozeman, MT 59717, USA
e-mail: Deborah.keil@montana.edu

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_10

ED_002330_00119699-00248

antinuclear antibodies, a marker of autoimmunity, increased with increasing PFOA serum concentration (Fletcher et al. 2009). In another study, there was no association between serum PFOA concentrations and immunoglobin levels in male workers (Costa et al. 2009). It is not until we explore the effects of PFOA and PFOS on children exposed *in utero* or post-natal development, that the epidemiology studies suggest a stronger link with immune dysfunction.

Most studies linking PFOA and PFOS to changes in immunity have been typically examined within the scope of maternal and child health. Specifically, perfluorinated contaminant blood levels and health of mothers and children have been monitored over time to learn about susceptibility to disease or vaccine titers. In one study, the relationship between maternal PFOA and PFOS exposure and hospitalization due to incidence of childhood infectious disease was examined. Fei and colleagues (2010) measured PFOA and PFOS in maternal blood during the first trimester of pregnancy in nearly 1,400 women from the Danish National Birth Cohort. Mean serum PFOA was 5.6 ng/ml and PFOS was 35.3 ng/ml. However, these data did not unequivocally support that prenatal exposure to PFOA and PFOS increased the risk of hospitalizations due to infectious diseases during early childhood. Limitations of the study include social and behavioral factors that may have influenced the correlation between PFCs and age-related immune responses.

The incidence of infant allergies, infectious disease and changes in IgE levels in cord blood have also been examined with regards to maternal PFC exposure. Okada et al. (2012) measured PFOS and PFOA in maternal serum after the second trimester (n = 343) and total IgE concentration in cord blood (n = 231). Maternal self-administered questionnaires indicated no significant association with maternal PFOA levels and incidence of infant allergies and infectious disease. However, cord blood IgE levels in female infants only, decreased significantly with increasing maternal serum PFOA. As a point of reference, mean maternal PFOA concentration was 1.4 ng/mL and IgE levels in cord blood was 0.62 IU/mL. In contrast to this study, Wang et al. (2011) reported that PFOA and PFOS concentrations in cord blood were positively correlated with cord blood IgE levels, but only in male offspring. However, following these children to the age of 2 revealed no association between PFCs and atopic dermatitis.

A sub-project of the Norwegian Mother and Child Cohort Study examined maternal serum concentrations of PFOA and PFOS and the antibody titers of 3-year old children (Granum et al. 2013). Average maternal serum concentrations were 1.1 ng/ml for PFOA and 5.6 ng/ml for PFOS. In 3 year old children, a positive association was observed between maternal PFOA and perfluorononanoic acid (PFNA) with number of episodes of common cold, as well as between PFOA and perfluorohexane sulfonate (PFHxS) with number of episodes of gastroenteritis.

Dong et al. (2013) investigated the relationship between perfluorinated compounds and incidence of childhood asthma. This was a cross-sectional study of 231 children age 10–15 years with physician-diagnosed asthma and 225 age-matched non-asthmatic controls recruited from two hospitals in Northern Taiwan. Serum was collected for measurement of ten perfluorinated compounds while absolute eosinophil counts, total IgE, eosinophilic cationic protein and asthmas were

assessed. Both PFOA and PFOS serum levels were significantly associated with biomarkers and asthma severity scores in the children. Serum levels of PFOS in asthmatic and non-asthmatic children were 45.5±37.5 and 33.4±26.4 ng/mL, respectively, while serum levels of PFOA in asthmatic and non-asthmatic children were 1.5±1.3 and 1.0±1.1 ng/mL respectively.

In a prospective study, a birth cohort of 587 singleton births during 1999–2001 from the National Hospital in the Faroe Islands examined children's antibody levels to diphtheria and tetanus vaccines in the context of perfluorinated compounds (Grandjean et al. 2012). Serum antibody concentrations were measured in mothers and children at age 5 years pre-booster, approximately 4 weeks after the booster, and at age 7 years. Previously, prenatal exposures to perfluorinated compounds were assessed in the mother during week 32 of pregnancy. Average maternal serum concentrations analyzed at 32 weeks of pregnancy were 3.2 ng/ml for PFOA and 27.3 ng/ml for PFOS. Serum concentrations were collected from the children at 5 years of age (mean of 4.06 ng/ml for PFOA and 16.7 mg/ml for PFOS) and serum antibody concentrations were collected at 5 and 7 years of age. Prenatal exposure to PFOA and PFOS were negatively associated with antibody titers against diphtheria. Maternal PFOS exposure was most strongly associated with responses in 5 year olds and postnatal exposure to both PFOA and PFOS were most strongly associated with responses in 7 year olds. The authors also examined deviations from the clinically protective level of 0.1 IU/L and reported that both pre- and postnatal exposure to PFOS and postnatal PFOA exposure were associated with increased odds of antibody titers being below clinically protective levels. The researchers learned that a twofold increase in levels of PFOS and PFOA at age 5 lead to a several fold decrease in protective antibodies against diphtheria and tetanus.

When gathering additional data, Grandjean and Budtz-Jørgensen (2013) estimated benchmark doses based on their 2012 study, under the assumption that previously published benchmark doses (BMDs) are not adequately protective of children or of the general public given potential suppression of immune responses. A BMD of 1.3 ng/ml for PFOS and 0.3 ng/ml for PFOA were determined. Additionally, they calculated a BMDL (benchmark dose level, or lower one-sided 95 % confidence limit of the BMD) of 1 μg/l and a reference dose (RfD) of 1 ng/l. The US EPA provisional health advisory for drinking water is 0.4 μg/l for PFOA and 0.2 μg/l for PFOS (2010/2015 PFOA Stewardship Program). As the latter is grounded on developmental and subchronic toxicity, these references levels are not likely to encompass immunotoxicity that are derived using serum levels (i.e., and RfD of 1 ng/l). If a BMD based on serum vaccine titers were acknowledged, then current limits may be several hundred-fold too high.

10.2 Immunotoxicity in Human Studies: *In Vitro*

Only a few studies investigate the direct, *in vitro* effects of perfluorinated compounds on human cells. Using donated blood from 11 volunteers, peripheral blood mononuclear cells (PBMCs) were isolated and tested for NK cytolytic activity

following *in vitro* exposure to PFOA or PFOS (Brieger et al. 2011). Viability of PMBCs was not affected, yet suppression of NK cytolytic activity and increasing TNF-α following lipopolysaccharide (LPS) stimulation was observed. Using a human promyelocytic cell line (THP-1), cellular mechanisms of PFC immunotoxicity have been examined by other researchers (Corsini et al. 2011, 2012, 2014). In addition to PFOS and PFOA, perfluorobutane sulfonic acid (PFBS), perfluorodecanoic acid (PFDA), perfluorooctanesulfonic acid (PFOSA), and a fluorotelomer (8:2 Telomer) all suppressed LPS-induced TNF-α production. PFOSA, PFOS, PFDA, and fluorotelomer affected IL-6 and IFN-γ release. All compounds except PFOA suppressed PHA-induced IL-10 release. These studies also report that PFOA and PFOS have different effects on immune cells and that PFOA-mediated suppression of cytokine production appeared to be dependent on PPARα activation whereas the actions of PFOS were independent of PPARα activation. Furthermore, leukocytes from female donors appeared to be more sensitive to PFCs than male donors, suggesting sex differences with regard to immune responses. These *in vitro* studies in the context of epidemiology studies, demonstrate that perfluorinated chemicals affect immunological cells by altering cytokine expression and these observations, although early in this investigation, can be linked mechanistically to alterations reported in decreased vaccine antibody titers reported in human studies.

10.3 Immunotoxicity in Rodent Models

Preliminary concerns about PFC immunotoxicity were based early reports that indicated reduction in relative spleen and thymus weights and suppression of the T cell dependent antibody response (TDAR). PFOA, PFOS, and ammonium perfluorooctanoate (APFO, a precursor compound to PFOA) in rodent models have demonstrated that these compounds suppress TDAR and, in the case of PFOS, suppress TDAR at doses within the range of exposures for the general human population (Peden-Adams 2008; reviewed in DeWitt et al. 2009, 2012). Since SRBC-specific IgM production is considered predictive of immunotoxicity and decreased host resistance (Luster et al. 1992, 1993; Selgrade 1999), these alterations in murine immune function are useful in extrapolating to human health risk (Selgrade 2007). Consequently, the focus of further research reports have concentrated on possible mechanisms of action related to this aspect of immune function.

PFOS and PFOA have both been shown to alter antigen-specific antibody production to a T cell dependent antigen, namely sheep red blood cells (SRBC). Using a B6C3F1 mouse model and SRBC challenge, PFOS decreased IgM production (as measured by the plaque-forming cell response) in both male and female mice (1.66 and 16.6 μg/kg/day, respectively, lowest observed adverse effect level [LOAEL] for each sex) at blood concentrations of 91.5 ± 22.2 ng/g and 666 ± 108 ng/g (mean ± SD) by 52 % and 50 %, respectively (Peden-Adams et al. 2008). Moreover, TDAR suppression due to PFC exposure has been supported with replication in at least four

reported studies (Peden-Adams et al. 2008; DeWitt et al. 2008; Zhang et al. 2013; Dong et al. 2009).

Keyhole limpet hemocyanin (KLH) antigenic challenge (T cell dependent antigen) has also been used to test adaptive immune function following exposure to PFCs. Conversely, KLH-specific IgG levels were not affected in female Sprague-Dawley rats following exposure to PFOS (Lefebvre et al. 2008). Depending on the methodology, plaque-forming cell or ELISA, it is not uncommon to observe different outcomes in these assays, even with two T cell dependent antigens (Loveless et al. 2007; White et al. 2007).

Adaptive immunity to a T cell independent antigenic challenge also varies following exposure to PFCs. Using TNP-LPS challenge, a T cell independent antigen, IgM antibody production was decreased at 0.334 mg PFOS/kg/d (334 μg/kg/day; 12). In a separate study with C57BL/6 female mice, the T cell independent antibody response also was suppressed after 15 days of drinking water exposure to 1.88 mg/kg of PFOA (DeWitt, unpublished data).

In addition to effects on adaptive immunity, PFOS and PFOA also effect cell populations in the bone marrow (Qazi et al. 2012). Specifically, a high dose, 10 day dietary exposure significantly reduced the total numbers of cells in the bone marrow including myeloid, pro/pre B, immature B and early mature B cells. Partial or complete restoration of the cell number occurred following 10 days of withdrawal of these compounds. At the lower dose of 0.002 %, only PFOA reduced the B-lymphoid cell population.

Quantitatively linking an immunological effect to onset of disease is key to improving species extrapolation and characterization of potential human health risks. The following studies demonstrate such a link. A LOAEL has been established at 0.5 mg/kg PFOS total dose (serum level = 666 ± 108 ng/g) in female B6C3F1 mice for decreased SRBC-IgM production (Peden-Adams et al. 2008). At comparable exposure levels, Guruge and colleagues (2009) reported susceptibility to influenza A-induced mortality. Taken together, these reports suggest that PFOS-induce humoral immune suppression at lower levels of exposure may be enough to compromise a host to disease onset. This is further corroborated by a study demonstrating that the PFC response may be predicative of susceptibility to influenza virus (Burleson and Burleson 2010) and recent studies in humans that indicate PFOS and PFOA may be associated with decreased responses to childhood vaccines (Grandjean et al. 2012; Granum et al. 2012).

As rodents are useful in characterizing immunotoxicity, these models are not without caveats. For instance, immunotoxicity outcomes vary between species and gender following exposure to PFOS and PFOA. Mice seem to be more sensitive to the effects of PFOS and PFOA as compared to rats (Loveless et al. 2008; DeWitt et al. 2008; Yang et al. 2002; Peden-Adams et al. 2008; Lefebvre et al. 2008). In both a rat and mouse model, males were more sensitive to the noted effects on antibody production (Peden-Adams et al. 2008; Lefebvre et al. 2008). In fact, a tenfold difference in the LOAEL (based on dose and approximately sevenfold difference based on serum PFOS concentrations) was reported between males and females (Peden-Adams et al. 2008). B6C3F1 mice exposed during gestation

demonstrated an increased male sensitivity to PFOS-induced suppression of IgM antibody production (Keil et al. 2008). As we learn more about possible mechanisms of action of PFCs, gender and species disparities may be explained in part by differences PPARα expression.

10.4 Mechanisms of Action in Immunotoxicity

The weight of evidence suggests that the primary mechanism of action for PFC-induced modulation of cellular processes is via the activation of PPARs (reviewed by Anderson et al. 2008; Butenhoff et al. 2012; Rosen et al. 2010). Both PFOS and PFOA bind to the peroxisome proliferator activated receptor alpha (PPARα). These transcription factors (PPARα and PPARγ) are ubiquitous and regulate gene expression by modulating lipid pathways, increasing permeability of mitochondrial membranes, affecting glucose regulation, cell proliferation, and inflammatory processes (Post et al. 2012; Starkov and Wallace 2002). Activation of PPARα by PFCs has been a major focus of mechanistic studies, with a greater role for PPARα as compared to PPARγ in mediating toxicity (Post et al. 2012; Takacs and Abbott 2007; Vanden Heuvel et al. 2006).

It is considered that immunotoxicity varies between species and gender due to the differential expression of these PPAR transcription factors in humans and in rodent models. Human hepatic PPARα expression is only one-tenth that of rodents (Kennedy et al. 2004). In general, males endure a longer half-life elimination rate as compared to females (Kudo et al. 2006).

Some studies have suggested other routes of toxicity for PFCs, however, many of these proposed pathways are not independent of the influence of PPAR. This is largely due to the fact that PPAR transcription factors are extensively expressed on lymphocytes, hepatocytes, cardiac cells, and microglia with the potential to affect numerous physiological pathways. Further examination of alternate mechanisms of actions by PFCs is certainly necessary to facilitate a more complete consideration of human health effects.

10.5 Proinflammatory Cytokines

Both PFOS and PFOA bind to the peroxisome proliferator activated receptor alpha (PPARα) and a significant number of PPARα agonists have been shown to reduce inflammation (Griesbacher et al. 2008). Therefore, it would seem that PFCs would reduce expression of cytokines or other related inflammatory markers. However, this is not the case. Increases in serum levels of the proinflammatory cytokines IL-6 and TNF-α associated with PFOS and PFOA exposure have been reported in mouse models. Qazi et al. (2009) reported increased basal serum concentrations of IL-6 in male C57Bl/6 mice, but not TNF-α following exposure to 400 mg/kg total dose

(0.02 % in diet for 10 days of either PFOS or PFOA). This pattern of increased basal serum IL-6 but not TNF-α was also reported by Dong et al. (2011) in male C57Bl/6 mice exposed to 50 and 125 mg/kg total dose of PFOS. Mollenhauer et al. (2011) reported increased serum IL-6 and decreased serum TNF-α at 1 mg/kg total dose following LPS injection and PFOS exposure for 28 days.

Proinflammatory cytokines are produced by peritoneal macrophages in the presence of PFCs. Following LPS challenge *in vitro* or *in vivo*, peritoneal macrophages responded by producing more TNF-α and IL-6 at PFOS concentrations ranging from 25 to 400 mg/kg total dose (Qazi et al. 2009; Dong et al. 2011). PFOA exposure also increases *ex vivo* TNF-α production after both *in vitro* and *in vivo* LPS stimulation at 400 mg/kg total dose (Qazi et al. 2009). Dong et al. (2011) observed increased *ex vivo* production of TNF-α and IL-6 from mixed spleen cell cultures at 125 mg PFOS/kg total dose while Qazi et al. (2009) reported similar results at 400 mg PFOS/kg total dose for TNF-α production, but not IL-6 production. Most PPARα agonists are known to reduce inflammation, yet this is not the case with PFCs. This is likely to suggest that PFCs possess multiple mechanisms of action on immune function that are not typical PPARα agonist mediated effects.

10.6 Conclusions

We have learned from rodent models that both PFOS and PFOA induce a dose-responsive suppression on adaptive immunity in mice. One of the primary differences between these two is that PFOA reduced the number of B and T cells (Yang et al. 2002), while PFOS induced suppression of antigen-specific antibody responses independent of significant reductions in B and T cell numbers (Dong et al. 2012; Peden-Adams et al. 2008; Keil et al. 2008). The mechanism(s) involved in PFC-induced suppression of adaptive immunity is likely linked with altered cytokine signaling that arises from interaction of PFCs with PPARα, but may also be related to the interaction of PFCs with other signaling molecules, such as NFκB (Corsini et al. 2012).

When extrapolating rodent studies to humans, it is concerning that suppression of adaptive immunity occurs at PFC exposure levels that are within a reasonable range for human exposure. Furthermore, the few human epidemiology studies currently available generally report that T-cell dependent production of antibody following vaccination is suppressed. This is further weighted with comparable observations in rodent models. However, mechanisms of action beyond PPARα-mediated effects require further examination. Human and rodent studies are far from complete and it is clear that we must learn more about mechanism(s) of immunotoxicity to reduce PFC-associated health effects and improve regulatory exposure limits.

ED_002330_00119699-00254

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Chapter 11
Effects of PFOA on Endocrine-Related Systems

Casey E. Reed and Suzanne E. Fenton

Abstract Perfluorooctanoic acid (PFOA) is an 8-carbon fully fluorinated chemical that has reported effects on endocrine-related systems in rodents, humans, and other species. Numerous endocrine organs may be targets for PFOA, including the brain, thyroid, pancreas, adipose tissue, ovary, uterus, testes, and breast. Developmental exposure effects have been reported on behavior, serum thyroid and gonadal steroid profiles, breast epithelial growth, and metabolic end points, such as serum insulin, leptin, and triglyceride levels and weight gain. Many of these PFOA-induced effects have been reported in two or more species. The mechanisms for these numerous effects are poorly understood and deserve further investigation to define the pathways that should be avoided as PFOA-replacement products enter the market.

Keywords PFOA • Mammary gland • Thyroid • Endocrine disruptor • Puberty • Lactation • Fertility

11.1 Introduction

Perfluoroalkyl acids (PFAAs) are chemicals with carbon chains that are completely fluorinated. There are a wide variety of PFAAs in the environment that could have potential endocrine effects, but there has been minimal research on most PFAAs; this chapter will focus on the endocrine-related effects of perfluorooctanoic acid (PFOA). PFOA is persistent, lipophobic, can bind proteins, and is highly detectable in wildlife and human serum (White et al. 2011a). This chapter will focus on developmental exposures and their long-term effects. There has not been enough research on the other PFAAs to include for this focus. The exact mechanism(s) of

C.E. Reed • S.E. Fenton, Ph.D. (✉)
National Toxicology Program (NTP) Laboratory, Division of the NTP, National Institute of Environmental Health Sciences NIEHS/NIH, DHHS, 111 TW Alexander Dr, Bldg 101, MD E1-08, Research Triangle Park, NC 27709, USA
e-mail: fentonse@niehs.nih.gov

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_11

ED_002330_00119699-00258

action for endocrine-related effects of PFOA has not been discovered; however it has been shown that a variety of target tissues and health outcomes from PFOA exposure are endocrine-related such as mammary gland, thyroid, and adipose tissue (Lau et al. 2003; Macon et al. 2011; White et al. 2007; Hines et al. 2009) (see Fig. 11.1).

There are key times in development where exposure to chemicals can have a long term effect. Developing infants from the fetal period to the prepubertal period are sensitive to environmental toxins because of the high growth rate (cell proliferation and differentiation) that takes place during this period (Birnbaum and Fenton 2003). Each time a cell proliferates or divides it offers an opportunity for mistakes in genomic repair, methylation, or programming to occur which may translate to growth of abnormal cells; thus the result may be cancer (Birnbaum and Fenton 2003). The placenta protects against the transmission of some compounds from the mother's circulation into fetal circulation, however PFOA can bypass this protective mechanism (Fei et al. 2007; Gutzkow et al. 2012; Inoue et al. 2004). Early-life exposures may predispose individuals to more chronic adverse effects than later life exposures when rapid cell division no longer occurs (Landrigan et al. 2002).

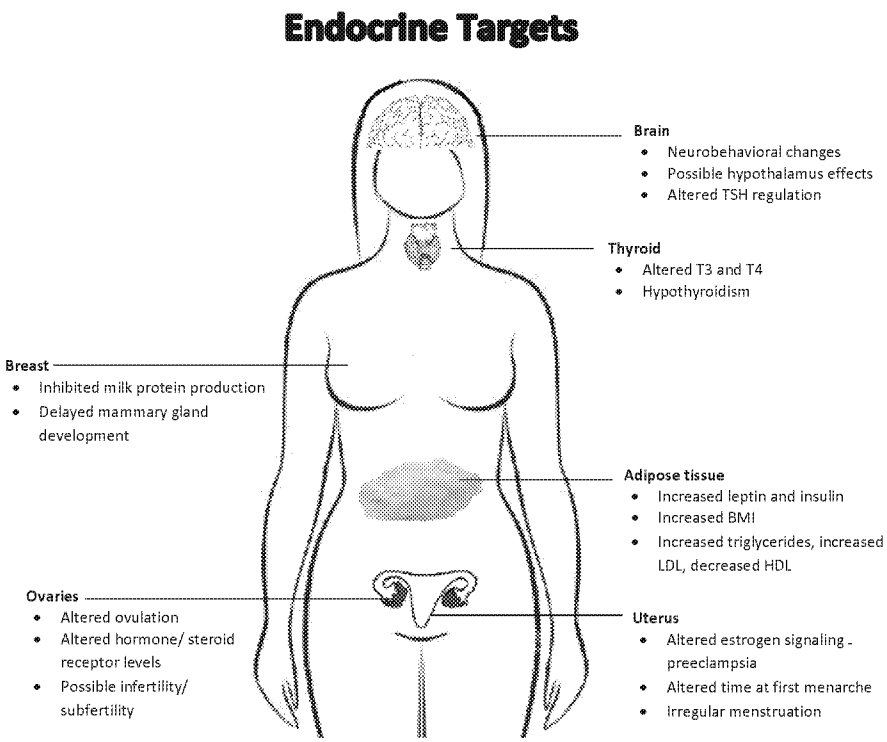

Fig. 11.1 Perfluorooctanoic acid (PFOA) is known to have effects on numerous endocrine targets. Effects of the chemical have been associated with brain, pancreas, thyroid, breast, adipose, ovarian and uterine effects in female rodent research models and in women for some of the listed outcomes. Endocrine effects have also been reported in males, but there is less data available

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Therefore, exposure to PFOA that occurs during gestation and through the time of puberty is concerning in that it may reprogram endocrine-related signaling and result in long-term adverse health effects.

PFOA has been the target of much animal and human epidemiological research. Animal research primarily utilized mice because the elimination kinetics of PFOA in mice is more similar than rats to that of humans. Rats have a shorter PFOA elimination half-life than mice, and demonstrate a rapid elimination in reproductive-aged females, resulting in differences in excretion between males and females that do not lend well to developmental toxicity studies (Fenton et al. 2009; Lau et al. 2007). There are numerous studies that have evaluated human PFOA exposures because it is found in more than 99 % of the general U.S. population through contamination of food, water, house dust, and air (Winquist et al. 2013). One particular study, the C8 Health Project (C8HP), focuses on highly-exposed residents of the Mid-Ohio Valley (Winquist et al. 2013). The C8HP includes two cohorts: one made up of community members 20 years and older and a separate group comprised of occupationally-exposed individuals (Winquist et al. 2013). These cohorts, and others like them around the world, provide vital insight into the human effects correlated with varying levels of PFOA exposure.

11.2 Gestational Exposure and Pregnancy Outcome

Adult PFOA exposure primarily occurs through intake of contaminated food and water while transplacental transfer and breast milk are important routes of exposure for fetuses and infants (Mondal et al. 2012; Haug et al. 2011; Fromme et al. 2010). PFOA has the highest placental passing ratio of all the PFAAs (0.89:1), indicating that it can pass easily through the placental barrier and into the fetal environment, and may result in fetal exposures that are equivalent to years of exposure by the mother (Liu et al. 2011). The transfer ratio of PFOA was two times higher than that of perfluorooctane sulfonate (PFOS), another highly studied PFAA (PFOS ratio was 0.54:1) (Liu et al. 2011).

One way to measure human gestational PFOA exposure is to analyze its concentration in umbilical cord blood and to compare that to maternal serum levels. Declining serum levels of PFOA can be detected in the first trimester of pregnancy in women (Javins et al. 2013), indicating an early life transfer of serum PFOA to the developing fetus. Maternal sera and cord blood measurements for PFOA are reported to be strongly correlated ($R > 0.5$; $p < 0.01$) (Kim et al. 2011), and one study found higher PFOA concentrations in cord blood than in maternal serum with a cord blood:maternal PFOA ratio of 1.3 (Midasch et al. 2007). Most studies, however, report higher PFOA levels in maternal serum than in umbilical cord blood with ratios ranging from 0.67 to 0.87 for paired samples (umbilical serum:maternal serum) (Fei et al. 2007; Hanssen et al. 2010; Inoue et al. 2004; Kim et al. 2011; Monroy et al. 2008; Needham et al. 2011). World-wide, PFOA has been detected in cord blood from the general population in the U.S., Canada, Germany, Norway,

Australia, South Africa, Korea, and Taiwan in concentrations ranging from 1.1 to 4.4 ng/ml (Post et al. 2012). These data provide strong evidence for transplacental transfer of PFOA between maternal and fetal circulation.

Developmental toxicology studies have found that PFOA has the potential to affect fetal growth and development. In studies designed to understand the transfer of PFOA from dam to fetus/neonate, CD-1 mice were given a single exposure to PFOA late in gestation (gestational day [GD] 17). This exposure did not affect the number of live fetuses, implantation sites, or weight of live-born pups on GD18 or post natal day (PND) 1 (Fenton et al. 2009). Interestingly, although dam serum concentrations were significantly greater than amniotic fluid levels collected the morning before birth, pups that were just a few hours old exhibited a significantly higher serum PFOA concentration than that of their dam due to the degree of placental transfer, and possibly from a short suckling period (Fenton et al. 2009). The levels of PFOA in mouse milk were 20–40 % that of maternal serum in early and late lactation when milk and blood volumes are low, relative to peak lactation, when milk PFOA levels were 10–20 % that in the maternal serum. Milk transfer of PFOA in rats (Hinderliter et al. 2005) and humans (Karrman et al. 2007) is reportedly less efficient than the mouse, with PFOA milk:maternal serum distribution ratios of 0.1 and 0.01, respectively.

There are a range of adverse developmental outcomes associated with PFOA in rodent models including decreased fetal weight and increased neonatal mortality (Lau et al. 2007; Olsen et al. 2009). PFOA effects in mice often occur in a dose-dependent manner. One study exposed pregnant mice to PFOA (doses from 1 to 40 mg/kg) from GD 1 through GD 17 (Lau et al. 2006). The exposures did not impact the number of implantations but there were significant increases in the number of full-litter resorptions starting at 5 mg/kg (Lau et al. 2006). There were no significant differences in live pup weights at doses at or equal to 10 mg/kg and significant prenatal losses were observed at the 20 mg/kg dose (Lau et al. 2006). Although most pups were born alive, there were increases in the incidences of stillbirth and neonatal mortality at doses of 5 mg/kg and higher ($p<0.05$) (Lau et al. 2006). At lower doses (1 and 3 mg/kg), there were significant changes in postnatal growth and development, particularly in the form of growth retardation and delayed eye opening (Lau et al. 2006). These doses are much higher than ordinary human exposures.

As stated previously, PFOA transfer to the developing fetus can be detected in the first trimester of pregnancy for humans (Javins et al. 2013). The first trimester marks a period of time where there is abundant fetal development where the nervous, cardiovascular, digestive, respiratory, renal, and endocrine systems are forming, which makes perturbations during this time period potentially catastrophic (Javins et al. 2013). Although there has yet to be a thorough investigation of the endocrine-disrupting effects of perfluorinated compounds, there are numerous endocrine-related outcomes that are correlated with PFOA serum levels during pregnancy and birth. Pregnancy is a time of particular interest not only because of potential developmental insults to the fetus, but also because it can result in adverse pregnancy outcomes. Preterm birth (before 37 weeks gestation), pregnancy-induced hyperten-

sion (PIH), and low birth weight (less than 2,500 g) are often described as adverse pregnancy outcomes, all of which are linked to endocrine system dysregulation. There was little evidence of an association between PFOA and preterm birth or low birth weight among the C8HP cohort (Darrow et al. 2013), but two studies found modest inverse associations between birth weight and maternal PFOA concentration (Apelberg et al. 2007; Fei et al. 2007). These results have not been repeated in other studies (Savitz et al. 2012; Monroy et al. 2008; Washino et al. 2009; Nolan et al. 2009). However, a recent meta-analysis was performed on all of the available data from animal and human studies related to birth weight and PFOA exposures. The in-depth analyses indicated a significant −0.023 g reduction in birth weight of offspring of non-human mammalian species for each unit (mg/kg birth weight/day) increase in PFOA dose (Lam et al. 2014). The human data analysis, with numerous studies analyzed together, resulted in 'sufficient' evidence of PFOA negatively affecting fetal growth (Lam et al. 2014). Prior to this meta-analysis, there was no summary of human data to support an association between PFOA exposure and clinically significant endpoints of growth restriction.

To date there is no evidence to link PFOA exposures with preterm delivery in women (Savitz et al. 2012; Fei et al. 2007; Hamm et al. 2010; Darrow et al. 2013); however one of the endocrine-related health outcomes that may lead to preterm delivery, PIH, has been associated with PFOA exposures. An association between increased maternal PFOA levels and PIH in the C8HP cohort was reported based upon log-transformed and categorical analyses (27 % increased odds with 95 % CI) (Darrow et al. 2013). Modest associations between serum PFOA and self-reported preeclampsia have been reported (Stein et al. 2009), but this finding associating PFOA and PIH was not evident in a study involving birth records (Savitz et al. 2012) or in a Norwegian cohort with background PFAA levels (Starling et al. 2014a). Savitz and colleagues (2012) have suggested that the maternal physiology is a more important determinant of pregnancy outcomes than the degree of PFOA exposure. Therefore maternal age, parity, and BMI all impact the absorption and elimination and can determine the amount of exposure the fetus receives from any chemical which makes causal associations difficult to verify (Savitz et al. 2012).

11.3 Lactation

Lactation is a physiological state that is regulated and maintained by the endocrine system and endocrine disruptors or chemicals that interact with the endocrine system can alter mammals' ability to lactate. Data indicate an association between the length of time a woman has lived near a source of PFAA contamination and the level of PFOA in her breast milk (von Ehrenstein et al. 2009). There is evidence that not only will PFOA be eliminated in milk to potentially affect the offspring, but it may also affect breast function. Animal models indicate that there are changes in maternal mammary gland structure after developmental PFOA exposure and that

PFOA exposure inhibits production of normal milk proteins, leading to increased pup mortality (White et al. 2011a, b; Lau et al. 2006). These data are further described in Chap. 8 in this book.

Many chemicals can pass through the maternal system and be transferred to the infant through breast milk. As previously mentioned, human studies have found that there is not only placental transfer of PFOA but that lactational transfer occurs and may provide the majority of infant PFOA exposure (Inoue et al. 2004; Apelberg et al. 2007; Monroy et al. 2008; Tao et al. 2008; Mondal et al. 2012; Fei et al. 2007; Fromme et al. 2010; Kim et al. 2011; Needham et al. 2011). Epidemiological studies focusing on the C8HP cohort found a higher child:maternal serum PFOA ratio in children (12 months old) whose mothers breast fed exclusively versus those who were breast and/or bottle fed (1.83 for breast feeding only and 1.14 for breast/bottle fed) (Mondal et al. 2012). Another study estimated that breast milk contributes over 83 % of infant PFOA exposure even though the concentration of PFOA measured in breast milk was low (Haug et al. 2011). A small survey of Italian mothers found that the highest levels of PFOA in milk came from primiparous women (Barbarossa et al. 2013). None of the findings were statistically significant because of the low number of participants, however it appears to indicate that PFOA concentrations in breast milk decrease after the first lactation and, therefore, first born infants may have higher exposures of PFOA (Barbarossa et al. 2013; Tao et al. 2008).

Exposure to PFOA via transplacental transfer and milk leads to an elevated body burden in humans and rodents. One study estimated that infant PFOA exposure through milk is 2,173 ng, which is much higher than the 183 ng PFOA received through gestational exposure (Liu et al. 2011). Higher exposure results in elevated PFOA body burden for infants, from birth through 6 months of age, compared to adults (Fromme et al. 2010). Mouse studies found a similar increased body burden from GD18 through PND8 and decreased between PND8 and 18 when milk intake decreased (Fenton et al. 2009). One study in particular has generated a great deal of useful data concerning elimination of PFOA into breast milk and the burden in the infant over time. Breastfeeding mothers in the C8HP were found to have a lower geometric mean PFOA concentration than non-breastfeeding mothers in the same cohort (Mondal et al. 2014). Consequently, the breastfed infants in the cohort had a higher PFOA concentration (geometric mean of 49 ng/ml) than non-breast fed infants (geometric mean of 22 ng/ml) (Mondal et al. 2014). Overall the serum PFOA concentration of breastfeeding mothers decreased 3 % per month of breastfeeding to culminate in an estimated 34 % decrease in maternal serum PFOA concentration after breastfeeding for 12 months (Mondal et al. 2014). The infants who were breastfed for 12 months had PFOA concentrations that were 141 % higher than their formula-fed counterparts (Mondal et al. 2014). Concerns regarding the comparison of findings between labs because of differences in limits of detection and accidental PFOA contamination by lab equipment (Mondal et al. 2012) may be minimal as a second recent study evaluating PFOA burden in adolescent girls in the San Francisco Bay and Greater Cincinnati areas also reported highly significant increases in serum PFOA and other PFAAs correlated with the duration that the child was breastfed (Pinney et al. 2014).

11.4 Puberty

Puberty is another vulnerable life stage where environmental influences have been linked with health problems. Severe pubertal delays may be a risk factor for infertility while moderate delays may predispose females to endometriosis, osteoporosis, and psychosocial issues (Lopez-Espinosa et al. 2011). Human epidemiological studies are inconsistent in regards to associations between PFOA exposures and pubertal timing. One study found that PFOA was associated with earlier puberty (Pinney et al. 2009); other studies find PFOA concentration to be associated with later time to first menstruation (Lopez-Espinosa et al. 2011; Kristensen et al. 2013); and yet another study found no relationship between pubertal timing and PFOA concentration (Christensen et al. 2011). It should be noted that there are specific differences in how pubertal timing is measured. Some studies measure pubertal attainment by breast development (Pinney et al. 2009), sex steroid hormone levels (Lopez-Espinosa et al. 2011), and self-reported age at menarche (Lopez-Espinosa et al. 2011; Christensen et al. 2011). Kristensen et al. (2013) studied a group of women who had in utero PFOA exposure. These women reached menarche 5.9 months later than a reference group with a lower PFOA exposure and found a statistically significant delay in menarche in relation to prenatal PFOA exposure ($p = 0.01$). There was no association between PFOA exposure and menstrual cycle length, reproductive hormone levels, or number of ovarian follicles among this cohort (Kristensen et al. 2013).

Mouse studies have found associations between PFOA concentration and altered ovarian function (altered hormone/steroid receptor levels), delayed vaginal opening at high doses, delayed mammary gland development (at the lowest doses tested), and histopathological changes in the reproductive tract that would indicate delays in pubertal timing (Yang et al. 2009; White et al. 2011a, b; Dixon et al. 2012; Zhao et al. 2012; Macon et al. 2011; Tucker et al. in press). These effects appear to be dependent on PFOA exposure level, timing of exposure, and there may be some effects that are dependent on strain sensitivity (Macon et al. 2011; Tucker et al. in press).

11.5 Subfecundity/Subfertility

Subfecundity, or prolonged time to conceive, is often a measure used to determine the fertility of females. Similar to other endocrine-related endpoints discussed earlier in this chapter, the epidemiological data related to PFOA and subfecundity are mixed. Two studies found an association between increased time to pregnancy and PFOA serum concentration (Whitworth et al. 2012; Fei et al. 2009), while another found no association (Vestergaard et al. 2012). The study by Whitworth et al. (2012) specified that the odds ratio for subfecundity was elevated only in parous women (OR for the highest quartile = 2.1), while no effect was seen in the

nulliparous women (OR for the highest quartile = 0.5). The proportion of women diagnosed with infertility (longer than 12 months without conceiving) in the Danish National Birth Cohort study was higher in the three higher quartiles of PFOA exposure versus the lowest quartile, indicating that PFOA may permanently alter an endocrine-related mechanism required for conception (Fei et al. 2009).

The mechanism of action by which PFOA alters female fertility is unknown, however it has been hypothesized that it may be an interaction with the hypothalamic-pituitary-ovarian axis, which in turn could trigger irregular menstruation, altered time of ovulation, or early spontaneous abortions (Fei et al. 2009). There is potential for the mode of action for increased time to pregnancy associated with high PFOA levels to include PFOA-induced irregular menstrual cycles (Fei et al. 2009). A report of longer menstrual cycles associated with the highest tertile of PFOA exposure was recently published using pooled estimates of over 1,600 women from Poland, Greenland, and Ukraine (Lyngso et al. 2014).

11.6 Thyroid

The thyroid gland is an integral part of the hormone regulatory system. It is needed for normal metabolic function. Thyroperoxidase catalyzes the transfer of iodine during thyroid hormone synthesis and PFOA decreases the activity of this enzyme in a cell-based system (Song et al. 2012). PFOA can also interfere with thyroid hormone levels and the sensitive feedback mechanisms they are associated with. Monkeys treated with PFOA were found to have decreased thyroid hormones thyroxine (T4) and triiodothyronine (T3), without the expected increase in thyroid stimulating hormone (TSH) (Calafat et al. 2007). This suggests that PFOA may block thyroid hormones from their binding proteins. Studies focused on individuals occupationally exposed to PFOA have had variable findings. PFOA exposure has ranged from no association with thyroid function (Olsen et al. 1998), to weakly positive changes in T3 (Olsen and Zobel 2007), to significant associations with elevated T4 and reduced T3 uptake (Huang et al. 2011). The C8HP recently published strong evidence for validated thyroid disease in relation to PFOA exposure estimates in combined cohorts of residents and workers in the Mid-Ohio Valley ($n > 32{,}000$ participants and $n > 4{,}000$ with reported disease) (Winquist and Steenland 2014). The trend for PFOA-related disease was more pronounced among women and absent in men, with hypothyroidism being the predominate disease. PFOA has also been linked to hypothyroidism in children (Lopez-Espinosa et al. 2012). A small and subclinical association between elevated serum free T4 and PFOA, without a concomitant decrease in TSH, was found in a cohort of Chinese adolescents and young adults (Lin et al. 2013). The group hypothesizes that PFAA exposure caused a syndrome of reduced thyroid responsiveness to thyroid hormone or that there may be TSH hypersecretion from the pituitary (Lin et al. 2013). The mechanism of interference between PFOA and the hypothalamic-pituitary-thyroid axis is still unclear.

There is a significant increase in serum T4 and reduction in T3 uptake in adults with 1 or more years of PFOA exposure (Knox et al. 2011). Using National Health and Nutrition Exposure Survey (NHANES data), TSH levels were found to increase with PFOA concentration, indicating that PFOA is associated with subclinical hypothyroidism in adults (Jain 2013). There was also a slight decrease in total T4 levels and no increase in total T3 in relation to PFOA concentration among this group (Jain 2013). In a separate analysis, NHANES data also indicated that there was an association between PFOA serum concentrations and self-reported thyroid disease (Melzer et al. 2010). Women in the highest PFOA exposure quartile (>5.7 ng/mL) were more than twice as likely to exhibit thyroid disease as women in the lower quartiles (<4 ng/mL) (Melzer et al. 2010). The significant effect in women was not recapitulated in men, although the trend was similar (Melzer et al. 2010). Because PFOA predominantly has thyroid effects on women, it has implications for pregnant mothers where thyroid hormone dysregulation can alter gene expression and development of the fetal brain (Javins et al. 2013). In utero thyroid levels are also involved in programming future body weight and therefore PFOA dysregulation of thyroid hormones may have implications for obesity later in life (Grun and Blumberg 2009).

11.7 Obesity/Fat Tissue/Lipid Metabolism

Obesity is a global problem that affects people of all ages and ethnicities. Over 10 years ago, researchers began suggesting that the rise in obesity is correlated with the marked increase in the number of industrial chemicals on the market (Baillie-Hamilton 2002) and that over-eating and less physical activity are not valid explanations for the epidemic. There are approximately 85,000 chemicals on the U.S. market. Of these, approximately 2 % have been tested and several have proved to be endocrine disrupting compounds. It is widely accepted that adipose tissue is an endocrine organ that produces hormones that act on other tissues in the body. The main hormones produced within adipose tissue are leptin, adiponectin, steroids, and resistin (Guerre-Millo 2002; Harwood 2012). Leptin is produced by white adipose tissue to regulate food intake, metabolism, and puberty progression (Gueorguiev et al. 2001). Low leptin levels or leptin resistance is related to adult overweight and obesity in a variety of animal models after developmental exposures to chemicals including PFOA (Newbold 2010).

A toxicology study in CD-1 mice investigated the metabolic effects of gestational and lactational PFOA exposure. They exposed mice to low doses of PFOA (0.01–5 mg/kg/day) during pregnancy and found that the doses of 0.01 mg/kg/day to 0.1 mg/kg/day induced elevated leptin, insulin, and body weight while the 1 mg/kg/day and 5 mg/kg/day doses caused decreased body weight after female offspring reached 10 weeks of age (Hines et al. 2009). Further, removing the ovaries prior to puberty prevented the body weight-related effects of the PFOA exposure indicating that the ovarian axis plays an important role in the PFOA-related metabolic effects (Hines et al. 2009). This study also dosed adult mice and found no

effect on body weight related to PFOA exposure which indicates that gestation and early life is a vital window for these PFOA effects (Hines et al. 2009).

A human study by Halldorsson and coworkers (2012) found maternal PFOA concentration to be positively associated with body mass index (BMI) and waist circumference among 20-year old female offspring of women who had PFOA exposure during their pregnancy ($p < 0.05$; $n = 345$). The women whose mothers were in the highest quartile of PFOA exposure had a BMI that was 1.6 kg/m^2 higher and a waist circumference that was 4.3 cm bigger than those females whose moms were in the lowest quartile (Halldorsson et al. 2012). There was no statistical difference between the males. Further, similar to the Hines study (2009), increased maternal PFOA levels were associated with increased insulin, leptin, and leptin-adiponectin ratio and inversely associated with adiponectin (Halldorsson et al. 2012).

One potential mechanism of action for these findings includes in utero PFOA exposure possibly interfering with ovary development or function which can lead to impaired estrogen synthesis (Hines et al. 2009). Another hypothesis is that PFOA interacts with peroxisome proliferator activated receptors (PPAR) alpha or gamma, signal transducers which are important in lipid metabolism in fat cells (Hines et al. 2009). Recent data utilizing prepubertal mammary tissue (mostly fat) suggests that PPAR gamma may indeed be an important modulator of effect following PFOA exposure (Macon et al. in press).

There are other important metabolic factors that are not directly weight-related, including cholesterol/triglyceride levels. Numerous studies have reported a positive association between PFOA levels and total cholesterol or LDL levels in humans (Nelson et al. 2010; Frisbee et al. 2010). A highly exposed occupational group not only had increased cholesterol in relation to PFOA exposure but they also exhibited increased triglycerides and lower HDL (the good cholesterol) (Olsen et al. 1998). The Norwegian Mother and Child Cohort Study, demonstrating low level exposures, found no evidence of an association between elevated triglycerides and PFAA concentrations in pregnant women (Starling et al. 2014b), suggesting that this may be an exposure-related effect.

In mice, however, serum cholesterol and PFOA are inversely correlated which indicates that there are different mechanisms of action for PFOA effects on cholesterol between mice and humans (White et al. 2011a; Quist et al. in press). Obesity and cholesterol-related heart disease are major contributors to adult morbidity so any modifying factors, such as preventing or limiting chemical exposures, could be potentially important in long term health and healthcare delivery systems.

11.8 Men

Much of the epidemiology and toxicology studies have focused primarily on female-related endpoints. Although fetal exposure to PFOA is inevitable there seems to be less focus on male outcomes. However, male mice who were developmentally exposed to ≥ 1 mg/kg/day PFOA may exhibit early onset puberty (Lau et al. 2006).

A longitudinal study of sons of women who were recruited during pregnancy was conducted by Vested et al. (2013) to determine if in utero PFOA exposure is related to semen quality and reproductive male hormone levels. The study found no relationship between the gestational PFOA exposure and abnormal spermatozoa morphology. This finding may be related to the fact that morphology and motility of spermatozoa are determined in adolescence and adulthood during sperm production. Two other studies focusing on PFOA and PFOS in combination found that the chemicals were negatively associated with the percentage of morphologically normal spermatozoa (Joensen et al. 2009; Toft et al. 2012). Statistically significant associations between PFOA and sperm count, sperm concentration, and LH were obtained after transforming data to obtain a normal distribution and correcting for confounders (Vested et al. 2013). There was a positive association between gestational PFOA exposure and LH and FSH in adulthood related to the idea that high gonadotropin concentrations are associated with low sperm concentration and sperm count (Vested et al. 2013; Appasamy et al. 2007; Gordetsky et al. 2012). Further studies need to be done to determine the male-specific effects of PFOA exposure, as the work by Vested et al. (2013) suggests that the fetal male reproductive system may be impacted by maternal PFOA exposures.

11.9 Conclusions

Although there has been little focus on PFOA as an endocrine disruptor, per se, there are numerous significant correlations between PFOA exposures and endocrine-related disease states (Fig. 11.1). Future studies should focus on modes of action in animal and human studies to identify the similar effect pathways. This will enable industry to design replacement chemicals that do not perturb those pathways and may in turn be a safer product.

Additional attention should be given to the timing of PFAA exposure and the disease end point, as the exposure that had the most impact on the end point may have been months, years or decades earlier, during a critical period of development for the endocrine-related tissue of interest. PFAA measurements made during meaningful life stages should be compared to latent disease end points. Further research is needed to determine the role of PFOA on many health outcomes, but the effects on the thyroid and adipose tissue are developing a weight of evidence to solidify a space for PFOA on the growing list of endocrine disrupting chemicals.

Acknowledgements This work was supported by the National Toxicology Program, National Institute of Environmental Health Sciences, NIH.

ED_002330_00119699-00268

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ED_002330_00119699-00273

Chapter 12
Carcinogenicity of Perfluoroalkyl Compounds

Gerald L. Kennedy and J. Morel Symons

Abstract This chapter reviews the information available on the carcinogenic potential of perfluoroalkyl acids in both animals and humans. Historically, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) have been the most widely used members of this chemical class making these the subject of the largest proportion of the reported studies. 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 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. Mechanistic studies suggest that the liver tumors seen with PFOA reflect the activation of PPARα while the mechanism for tumor formation in the testis and pancreas is less clear. 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 the 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. It should be noted that human exposures to these chemicals is currently quite low and appears to be decreasing.

Keywords PFOA • PFOS • Rodent carcinogenicity • PPAR alpha activation • Worker epidemiology • Community epidemiology

G.L. Kennedy (✉)
Consultant to DuPont, Wilmington, DE 19805, USA

515 Wissfire, Wilmington, DE 19803, USA
e-mail: gkdomer64@aol.com

J.M. Symons
DuPont Epidemiology Program, Newark, DE 19714, USA

© Springer International Publishing Switzerland 2015

J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_12

ED_002330_00119699-00274

This chapter will cover the published information relating to the potential carcinogenic activity of perfluoroalkyl acids. The reader will quickly notice that the information cited comes mainly from one member of this family of compounds, perfluorooctanoic acid (PFOA). A natural conclusion might be that this is the key member of the family in terms of potential carcinogenic activity. However, the focus on this chemical comes from the effort to more completely describe potential hazards of this particular chemical because it, like many members of the family, is capable of entering the human body and has attenuated elimination kinetics. Animal studies on PFOA, perfluorooctane sulfonate (PFOS), and perfluorohexanoic acid (PFHxA) evaluating carcinogenic potential have been conducted while other members of this chemical class have not been studied. Industrial use of PFOA and PFOS has resulted in occupationally exposed workers who have been studied for cancer mortality and, less frequently, cancer incidence. Community members living near a chemical plant in West Virginia using PFOA were studied because of exposure through PFOA-contaminated water. This activity resulted in an evaluation of the cancer profile in these surrounding communities. This type of information was designed to look at the potential impact of PFOA on these communities where exposures were greater than seen in other communities but not as great as exposures in workers making and using the chemical.

It might be tempting to look at the structural similarities of these chemicals and use results from one member of the group to predict biological activities of others. Indeed, it has been suggested that, similar to the approach taken for polychlorinated biphenyls, dioxins, and dibenzofurans, the use of Toxic Equivalency factors be employed for risk assessment purposes. Scialli et al. (2007) used data from four different perfluoroalkane acids (PFOS, PFOA, perfluorobutanesulfonate-PFBS, and perfluorodecanoic acid-PFDA) where tests were available on the same species using essentially the same designs, and constructed dose-response curves which could be modeled for concordant endpoints. Scialli and colleagues were unable to identify a scaling system that gave values consistently within an order of magnitude for the same compounds and concluded that combining exposure levels of perfluoroalkane acids for risk assessment was not supportable. A caution to this conclusion was that with additional data being made available, this position could be re-evaluated. Peters and Gonzales (2011) also looked at the appropriateness of using toxic equivalency factors for perfluoroalkyl chemicals and also concluded that the use of such an approach is likely unsuitable. Four facts which do not support predicting the effect of one perfluoroalkyl chemical by using the results from another are that, first, on a mechanistic basis, the effects of these chemicals are modulated by more than one receptor. Second, where comparative data are available, the induced effects are quite discordant. Third, very limited information has been published to evaluate either additivity or synergism with these chemicals. Fourth, the lack of solid data does not allow application of additivity. Importantly, the lack of a strong data base for many of the commonly used commercial perfluoralkyl chemicals seriously limits evaluation. Peters and Gonzales (2011) also presents inherent limitations that would need to be overcome including bioavailability and pharmacokinetics, understanding of the target genes the mediate toxicity, influence of species differences,

identification of potential nonadditive effects, and influence of endogenous chemicals that could modify the effect(s) of these chemicals.

Thus, the reader is cautioned to apply the information presented for a specific chemical to that chemical and not extend the findings (or non-findings) to other perfluoroalkanes. Also, because the reader will note that material covered here is predominantly derived from studies of PFOA, and to a lesser degree studies of PFOS, it must be remembered that this reflects more accurately their use rather than their potential for biologic activity among members of this chemical family. A final introductory note is that when evaluating the human information, those individuals exposed to greater amounts of chemical would be expected to produce a greater, rather than a lesser, chance of response. Therefore, studies in those working directly with the material would have the greatest exposure and would be most likely to respond.

12.1 Animal Studies

12.1.1 Bioassays with APFO

A limited number of long-term studies looking at the carcinogenic potential of PFCs have been published. With APFO (the ammonium salt of PFOA), two long-term feeding studies were conducted in rats (Biegel et al. 2001; Butenhoff et al. 2012). Two-year studies were also conducted in rats fed PFOS (Thomford 2002) and PFHxA (Klaunig et al. 2014). Although the dosing period was only 6 months, studies in monkeys were conducted with PFOA (Butenhoff et al. 2002) and PFOS (Seacat et al. 2002) which included looking at a variety of endpoints associated with long term outcomes which could be linked to cancer (Butenhoff et al. 2002).

For APFO, rats of both sexes were fed either 30 or 300 ppm (approximately 1.5 and 15 mg/kg) for 2 years (Butenhoff et al. 2012). A significant increase in Leydig cell tumors of the testes was seen in the males fed 300 ppm. No increase in tumor incidence of any other tissues or organs was seen in the males fed 30 ppm or in both groups of females (Table 12.1). The conclusion of the original study was that there was no increase in the incidence of proliferative lesions in the mammary gland in the APFO-treated rats above the historical control and normal expected background incidence from the published literature for female Sprague-Dawley rats. However, the incidence of fibroadenomas in the mammary gland was increased in the high-dose group when compared to the concurrent controls; therefore, a Pathology Working Group (PWG) review of this tissue was conducted using current diagnostic criteria. The consensus reached by the PWG was that the incidence of mammary gland neoplasms (lobular hyperplasia, fibroadenoma, and adenoma) was not affected by chronic dietary administration of APFO, and no increase in proliferative lesions in that tissue were produced (Hardisty et al. 2010). The primary difference between the original reported

Table 12.1 Incidence of neoplastic microscopic findings for male and female rats in either control groups fed 30 ppm or 300 ppm APFO in their diet for 2 years

Organ/lesion	Dietary dose group (ppm APFO)					
	Males			Females		
	0	30	300	0	30	300
Adrenal						
Pheochromocytoma, benign	2/49 (4)[a]	4/50 (8)	4/50 (8)	2/50 (4)	0/50 (0)	0/49 (0)
Pheochromocytoma, malignant	0/49 (0)	1/50 (2)	0/50 (0)	0/50 (0)	0/50 (0)	1/49 (2)
Liver						
Hepatocellular adenoma	0/49 (0)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)
Hepatocellular carcinoma	3/49 (6)	1/50 (2)	5/50 (10)	0/50 (0)	0/50 (0)	1/50 (2)
Mammary gland	–	–	–	7/46 (15)	14/45 (31)	5/44 (11)
Adenocarcinoma	–	–	–	3/46 (7)	0/45 (0)	0/44 (0)
Adenoma	–	–	–	1/46 (2)	0/45 (0)	0/44 (0)
Carcinoma	–	–	–	10/46 (22)	19/45 (42)	21/44 (48)*
Fibroadenoma	–	–	–	0/46 (0)	0/45 (0)	1/44 (2)
Lymphangiosarcoma	–	–	–			
Reevaluation by PWG[b]						
Adenocarcinoma				9/50 (18)	16/50 (32)[c]	5/50 (10)
Adenoma				1/50 (2)	0/50 (0)	0/50 (0)
Fibroadenoma				16/50 (32)	16/50 (32)	20/50 (40)
Fibroadenoma (multiple)				2/50 (4)	6/50 (12)	3/50 (6)
Pituitary						
Adenoma	17/48 (35)	17/47 (36)	13/46 (28)	33/46 (72)	39/47 (83)	36/50 (72)
Testes/epididymis						
Leydig cell adenoma	0/49 (0)	2/50 (4)	7/50 (14)*	–	–	–
Thyroid						
C-cell adenoma	0/43 (0)	2/47 (4)	4/47 (9)	1/50 (2)	0/45 (0)	0/41 (0)
C-cell carcinoma	2/43 (5)	0/47 (0)	0/47 (0)	0/50 (90)	0/45 (0)	0/41 (0)

*Statistically significant different from controls ($p \leq 0.05$)
[a]Number observed/number examined (%)
[b]Hardisty et al. (2010)
[c]The incidence in the groups sharing this footnote were statistically significantly different from each other ($p < 0.01$, Hardisty et al. 2010)

finding and the PWG involved classifying lesions originally noted as lobular hyperplasia as fibroadenomas and this occurred mainly in the control group.

A dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats sacrificed at 2 years (Mann and Frame 2004). The significance was unknown and there was no progression to tumors. Using more recently published nomenclature, these lesions were diagnosed as gonadal hyperplasia or tubular adenoma and no statistically significant increase in hyperplasia and adenomas was seen in the PFOA-treated rats. There was some evidence for an increase in stromal size in the 300 ppm group but the total number of rats with either adenoma or hyperplasia was 12, 16, and 17 in the 0, 30, and 300 ppm groups respectively which does not suggest a risk for tumor development.

To investigate the time course and mechanism of action of APFO, a 2-year feeding study in rats was conducted with a number of interim sacrifices to measure potential treatment related changes as a function of exposure time (Biegel et al. 2001). To match the exposure conditions in an earlier chronic study in rats (Butenhoff et al. 2012), a single test group exposed to 300 ppm was used along with a group pair-fed to the 300 ppm group to detect any possible influence of changes in feeding amounts. Increase in the incidence of adenomas in the liver, pancreas, and testis were seen in male rats receiving 300 ppm (equivalent to a daily dose of approximately 15 mg/kg) as shown in Table 12.2. Hyperplasia of both the pancreas and the testis was also increased. Cell proliferation was seen in the pancreas but not in either the liver or the testicular Leydig cells (Biegel et al. 2001).

The above tumor triad was produced in rats by clofibrate (Svoboda and Azarnoff 1979), HCFC-123 (Malley et al. 1995), gemfibrozil, and diethyl-hexyl phthalate (DEHP). Trichloroethylene (TCE) produced both liver and Leydig cell tumors (Cook et al. 1999; David et al. 2000; Voss et al. 2005). Nafinopen (Cook et al. 1999) produced both liver and pancreatic tumors. Other chemicals causing Leydig cell tumors in rats include clofibrate, gemfibrozil, methyl clofenazide, perchloroethylene, and TCE (Cook et al. 1999). In mice, estradiol exposure leads to Leydig cell tumors while estrogenic compounds do not induce testicular tumors in rats (Cook

Table 12.2 Incidence of liver, testes, and pancreas tumors in rats fed 300 ppm APFO in the diet for 2 years

		Groups:	Control	Pair fed	PFOA
Tumor	Cancer				
Liver	Adenomas		2/80[a]	1/79	10/76*
	Carcinomas		0/80	2/79	0/76
Testes	Adenomas		0/80	2/78	8/76*
(Leydig cell)	Hyperplasia		11/80	26/78*	35/76*
Pancreas	Adenomas		0/80	1/79	7/76*
	Carcinomas		0/80	0/79	1/76
	Hyperplasia		14/80	8/79	30/76*

*Statistically significant different from controls ($p \leq 0.05$)
[a]Number affected/number of rats tested

et al. 1999). In F344 rats there is an age-related increase in serum estradiol which correlates with Leydig cell hyperplasia and tumor formation (Grunewald et al. 1992).

Pancreas acinar cell tumors are modified by steroid concentrations, growth factors, cholecystokinin (CCK), and dietary fat (Longnecker 1983, 1987; Longnecker and Sumi 1990). CCK is a growth factor found in the gut mucosa which is released by the presence of food in the duodenum, then binds to pancreatic tumor cell receptors to release pancreatic secretions including chymotrypsin. It has been hypothesized that PFOA increases fat content in the gut by enhanced excretion of cholesterol and triglycerides resulting in hyperplasia and adenomas.

To look further at the induction of Leydig cell adenomas by APFO, male rats were treated by oral gavage with either 1, 10, 25, or 50 mg/kg for 14 days along with a group of pair-fed controls to the 50 mg/kg rats (Cook et al. 1992). A decrease in the rate of body weight gain was seen at 10 mg/kg and higher, and, since the body weights of the group of pair-fed and its control group were similar, this was attributed to decreased food intake. At the top two doses, accessory sex organ weight was decreased while testes weights and histopathology were unchanged. Serum estradiol levels were increased at 10 mg/kg and higher being 2.7 times control levels at 50 mg/kg. Serum testosterone concentrations decreased (3.2, 1.6, 1.6, 1.2, 0.8, and 0.7 ng/dl in rats receiving 0, 1, 10, 25, 50, and 50 mg/kg pair-fed respectively). Similarly, interstitial cell testosterone levels were lower in the APFO-treated rats with the greatest effect seen at 50 mg/kg in the pair-fed group. Liver weights at 10 mg/kg and higher were increased and beta oxidation also increased from 8 to 11 times in a dose-related fashion (Cook et al. 1992).

In a series of studies to determine the level of the testosterone lowering lesion, rats were given 50 mg APFO/kg for 14 days followed by treatment with human chorionic gonadotropin, gonadotropin-releasing hormone (GnRH), or naltrexone. Human chorionic gonadoptropin (hCG) affects lesions in the steroidogenic pathway by binding to luteinizing hormone (LH) receptors on Leydig cells to stimulate testosterone synthesis. GnRH affects lesions at the adenohypophysis by stimulating LH release from gonadotropin. Naloxone affects lesions at the hypothalamus by enhancing GnRH release by removal of inhibitory action of opiate neurotransmitters on GnRH controlling neurons. Only hCG led to a 50 % decrease in serum testosterone suggesting the lesion was at the testes modifying the conversion of 17 alpha to androstenedione. No changes seen with GnRH treatment suggests that the lesion was not at the pituitary level, and, for naltrexone, the lack of change suggests that the lesion was not at the hypothalamus level.

In a 6-month study in which cynomolgus monkeys were given daily doses of either 3, 10, or 30/20 (dose reduced to 20 mg/kg after 2 weeks) mg APFO/kg, the effects on biological markers associated with the hepatic, pancreatic, and testicular responses (seen in rats dosed with APFO and other peroxisome proliferating chemicals) were evaluated (Butenhoff et al. 2002). There was no increase in peroxisomal proliferation as measured by palmitoyl CoA oxidase activity. The approximately twofold increase seen at the high dose reflects that this species is not particularly responsive to peroxisome proliferating compounds. No changes in reproductive

hormone levels were seen as estradiol, testosterone, and cholecystokinin concentrations in each monkey were unaltered over the course of the experiment. No evidence of cholestasis as indicated by changes in bile acids, bilirubin, or alkaline phosphatase, was observed. Cell proliferation in the liver, pancreas, or testes, as demonstrated by replicative DNA synthesis, was not affected by APFO treatment. Although the study duration was only 6 months, biological markers associated with responses in the three tissues shown to result in adenomas in the rat were not affected.

12.1.2 Bioassay with PFOS

A 2-year study with PFOS fed to male and female rats at concentrations of 0, 0.5, 2, 5, and 20 ppm (dosing equivalents of 0, 0.02, 0.10, 0.25, and 1.1 mg/mg respectively) was conducted (Thomford 2002). An extra group fed 20 ppm for 1 year followed by a 1 year recovery with no PFOS added to the diet was employed. The incidence of hepatocellular adenomas in male rats showed a positive trend with 7/60 (11.7 %) found in the high-dose group compared to 0/60 (0 %) in the control group. In females, the incidence of hepatocellular adenomas was also increased with 5/60 (8.3 %) observed in the high-dose group compared to 0/60 in the control group. In addition, the only hepatocellular carcinoma in this study occurred in this group (Table 12.3).

Among males fed 20 ppm PFOS for 1 year with 1 year recovery, the incidence of thyroid follicular cell adenomas appeared to be increased. This was not observed in males fed 20 ppm continuously for 2 years or in females.

Another non-dose related observation was the apparent increase in mammary gland tumors in the group fed 0.5 ppm. Combining rats with either a mammary adenoma or a carcinoma, the incidence in all groups including the controls was relatively high. None of the remaining tissues or organs had tumor incidences that could be related to the feeding of PFOS. Further, although some of the incidence values in some of the test groups appear greater than those in the control group, the lack of a dose-response allows only a suggestion of carcinogenicity in the rat.

In a 6-month study in which cynomolgus monkeys were given daily doses of either 0.03, 0.15, or 0.75 mg potassium PFOS/kg, the effects on biological markers associated with the hepatic, pancreatic, and testicular responses seen with APFO in rats were evaluated (Seacat et al. 2002). Hepatic peroxisome proliferation measured by palmitoyl CoA oxidase activity was increased in the females given 0.75 mg/kg but the response was less than the twofold change typically associated with biological significance. No effects on cell proliferation were seen in either the liver, pancreas, or testes using the proliferating cell nuclear antigen immunohistochemistry cell labeling index.

Table 12.3 Incidence of neoplastic microscopic findings for rats fed PFOS for 2 years

	Dietary PFOS (ppm)	0	0.5	2	5	20	20[a]
Males							
Tumors							
Liver-hepatocellular adenoma		0/60	3/50	3/50	1/50	7/60*	0/40
Thyroid							
Follicular cell adenoma		3/60	5/49	4/50	4/49	4/59	9/39*
Follicular cell carcinoma		3/60	1/49	1/50	2/49	1/59	1/39
Females							
Liver							
Hepatocellular adenoma		0/60	1/50	1/49	1/50	5/60*	2/40
Hepatocellular carcinoma		0/60	0/50	0/49	0/50	1/60	0/40
Thyroid							
Follicular cell adenoma		0/60	0/50	0/49	2/50	1/60	1/40
Follicular cell carcinoma		0/60	0/50	0/49	1/50	0/60	0/40
Mammary gland							
Adenoma		23/60	30/50*	22/48	26/50	15/60	16/40
Carcinoma		11/60	12/50	11/50	11/50	14/60	10/40
Combined adenoma and carcinoma		29/60	36/50	31/48	29/50	24/60	17/40

From Thomford (2002)
*$p<0.05$
[a]Fed PFOS for 1 year, control diet for 1 year

12.1.3 Bioassays with Other Polyfluorinated Compounds

A 2-year rat study was conducted to evaluate both the chronic toxicity and potential carcinogenicity of perfluorohexanoic acid (PFHxA) (Klaunig et al. 2014). Male rats were given daily gavage doses of either 0 (control), 2.5, 15, or 100 mg PFHxA/kg for 104 weeks. Female rats were given daily doses of either 0, 5, 30, or 200 mg PFHxA/kg. No increase in neoplasms related to treatment of PFHxA at any of the three dosage levels examined was seen in either male or female rats (Table 12.4).

In a TSCA 8(e) notification, a rat oral gavage study was conducted with 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propionic acid, ammonium salt (CAS 62037-80-3) in which female rats were treated with doses of either 1, 50, or 500 mg/kg daily for 23Months (Anand 2013). Doses for male rats were 0.1, 1, and 50 mg/kg and were treated for 24 months. Although actual incidence numbers were not given, an increase in liver adenomas was reported in female rats given 500 mg/kg. This result was not reported for females at the two lower doses. Non-neoplastic liver

Table 12.4 Neoplastic findings in rats dosed with PFHxA for 2 years

Incidence of neoplastic findings[a]										
				Males				Females		
		Dose (mg/kg)	0	2.5	15	100	0	5	30	200
Tissue	Lesion									
Adrenal cortex	Adenoma		0/60	0/60	0/60	1/70	2/60	1/60	0/60	2/70
	Adenocarcinoma		1/60	0/60	1/60	0/70	0/60	0/60	2/60	0/70
Adrenal medulla	Pheochromocytoma		4/60	3/60	2/60	1/70	1/60	1/60	2/60	1/70
Kidney	Tubular adenoma		0/60	2/60	0/60	0/70	0/60	0/60	0/60	0/70
	Tubular carcinoma		1/60	0/60	0/60	0/70	1/60	0/60	0/60	0/70
Liver	Adenoma		1/60	0/60	1/60	1/70	0/60	0/60	1/60	2/70
	Carcinoma		2/60	0/60	1/60	0/70	2/60	0/60	1/60	0/60
Brain	Astrocytoma		0/60	3/60	4/60	0/70	1/60	0/60	1/60	1/70
Pancreas	Ascinar adenoma		0/60	0/60	1/60	0/70	0/60	0/60	0/60	0/70
	Islet cell adenoma		9/60	10/60	5/60	4/70	4/60	2/60	3/60	4/70
	Islet cell carcinoma		0/60	2/60	1/60	0/70	2/60	0/60	0/60	1/70
Pituitary	Pars distalis-adenoma		32/60	38/60	33/60	29/70	50/60	51/60	54/60	55/70
	Pars distalis-carcinoma		0/60	0/60	0/60	0/70	1/60	0/60	0/60	2/70
	Pars intermedia-adenoma		12/60	16/60	16/60	16/70	0/60	1/60	0/60	1/70
	Pars intermedia-carcinoma		0/60	0/60	2/60	0/70	0/60	0/60	0/60	0/70
Prostate	Adenoma		1/60	1/60	0/60	0/70	–	–	–	–
Mammary gland	Fibroadenoma		1/60	0/60	0/60	0/70	14/60	17/60	18/60	20/70
	Adenoma		0/60	0/60	0/60	0/70	2/60	2/60	3/60	6/70
	Adenocarcinoma		0/60	1/60	1/60	1/70	12/60	9/60	14/60	10/60
Skin	Fibroma		0/60	0/60	0/60	1/70	1/60	0/60	0/60	0/70
	Papilloma		1/60	0/60	0/60	2/70	0/60	0/60	0/60	0/70

(continued)

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Table 12.4 (continued)

Incidence of neoplastic findings[a]										
				Males				Females		
		Dose (mg/kg)	0	2.5	15	100	0	5	30	200
Tissue	Lesion									
Testes	Interstitial cell		1/60	1/60	1/60	0/70	–	–	–	–
Thyroid	C-cell-adenoma		10/60	5/60	3/60	5/70	9/60	4/60	14/60	7/70
	C-cell-carcinoma		0/60	0/60	0/60	0/70	0/60	1/60	0/60	0/70
	Follicular cell-adenoma		3/60	4/60	2/60	4/70	1/60	3/60	2/60	2/70
Uterus	Leiomyoma		–	–	–	–	1/60	0/60	0/60	0/70
	Endometrial polyp		–	–	–	–	2/60	1/60	3/60	2/70
	Endometrial adenocarcinoma		–	–	–	–	0/60	0/60	1/60	0/70

[a]Number affected/number tested

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changes were reported in female rats given either 50 or 500 mg/kg. In males, marginal increases in interstitial cell tumors of the testis and acinar cell tumors of the pancreas were reported. No increase in liver adenomas was reported. Non-neoplastic liver lesions including hypertrophy, degeneration, and necrosis were reported in males given 50 mg/kg but not either 0.1 or 1 mg/kg.

12.1.4 Initiation/Promotion Studies

Both the ammonium and sodium salts of PFOA have been evaluated in a battery of genotoxicity tests (Butenhoff et al. 2014). Although PFOA is a hepatocarcinogen, the weight of evidence from these studies supports the position that PFOA is non-genotoxic and non-mutagenic. Consistent with PFOA being a non-genotoxic hepatocarcinogen, initiation-promotion studies have demonstrated that PFOA is an initiator of liver tumors.

In an initiation promotion study, rats were initiated with diethylnitrosamine (DEN), fed 2-acetylaminofluorene (AAF), and given a single dose of carbon tetrachloride (CCl_4) (Abdellatif et al. 1990). Following this, a group of 12 rats were fed a diet containing 150 ppm APFO for 7 months. Rats were then sacrificed for microscopic examination of the liver. The incidence of hepatocellular carcinomas was 33 % in the APFO fed rats compared to 0 % in the controls. APFO produced an increase in hydrogen peroxide (H_2O_2), catalase, and fatty acid beta-oxidation while having no effect on glycolate oxidase (leads to production of H_2O_2) and urate oxidase (an enzyme not found in humans) and serum triglyceride levels. The cancer effect was attributed in the overproduction of H_2O_2, an effect commonly seen with peroxisome proliferators.

Many peroxisome proliferators have been shown as promoters of liver tumors in rodents including WY-14,643, nafenopin, dichlorophenyl, trichloroethylene, and DEHP (Cook et al. 1999). As mentioned, it has been hypothesized that DNA damage is mediated by a reactive molecular species derived from H_2O_2 generated by peroxisomes during beta-oxidation of fatty acids. Following PFOA exposure, peroxide is observed in rat livers as the result of beta-oxidation. Double bond conjugation and peroxidation of membrane lipids leads to lipofuscin accumulation suggesting oxidative damage.

In a study, a single dose of 200 mg/kg DEN followed 2 weeks later by feeding of 0.03 % AAF for 2 weeks (reference group) or DEN followed 2 weeks later by a single 2 mg/kg CCl_4 dose followed by feeding of 150 ppm PFOA for 2 weeks was conducted (Nilsson et al. 1991). Hepatocellular carcinomas were found in 3 of 12 PFOA-fed rats while 0/12 were seen in the reference group. Focal nodules were seen in both groups, six in the reference group and eight in the PFOA-fed group. Liver weight increases and well as increase acyl Coenzyme A, dicarbonyl Coenzyme A, catalase, and decreased triglyceride levels were seen.

A study of rainbow trout with up to 9 months of chronic exposure to PFOA alone did not produce an increase in liver tumor incidence (Tilton et al. 2008). Trout,

initiated with aflatoxin B1 in water at 0.01 ppm for 30 min, control water for 3Months, then PFOA at either 200 or 1,800 ppm (equivalent to either 5 or 50 mg/kg) for 6 months, showed a very weak promotion effect. Aflatoxin alone resulted in 36 % of the trout developing liver tumors. In aflatoxin B1 initiated trout, the liver tumor incidence in the 200 ppm PFOA group was 34 % while for those given 1,800 ppm PFOA, it was 71 %. The multiplicity of tumors at the higher dose of PFOA was increased with 10 % having six or more tumors. In this experiment, the PPARα agonist DEHP lead to a 100 % liver tumor incidence while no increase was seen with clofibrate.

12.1.5 Studies on the Mechanism of Action

The liver is the main target for perfluoroalkyl compounds in animals. Liver toxicity in rodents results from the ability of these compounds to activate the peroxisome-proliferator-activated-receptor (PPARα), a member of the nuclear receptor superfamily. Studies of PPARα in various species have shown the rat and mouse to be the most sensitive species in response to PPARα agonist, hamsters are moderately responsive, and guinea pigs, primates, and humans are less responsive. Activation of the receptor in rodents initiates a characteristic sequence of biochemical and morphological events mainly in the liver. These events include marked hepatocellular hypertrophy due to an increase in both the number and size of the peroxisomes, a large increase in peroxisomal fatty acid beta-oxidation, an increase in CYP450 mediated gamma-hydroxylation of lauric acid, and alterations in lipid metabolism. PPARα regulates lipid homeostasis through the modulation of expression of genes involved in fatty acid uptake, activation, and oxidation. Both PFOA and PFOS are relatively weak ligands compared to the naturally-occurring long-chain fatty acids such as linoleic and alpha-linoleic acid (Vanden Heuvel et al. 2006).

PFOA appears to induce liver tumors via binding to the PPARα nuclear receptor resulting in peroxisome proliferation and increased liver mitogenesis (Biegel et al. 2001; Maloney and Waxman 1999; Pastoor et al. 1987). The key events following PPARα ligands activating PPARα involve regulation of the transcription of genes involved in peroxisome proliferation, cell cycle/apoptosis, and lipid metabolism. This leads to perturbations in cell proliferation, apoptosis, and peroxisome proliferation. Suppression of apoptosis along with a stimulation of cell proliferation allows DNA-damaged cells to persist and proliferate giving rise to preneoplastic foci. Clonal expansion then leads to tumor formation.

A number of events have an influence on this process. Peroxisome proliferation may lead to oxidative stress which could cause indirect DNA damage or by stimulation of cell proliferation. PPARα ligands also inhibit gap junction intercellular communication and stimulate non-parenchymal hepatic Kupffer cells, both of which could induce cell proliferation. The evidence for these key events from PPARα activation to selective clonal expansion to yield liver tumors is quite convincing (Klaunig et al. 2003).

PFOA has been demonstrated to activate PPARα (Pastoor et al. 1987; Maloney and Waxman 1999). In PPARα knockout mice, PFOA did not increase beta-oxidation unlike that readily produced in wild-type mice (Yang et al. 2002). PFOA induction of hepatomegaly, peroxisomal beta-oxidation, microsomal 1-acylglycerophosphocholine acetyltransferase, and cytosolic long-chain acyl CoA hydrolase can be blocked in castrated male rats showing the effect to be related to the elimination rate (Kawashima et al. 1995). Several key endpoints which could be the initiating effect leading to liver tumors, (and possibly pancreas and testicular tumors) were shown to be modified by PFOA (Liu et al. 1996). These include increasing liver weight, hepatic beta-oxidation, hepatic aromatase (CYP19A1), and hepatic total cytochrome P450. These changes were observed in the 2-year rat study with PFOA (Biegel et al. 2001).

The induction of Leydig cell tumors by PFOA is postulated to be due to a hormonal mechanism whereby PFOA inhibits testosterone biosynthesis and increases serum estradiol levels via induction of hepatic aromatase activity (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996). This mechanism appears to be influenced and perhaps mediated by PPARα. The induction of pancreatic acinar cell tumors is postulated to be secondary to the liver effects, specifically a sustained increase in plasma cholecystokinin (CCK) secondary to reduced bile flow or altered bile acid composition resulting in an indirect inhibition of trypsin. An indirect inhibition of trypsin by WY-14,643 (a strong PPARα activator) results in an increase in CCK levels (Obourn et al. 1997).

Like some other PPARα agonists, PFOA induces hepatocellular adenoma, Leydig cell adenomas, and pancreatic acinar cell adenomas in rats. Although humans possess PPARα at sufficient levels to mediate the hypolipidemic response to therapeutic fibrate drugs, there are enough qualitative and quantitative differences between the response of the human liver to PPARα agonists relative to the response of the rat liver. These differences include gene promoters, receptor activities, and receptor levels that make the mode of action for liver tumors unlikely to be operative in humans. There is inadequate evidence to link PPARα and the induction of either Leydig cell adenomas or pancreatic acinar cell adenomas. Additionally, there is insufficient evidence to link other mode-of-actions with PFOA-induced testicular or pancreatic adenomas.

12.1.6 Ancillary Information

The occurrence of liver tumor with two other peroxisome proliferators, WY14643 and DEHP, was studied in both in wild-type and PPARα null mice (Ito et al. 2007). Mice fed DEHP at 12,000 ppm for 6 months developed liver enlargement, an increased number of peroxisomes, and eosinophilia, a series of findings not seen in the PPARα null mice. In this report, groups of Sv/129 mice, either null or wild type, were fed either 100 or 500 ppm DEHP from 3 weeks of age to 23Months. The incidence of hepatocellular adenomas (and possibly carcinomas) was slightly

increased in the null mice. Inflammatory cell infiltration and 8-OHdG levels (oxidative stress) were higher in null mice than wild type- both elevated from controls suggesting that oxidative stress may lead to induction of inflammation, expression of proto-oncogenes, and an increase in tumors in null mice. Thus, different mechanisms were shown to induce hepatocellular tumors in wild-type and PPARα null mice (Ito et al. 2007; Takashima et al. 2008). The mechanistic hypothesis included that either oxidative stress from increased beta-oxidation induced by peroxisome proliferators produces excess ROS leading to DNA damage and cancer or an imbalance in hepatocyte growth control reflected by increased cell proliferation and suppression of apoptosis disrupting hepatocyte growth control. The authors concluded that most likely both contributed.

To look at the activity of aromatase as a mechanism for the increased estradiol observed, rats were given oral gavage doses of 0, 0.1, 2, 20, or 40 mg APFO/kg for 14 days with a pair-fed group matching the top dose (Liu et al. 1996). Both testicular and hepatic aromatase activity along with body weights, liver weights, microsomal protein, and estradiol were measured. Aromatase activity in the liver was increased by up to 16 times but no significant effect on this testicular enzyme was seen. Body weight decreases were seen as well as increased liver weight along with increased hepatic beta-oxidation, cytochrome P450 activity, and protein content of microsomes. A doubling of serum estradiol was seen along with a linear correlation between serum estradiol and hepatic aromatase.

12.1.7 PFOA as an Anti-tumorigenic Agent

Some PPARα ligands have been shown to possess anti-tumorigenic properties, such as suppression of growth of several types of human cancer cells *in vitro* and inhibition of carcinogenesis *in vivo* making PPARα a potential candidate for cancer therapy (Pozzi and Capdevila 2008). PPARα ligands such as fibrates, which cause tumors in rodents, are commonly used therapeutically in humans with no evidence of carcinogenicity (Peters et al. 2005).

A Phase 1 clinical trial was conducted to assess the tolerability, safety, and pharmacokinetics of APFO administered orally once a week to human patients (Macpherson et al. 2010). A total of 42 patients, who had both advanced refractory solid tumors and clinically normal liver and kidney function were enrolled. Dose escalation, starting with a dose of 50 mg once a week followed a standard 3+3 design until dose-limiting toxicity was observed in two or more patients. The largest group of patients presented with colorectal cancer (N=16) with pancreas, esophageal, and kidney cancers, each represented by two of more patients. Doses of up to 1,200 mg were tested without producing clinical changes in either the liver or kidney; thus, the goal of finding a dose-limiting toxicity was not attained. As a practical matter, APFO was given in 50 mg capsules so those given the highest dose tested needed to take 24 capsules orally. Although the study was not designed to evaluate efficacy, stable disease at 12 weeks or greater was observed in eight of the

first 37 patients enrolled and included a case with anaplastic thyroid at 40 weeks, one with pancreatic cancer at 35 weeks, and one with cervical cancer at 34 weeks. Further studies have not been conducted at this time.

The proposed mechanism for the anti-tumorigenic response is through inhibition of PIM-1 kinase. PIM proteins belong to a family of serine and threonine kinases which play a role in cell cycle regulation and have a potent anti-apoptotic activity. Increased expression of PIM kinase is associated with malignant subtypes of leukemia and lymphoma (Adam et al. 2006; Cohen et al. 2004; Brault 2010) and a number of solid tumors including pancreatic (Li et al. 2006; Chen et al. 2009; Reiser-Erkan et al. 2008), colorectal (Popivanova 2007), esophageal (Beier et al. 2007), and prostate (Chen et al. 2005; Mumenthaler et al. 2009; Roh et al. 2008) cancers.

APFO has been tested in four human tumor xenograft models, HT-29 (colon), PC3 (prostate), PANC-1 (pancreatic), and HepG2 (liver) (Elcombe et al. 2011). Anti-tumor effects were detected in all models. No significant toxicity was observed in the treated mice although there was liver weight enlargement and some evidence of changes in liver enzyme function. The effect of PFOA on HT-29 (colon adenocarcinoma) tumors was assessed in nude mouse xenografts. Mice were inoculated with a tumor cell suspension on each flank and tumors were allowed to grow for 16 days. APFO was given by intraperitoneal injection of 25 mg/kg 3 times a week for 4 weeks. The HT-29 tumor volumes at 30 days were 280 mm^3 in the saline injected controls compared to 175 mm^3 in the APFO treated mice. Relatively few animals were used in each group but a suggestion of anti-tumor effect was noted. In a parallel experiment using a prostate tumor cell line PC3, APFO intraperitoneal doses of either 5, 15, or 25 mg/kg were used. All of the APFO mice showed decreased tumor volume with a volume of 10 mm^3 in the highest APFO group compared to 50 mm^3 in the saline-treated controls.

Two other xenograft models were tested with similar results. Using the human pancreatic cell line PANC-1 (a slow growing tumor in vivo), a fourfold increase in size over a 90-day test period was seen in the controls compared to a 2.5-fold increase in mice receiving 25 mg/kg APFO. Tumor weights were 0.5 g in the APFO-treated mice compared to 1.2 g in the controls. A lesser response was seen in a test using a xenograft model of liver carcinoma in cell line HepG2. After 24 days of test, APFO-treated mice had a tumor volume of 1,000 mm^3 and a weight of 1.5 g while the controls had a volume of 1,200 mm^3 and a weight of 1.8 g.

12.2 Studies Involving Exposed Humans

12.2.1 General Epidemiologic Concepts

For the purpose of this review, risk estimates reported by epidemiologic studies are described as measures of potential associations between cancer, either as all cancers or for specific diagnostic types, and PFOA and other perfluorinated alkyl acids

(PFAAs) including PFOS. For mortality studies, typically reported for occupational cohorts, the Standardized Mortality Ratio (SMR) is estimated as the ratio of observed number of cancer deaths among a study group relative to an expected count of cancer deaths estimated from a defined reference population rate (Checkoway et al. 2004). In addition, relative risk (RR) estimates evaluate the probability of a cancer death or diagnosis among those assigned to a higher exposure category relative to those persons classified as less exposed. A related measure of RR is the odds ratio (OR) which is a measure of association based on the same relative comparison of exposure groups and describes the odds of having cancer among exposed cases relative to the odds of not having cancer among exposed controls (Gordis 2009). Finally, several studies report the hazard ratio (HR) which is estimated using a proportional hazards (PH) model, usually the Cox PH model. The HR is the ratio of the rate of cancer events between different levels of exposure using time to the event (*i.e.*, cancer diagnosis or death) as the time-scale variable. An increased HR indicates an earlier occurrence of the event among the exposed group relative to the reference group assuming that the underlying hazard rates are proportional for the two groups (Kleinbaum 1996).

For all measures of risk, estimates are presented with the reported 95 % confidence interval (CI) as a standard convention. In addition, statistical significance of risk estimates is interpreted based on the lower and upper values of the 95 % CI and the corresponding p-value for the association. Risk estimates measuring association between exposure and cancer are considered to be significant when the 95 % CI does not include 1.0 in its range, consistent with $p<0.05$. Associations that have a reported 95 % CI that does include the value 1.0 (*i.e.*, $p>0.05$), cannot exclude random chance as an explanation for the measured association. Many published studies emphasize observed increased risk estimates that are not statistically significant when describing and interpreting results; however, these estimates are not indicative of a valid, non-random increase in risk any more than non-significant risk estimates less than 1.0 point to a possible lowering of risk associated with exposure.

Studies may categorize exposure for study participants by defining a classification approach based on subjective levels of exposure potential or by applying a quantitative distribution such as quartiles. These studies may also present a test for trend, usually indicated by a p-value for an analysis of the ordered categorical risk estimates. In many cases, the trend test is based on the assumption of a monotonic relationship between exposure category and outcome as evaluated by a linear regression model. The p-value from such tests conflates the test of significance for the slope coefficient from a regression model with an assumed monotonic dose-response without estimation of the actual exposure-response relationship at biologically plausible exposure values (Maclure and Greenland 1992). In particular, this approach is problematic when applied to a naive method such as percentile classification (*i.e.*, quartiled exposure groups) when exposure is within a very narrow range of values (Greenland 1995). Caution should be taken into account when interpreting studies that report non-significant categorical associations but rely on a significant trend test p-value for inference of an association.

12.2.2 Occupational Studies – PFOA

A number of studies have looked at the potential carcinogenic effects of PFOA in exposed persons, particularly exposed chemical workers. These studies include workers based in manufacturing plants using the chemical for industrial purposes with occupational exposure to PFOA estimated by a job exposure matrix (JEM) approach. The occupational cohorts studied have involved industrial facilities of the 3M Corporation (manufacturing plants at Cottage Grove, Minnesota and Decatur, Alabama), and DuPont (a polymer production facility, the Washington Works plant, located in Washington, West Virginia). The DuPont plant primarily used APFO in polyethylene production processes. A separate cohort study for tetrafluoroethylene (TFE) synthesis and polymerization workers comprised workers at six facilities operated by four companies including employees from the DuPont Washington Works facility. In addition, there are a series of studies among a community population who were residents of 6 water districts in the Mid-Ohio River Valley in Ohio and West Virginia exposed to drinking water contaminated with PFOA. Exposure assessment for the Mid-Ohio River Valley community studies included both measurement of concentrations in blood serum samples as well as cumulative estimates of drinking water exposure determined by environmental fate and transport modeling. A third group of studies include individual population-based studies of various human cancers among persons with general background levels of exposure to PFOA as measured by serum concentrations taken from biologic samples.

A proportional mortality analysis among 3M plant workers exposed to industrial fluorochemicals including primarily PFOA and PFOS at the Cottage Grove plant was reported (Ubel et al. 1980). A total of 3,688 employees employed during the years 1948 to 1978 were included in the cohort and 180 deaths were recorded through the end of follow-up (159 males and 21 females) of which 177 were matched with death certificates providing information as to underlying cause. The number of female deaths was considered to be too few for meaningful statistical analysis. Among male workers, observed mortality counts agreed with expected numbers for specific causes of death due to cancer. This study provides limited evidence to evaluate the potential association between PFOA and PFOS exposures and cancer mortality with no notable increases observed among fluorochemical workers at the Cottage Grove plant.

A subsequent retrospective cohort mortality involving 2,788 males and 749 females employed at the Cottage Grove plant from 1947 to 1983 was reported (Gilliland and Mandel 1993). Inclusion in the PFOA-exposed category was based on any job history in the Chemical Division for 1 month or more while the unexposed category comprised workers who either never worked in Chemical Division or did so for less than 1 month. Vital status was ascertained through 1989 for the cohort and expected mortality numbers were estimated from United States (U.S.) and Minnesota population rates. For all female employees, the overall cancer SMR was 0.71 (95 % CI: 0.42, 1.14) with no significant increase for any specific cancer

type. The overall cancer SMR for all male employees was 1.05 (95 % CI: 0.86, 1.27) with no significant increase for any single cancer type.

Among the 1,339 male workers who worked at least 1 month or more in the Chemical Division, no significantly increased SMRs were reported for cancers of the gastrointestinal tract including specific results for the colon and pancreas, respiratory tract including the lung, testis, bladder, or lymphopoietic system including leukemia. The authors note that for prostate cancer deaths, workers in the Chemical Division had an SMR of 2.0 (95 % CI: 0.6, 4.6) for four observed deaths compared to approximately two deaths that were expected based on Minnesota White male mortality rates. Among these four cases, only one of the employees appears to have worked directly in the PFOA production building (Olsen et al. 1998).

Gilliland and Mandel (1993) included the use of an internal cohort of non-Chemical Division workers considered to be non-exposed as a comparison group to minimize the potential for the healthy worker effect, a bias widely noted when observed mortality is lower for occupational cohorts relative to expected mortality based on general population rates (Monson 1986). The authors applied a proportional hazards regression model to estimate the HR for all cancer deaths and for prostate cancer deaths among male employees for four occupational metrics: year and age of first employment, duration of employment, and months spent in the chemical division. Although, all cancer deaths were not significantly increased with increasing number of months in the Chemical Division, the rate of prostate cancer death was significantly increased for each month spent in the Chemical Division. The estimate of the HR for each year in the chemical division associated with prostate cancer mortality was 1.13 (95 % CI: 1.01, 1.27); however, the authors note that this finding is based on a small number of cases and could be biased by unmeasured confounders as occupational exposure to PFOA or PFOS was not estimated for any worker.

An updated mortality study in a cohort of 3,993 employees at the Cottage Grove plant was reported (Lundin et al. 2009). Three general categories of PFOA exposure were identified: ever definite exposure (primarily jobs in electrochemical fluorination), probable occupational exposure (jobs in other Chemical Division areas where exposure was possible but assumed to be lower and transient), and no or minimal exposure (jobs in the Non-Chemical Division of the plant). No increase in the SMR for deaths from all cancers was seen in any of the three groups. The all cancer SMRs were 0.9 (95 % CI: 0.5, 1.4), 0.9 (95 % CI: 0.8, 1.1), and 0.8 (95 % CI: 0.6, 1.0) in the ever definite exposure group, the probable exposure group, and the minimal exposure group, respectively. SMRs for cancers of the biliary passages and liver; pancreas; respiratory cancers of the trachea, bronchus, and lung; and bladder and other urinary organs showed no evidence of exposure-related associations. The prostate cancer SMRs were 2.1 (95 % CI: 0.4, 6.1), 0.9 (95 % CI: 0.4, 1.8), and 0.4 (95 % CI: 0.1, 0.9) in the ever definite exposure group, the probable exposure group, and the minimal exposure group, respectively.

Lundin and co-authors (2009) created additional exposure categories: high exposure (included workers with definite exposure for 6 months or more), moderate exposure (included workers with probable exposure or those with definite exposure for less than 6 months), and low exposure (included workers primarily in the

nonchemical division of the plant). Prostate cancer mortality was significantly increased among workers in the high exposure group (HR = 6.6, 95 % CI: 1.1, 37.7, two deaths) with a non-significant increase estimated for the moderate exposure group (HR = 3.0, 95 % CI: 0.9, 9.7, ten deaths) when compared to the low exposure group (four deaths). For the combined high and moderate exposure groups, the HR was 3.2 (95 % CI: 1.0, 10.3, 12 deaths) when compared to the low exposure group. Interpretation of the relative risk estimates for prostate cancer mortality is complicated by a deficit of prostate cancer mortality in the low exposure group which was assigned as the referent group. Workers in this exposure category had an abnormally low occurrence of prostate cancer death as indicated by a significantly reduced SMR when compared to expected prostate cancer deaths based on the Minnesota male population (SMR = 0.4, 95 % CI: 0.1, 0.9). The authors cautioned that the prostate cancer risk should be elucidated using incident cases, rather than deaths from the disease.

In the most recent report from this cohort, both cancer mortality and incidence were assessed for two groups of 3M workers comprising 9,027 total employees (Raleigh et al. 2014). The cohort included 4,668 workers with potential occupational exposure to PFOA at the Cottage Grove plant and 4,359 workers with no occupational exposure to PFOA at a non-related production facility in St. Paul, Minnesota. Mortality and cancer incidence for this combined cohort were determined from linkage of workers with the National Death Index and with cancer registries for the states of Minnesota and Wisconsin. Industrial hygiene data and expert evaluation were used to create a task-based JEM to estimate cumulative PFOA exposure. SMRs were estimated using expected mortality numbers based on Minnesota population mortality rates. HRs for time-dependent cumulative PFOA exposure were estimated from an extended Cox PH model. Outcomes of *a priori* interest included mortality and incidence for cancers of the liver, pancreas, testes, kidney, prostate, and breast.

Observed mortality counts in the PFOA-exposed cohort were less than the numbers expected for deaths based on Minnesota residents resulting in SMRs less than 1.0 for all listed cancers (Table 12.5). When assessing selected causes of deaths based on cumulative PFOA exposure categorized by quartiles, the HRs for mortality from cancer outcomes of interest did not show an association with increasing exposure. Similarly, there was little evidence that incident cancers were associated with PFOA exposure (Table 12.6). Compared to the non-exposed population of workers from the St. Paul facility, there were no significant HRs observed for incident cancers in the combined two highest exposure quartiles of PFOA among workers at the Cottage Grove plant. No association was observed between PFOA exposure and incident cases of kidney, prostate, or breast cancer when analyzed by quartile of cumulative exposure. The authors conclude that this analysis did not support an association between occupational exposure and cancer mortality or incidence but caution that for some of the cancers of interest, the study had limited ability to detect a precise association due to small numbers of cases.

Cancer mortality among workers at the DuPont Washington Works plant has been reported by Leonard et al. (2008) who conducted a study with the primary

Table 12.5 Standardized mortality ratios (SMR) for selected causes of death for the Cottage Grove and Saint Paul cohorts

	Cottage Grove plant		Saint Paul plant	
Cause	Obs	SMR (95 % CI)	Obs	SMR (95 % CI)
All causes	1,125	0.85 (0.80, 0.90)*	1,829	0.98 (0.94, 1.03)
All cancers	332	0.87 (0.78, 0.97)*	514	1.04 (0.95, 1.13)
Liver cancer	8	0.81 (0.35, 1.59)	7	0.55 (0.22, 1.14)
Pancreatic cancer	18	0.85 (0.50, 1.34)	30	1.09 (0.74, 1.56)
Prostate cancer	24	0.83 (0.53, 1.23)	48	1.03 (0.76, 1.37)
Kidney cancer	6	0.53 (0.20, 1.16)	18	1.23 (0.73, 1.95)
Breast cancer	11	0.82 (0.41, 1.47)	15	1.39 (0.78, 2.29)
Bladder cancer	8	0.89 (0.38, 1.76)	8	0.62 (0.27, 1.22)
Diabetes mellitus	27	0.76 (0.50, 1.11)	64	1.42 (1.09, 1.81)*
Ischaemic heart disease	248	0.84 (0.74, 0.95)*	444	0.95 (0.87, 1.05)
Cerebrovascular disease	57	0.81 (0.61, 1.05)	112	1.02 (0.84, 1.23)
Chronic renal disease	14	1.09 (0.60, 1.84)	13	0.72 (0.38, 1.24)

From Raleigh et al. (2014)
*Statistically significant ($p \leq 0.05$)

Table 12.6 Hazard Ratios for selected cancers comparing APFO exposure quartiles to the referent population

	Exposure quartile									
	Referent		Q1		Q2		Q3		Q4	
Cancer	Obs	HR(95 % CI)	Obs	HR(95 % CI)	Obs	HR(95 % CI)	Obs	HR(95 % CI)	Obs	HR (95 % CI)
Prostate cancer	253	1 (referent)	42	0.80 (0.57, 1.11)	42	0.85 (0.61, 1.19)	49	0.89 (0.66, 1.21)	55	1.11 (0.82, 1.49)
Kidney cancer	19	1 (referent)	4	1.07 (0.36, 3.16)	4	1.07 (0.36, 3.17)	4	0.98 (0.33, 2.92)	4	0.73 (0.21, 2.48)
Pancreatic cancer	15	1 (referent)	Combined with Q2		1	0.13 (0.02, 1.03)	Combined with Q4		9	1.36 (0.59, 3.11)
Bladder cancer	43	1 (referent)	7	0.81 (0.36, 1.81)	6	0.78 (0.33, 1.85)	15	1.50 (0.80, 2.81)	12	1.66 (0.86, 3.18)
Breast cancer	28	1 (referent)	8	0.36 (0.16, 0.79)	8	0.65 (0.29, 1.42)	14	1.47 (0.77, 2.80)	4	0.85 (0.29, 2.46)

From Raleigh et al. (2014)
Referent population = Saint Paul, MN plant

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Table 12.7 Cancer mortality for DuPont Washington works employees compared to three external reference populations

	WW cohort	US population			WV population			DuPont region I workers		
Cause of death	O	E	SMR	CI	E	SMR	CI	E	SMR	CI
All malignant neoplasms	234	315	0.74	0.65, 0.84*	340	0.69	0.60, 0.78*	229	1.02	0.89, 1.16
Liver	8	8.1	0.99	0.43, 1.96	6.9	1.15	0.50, 2.27	5.5	1.45	0.63, 2.86
Pancreas	11	15.4	0.71	0.36, 1.28	13.7	0.80	0.40, 1.43	11.2	0.98	0.49, 1.76
Breast	2	3.7	0.55	0.07, 1.97	3.5	0.57	0.07, 2.05	2.8	0.70	0.09, 2.54
Prostate	12	23.2	0.52	0.27, 0.91*	20.9	0.58	0.30, 1.00	18.4	0.65	0.34, 1.14
Testes	1	1.2	0.87	0.02, 4.84	1.3	0.76	0.02, 4.22	0.6	1.70	0.04, 9.46
Kidney	12	7.9	1.52	0.78, 2.65	7.9	1.51	0.78, 2.64	6.6	1.81	0.94, 3.16
Thyroid/other endocrine glands	3	1.0	3.12	0.64, 9.12	1.1	2.86	0.59, 8.35	0.5	6.29	1.30, 18.37*

From Leonard et al. (2008)
O observed, *E* expected, *SMR* standardized mortality ratio, *CI* = 95 % confidence interval
*Statistically significant ($p \leq 0.05$)

objective to determine if mortality from ischemic heart disease was increased in a cohort based on a previous association between PFOA exposure and increased lipids (Sakr et al. 2009). The secondary objective of the study was to examine a broad range of other causes of mortality including cancer outcomes. The cohort included 6,027 individuals working at the plant from January 1, 1948 through December 31, 2002, the end date for mortality ascertainment. SMRs were estimated based on three reference population rates: the U.S. population, the West Virginia population, and an eight-state regional population of over 74,000 DuPont employees with no work history at the Washington Works facility. Similar to retrospective cohort study of the Cottage Grove plant, all Washington Works employees were considered to have PFOA exposure even though only 23 % of 1,025 workers who participated in a previous health survey had work assignments in PFOA areas of the plant (Sakr et al. 2007).

All cancer mortality was significantly lower among the workers compared to the U.S. and the West Virginia population rates and was no different from the DuPont employee reference rates (Table 12.7). For specific cancer mortality outcomes, no significant increases were reported for observed deaths due to liver, pancreas, testicular, prostate, or breast cancers. For kidney cancer (12 deaths observed through 2002), SMRs for workers were 1.52 (95 % CI: 0.78, 2.65) when compared to the US reference rate; 1.51 (95 % CI: 0.78, 2.64) compared to the West Virginia reference rate; and 1.81, (95 % CI: 0.94, 3.61) compared to the DuPont regional worker

reference rate. Bladder cancer mortality (seven deaths observed through 2002) was similar to expected numbers based on the U.S. and West Virginia population rates and resulted in an SMR of 1.30 (95 % CI: 0.52, 2.69) when compared to the DuPont regional worker reference rate. Of interest is that the SMR for prostate cancer was significantly decreased compared to the U.S. population (SMR = 0.52, 95 % CI: 0.27, 0.91), and prostate cancer SMRs were reduced compared to West Virginia residents (SMR = 0.58, 95 % CI: 0.30, 1.00) and the DuPont regional employees (SMR = 0.65, 95 % CI: 0.34, 1.14).

An update of mortality ascertainment for the Washington Works cohort extended through 2008 was reported by Steenland and Woskie (2012). The updated study observed an increase in the total number of deaths in the cohort from 806 to 1,084 during the six additional years of follow-up through December 31, 2008. This update also analyzed cancer mortality based on occupational exposure to PFOA. Using a job exposure matrix developed by Kreckmann et al. (2009), workers were assigned to one of eight job category and job group combinations for estimation of cumulative PFOA exposure (Woskie et al. 2012). Modeled serum PFOA levels among workers in each job category and group combination were correlated with measured levels by job category overall and across time to derive cumulative exposure estimates for 5,791 workers with sufficient work history records. Cumulative exposure was categorized by a quartile distribution with the lowest quartile assigned as the referent category for analyses.

The SMR for total cancer mortality did not differ significantly for plant workers in any quartile of estimated cumulative serum PFOA when compared to the DuPont regional employee reference rates (SMR = 0.93, 95 % CI: 0.83, 1.04) and was significantly lower than expected based on the U.S. reference rate (SMR = 0.74, 95 % CI: 0.66, 0.83). Although six additional years of mortality ascertainment were added, the number of kidney cancer deaths (12) was equal to the number reported by Leonard et al. (2008) as no kidney cancer deaths occurred among cohort members from 2003 to 2008. The SMR for kidney cancer among all workers combined was 1.28 (95 % CI: 0.66, 2.24) while the SMR for the highest quartile (quartile 4) of cumulative PFOA exposure was significantly increased (SMR = 2.66, 95 % CI: 1.15, 5.24) with no significant increase observed for the other exposure quartiles (quartiles 1, 2, and 3). For mesothelioma, a significant positive exposure-response trend was observed when compared to other DuPont regional workers based on six deaths (SMR = 2.85, 95 % CI: 1.05, 6.20) with five deaths observed in the highest quartile of PFOA exposure (SMR = 6.27, 95 % CI: 2.04, 14.63). The authors state that the increased SMR for mesothelioma did not appear to be specific to PFOA exposure and suggested that it was the result of co-exposure to asbestos among workers that was highly correlated with estimates of cumulative PFOA exposure.

In addition, Steenland et al. (2015) presented additional analyses based on medical record review among 3,713 workers at the Washington Works facility. Eighteen disease outcomes with incident cases greater than or equal to 20 were analyzed. Among the four incident cancer outcomes reported, prostate cancer showed a positive non-significant trend (p-value for categorical trend test = 0.11, 129 cases). Bladder cancer had a significant negative trend such that higher PFOA exposure

Table 12.8 Mortality by cumulative exposure to APFO (unit–years) among 4,773 male workers ever exposed to TFE, 1950–2008

Cumulative exposure to APFO (unit-years)				
Cause of death				
	Never exposed	Low (<16)	Medium (16–138)	High (139+)
	Obs/Exp	Obs/Exp	Obs/Exp	Obs/Exp
	SMR (95 % CI)	SMR (95 % CI)	SMR (95 % CI)	SMR (95 % CI)
All causes	101/132.3	178/243.3	178/220.9	178/225.2
	0.76 (0.62, 0.93)*	0.73 (0.63, 0.85)*	0.81 (0.69, 0.93)*	0.79 (0.68, 0.92)*
All cancer	28/40.1	51/65.8	53/65.4	55/70.3
	0.70 (0.46, 1.01)	0.78 (0.58, 1.02)	0.81 (0.61, 1.06)	0.78 (0.59, 1.02)
Esophageal cancer	0/1.3	4/2.5	4/2.6	3/2.6
	–	1.62 (0.44, 4.14)	1.54 (0.42, 3.93)	1.16 (0.24, 3.39)
Liver cancer	1/1.4	1/1.4	2/1.6	4/1.9
	0.72 (0.02, 4.02)	0.70 (0.02, 3.87)	1.25 (0.15, 4.52)	2.14 (0.58, 5.49)
Pancreatic cancer	3/1.8	0/3.2	4/3.1	6/3.3
	1.66 (0.34, 4.84)	–	1.30 (0.35, 3.33)	1.84 (0.67, 4.00)
Lung cancer	10/13.3	20/21.9	16/21.3	13/23.9
	0.75 (0.36, 1.39)	0.91 (0.56, 1.41)	0.75 (0.43, 1.22)	0.54 (0.29, 0.93)*
Kidney and other urinary organs cancer	0/1.0	3/1.9	3/2.0	4/2.0
	–	1.57 (0.32, 4.59)	1.50 (0.31, 4.39)	2.00 (0.54, 5.12)
Leukemia	1/1.3	4/2.4	3/2.2	4/2.2
	0.79 (0.02, 4.40)	1.64 (0.45, 4.20)	1.35 (0.28, 3.94)	1.85 (0.50, 4.74)

Reference: National Rates
From Consonni et al. (2013) supplement
*Statistically significant ($p \leq 0.05$)

quartiles had a lower relative risk for this incident disease (p-value for log cumulative exposure trend = 0.04, 29 cases). No significant trend tests were reported for either colorectal cancer (41 cases) or melanoma (41 cases) (Steenland et al. 2015).

A retrospective cohort mortality study including 5,879 male workers from six tetrafluoroethylene (TFE) production sites in Europe and the U.S. was reported (Consonni et al. 2013). Occupational TFE exposure was the main focus with cumulative exposure to PFOA estimated using an exposure matrix that was highly correlated with TFE exposure estimates (Sleeuwenhoek and Cherrie 2012). The TFE study sites differed for duration of ascertainment period with an average of 25 years of follow-up overall. Among 4,205 workers classified as ever having occupational exposure to PFOA among those workers with TFE exposure, the SMR for all cancer deaths was significantly reduced (SMR = 0.79, 95 % CI: 0.67, 0.92) compared to an expected number estimated from national rates (Table 12.8 – Consonni

et al. 2013 supplement). For workers categorized in the highest tertile of cumulative PFOA exposure, there was no significant increased SMR for cancers of the esophagus, liver, pancreas, lung, or kidney, or for leukemia. The authors conclude that no exposure-response trend was observed for any of these outcomes and the study was limited by the inability to separate the potential effects from either PFOA or TFE.

12.2.3 Occupational Studies – PFOS

All available epidemiologic studies of cancer risk and occupational exposure to PFOS have been conducted among the employee cohort at a 3M facility in Decatur, Alabama that manufactured PFOS-based fluorochemicals in its chemical division from 1961 to 2002. Because the Decatur plant primarily manufactured PFOS-based chemicals, it has been studied only with respect to PFOS exposure; however, PFOA is a residual by-product of PFOS production. Therefore, chemical workers were potentially exposed to PFOA as well as other chemicals (Sigurdson et al. 2003).

A retrospective cohort mortality study of individuals who worked at least 1 year at the 3M facility in Decatur, Alabama was reported (Alexander et al. 2003). The site contained two plants, one producing specialty chemicals and the other making a specialty film. Perfluorooctanesulfonyl fluoride (POSF) is the major fluorochemical produced at this plant. POSF-based products can be metabolized to PFOS in humans. A cohort of 2,083 employees with 1-year or more of employment was classified as either non-exposed, low exposed, or high exposed based on biological monitoring data for PFOS and work site. A previous study reported that the mean concentration of PFOS in chemical plant workers was approximately 900 ppb while the mean PFOS concentration in film plant workers was approximately 100 ppb. The authors assigned all workers in the film plant to the non-exposed group while the low and high exposure groups included workers at the chemical plant categorized by their potential for exposure to POSF based on job role.

A total of 39 cancer deaths occurred in the cohort through 1997. For all three groups, observed cancer mortality was lower than that expected based on general population rates. SMRs for all cancer deaths were 0.84 (95 % CI: 0.50, 1.32, 18 deaths), 0.52 (95 % CI: 0.19, 1.44, 6 deaths), and 0.73 (95 % CI: 0.41, 1.21, 15 deaths) for the high, low, and non-exposed groups, respectively. For bladder cancer, three deaths occurred in the cohort (SMR = 4.81, 95 % CI: 0.99, 14.06) with all three cases having at least 1 year in a high exposed job. The authors conclude that bladder cancer mortality in this study could not be attributed to fluorochemical exposures due to the small number of cases and the possibility for unknown exposures to other substances that are potential bladder carcinogens either at work or due to lifestyle factors such as smoking (Alexander et al. 2003).

A follow-up study was conducted to determine whether bladder cancer mortality among workers with high potential workplace exposure to POFS-based fluorochemicals was representative of the overall bladder cancer experience of the cohort

(Alexander and Olsen 2007). Exposures to PFOS were estimated from work history and weighted using biological monitoring data. Categories of exposure included: no direct workplace exposure (serum PFOS concentrations between 100 and 290 ppb), job assignments with low potential for exposure (serum concentrations between 390 and 890 ppb), and job assignments with high potential for exposure (serum concentrations between 1,300 and 1,970 ppb). Mortality ascertainment was extended through 2002 with two additional deaths due to bladder cancer observed. In addition 1,400 of 1,845 cohort members responded to a questionnaire administered in 2002 with six bladder cancer cases reported. Of these, two were validated by medical records and four were not confirmed due to lack of consent for medical record review. Combining the 11 bladder cancers for an incidence rate analysis, the authors estimated a standardized incidence ratio (SIR) for all workers of 1.41 (95 % CI: 0.79, 2.33). No SIR based on stratification by exposure potential and duration of employment in a high exposure group was significantly increased. The authors conclude that the incidence of bladder cancer in workers is similar to that of the US population.

Health claims data for 652 chemical division employees (PFOS exposed) were analyzed against claims for 659 film division workers (non-PFOS exposed) at the Decatur plant (Olsen et al. 2004). Health claims were grouped into episodes of care defined as sets of one or more claims records which could be categorized into a discrete disease diagnosis. Two analyses were conducted: one comparing the all chemical workers to all film workers, and a second analysis of 211 workers with high exposure jobs in the chemical division compared to 345 workers who had similar jobs in the film division without POSF exposure for at least 10 years. Episodes of care were compared to similar health claims for approximately 20,000 manufacturing workers of the 3M Company in the U.S. No difference in the number of episodes of care per year was seen for those in the chemical division (average 2.7 per year) compared to those in the film division (average 3.0 per year). Relative risk (RR) was estimated for the ratio of episodes of care for specific diagnoses. For prostate cancer, five episodes were seen in the chemical division compared to 3.1 expected based on company-wide rates. The film division had one prostate cancer episode compared to 4.7 expected. Overall, the results of this study appear to show that the risk of cancer in the chemical division workers exposed primarily to PFOS was no different than that of the film plant workers. For bladder cancer, no episodes of care were recorded for chemical division workers during the period of the study.

A separate study of self-reported health conditions including cancer diagnoses among 1,400 workers at the Decatur facility was conducted for responses from the 2002 questionnaire (Grice et al. 2007). PFOS-exposure groupings were based assignments made previously (Alexander and Olsen 2007). Cancer diagnoses were validated by medical record review and included 12 cases of colon cancer, 8 cases of melanoma, and 22 cases of prostate cancer. No significant association between these cancers and any of the PFOS-exposure categories was observed.

12.2.4 Studies in a Community with PFOA-Contaminated Drinking Water

The C8 Health Project, a cross-sectional survey and biomarker study in 2005 and 2006 among 69,030 residents of the mid-Ohio Valley, was conducted in response to a legal settlement from a class-action lawsuit against DuPont (Frisbee et al. 2009). The aim was to investigate the potential human health effects of PFOA exposure from contaminated drinking water. Among the series of studies conducted to address this aim, a cancer-registry based case-control study was reported assessing the relationship between PFOA exposure via drinking water and cancer in residents living in the 6 water districts with contaminated drinking water and 13 adjacent counties surrounding the DuPont Washington Works plant (Vieira et al. 2013). Data on incident cases of 18 types of cancer diagnosed from 1996 through 2005 in five Ohio and eight West Virginia counties reported to the state cancer registries for Ohio and West Virginia were used. The study included 7,869 cancer cases in Ohio and 17,238 cancer cases in West Virginia. Serum PFOA levels were estimated using combined environmental, exposure, and pharmacokinetic models and were based on residential water district at the time of diagnosis (Shin et al. 2011). For comparative analyses, the authors fit logistic regression models to estimate the adjusted odds ratio (OR) for specific types of cancer cases using incident cancers from all other cancer categories as controls after excluding cases of kidney, pancreatic, testicular, and liver cancers. These cancer types were excluded from control groups due to the previous reports of associations with PFOA.

A positive association was found between kidney cancer and either the high or very high exposure categories with ORs of 2.0 (95 % CI: 1.3, 3.2) and 2.0 (95 % CI: 1.0, 3.9) for the high and very high categories, respectively. Among the nine cases in the very high exposure group stratified by sex, the association was observed for women (OR = 3.5, 95 % CI: 1.4, 8.3, six cases), but not for men (OR = 1.0, 95 % CI: 0.3, 3.4, three cases). For testicular cancer, there was a small number of cases overall (n = 18) with ORs above 1.0 reported for the very high exposure category (OR = 2.8, 95 % CI: 0.8, 9.2, six cases) and the Little Hocking water district which had the highest estimated exposure to PFOA (OR = 5.1, 95 % CI: 1.6, 15.6, eight cases). However, no exposure –response pattern was observed as the ORs for the low to high exposure categories and the other water districts were all non-significant and less than 1.0. Associations in the very high exposure group were also noted for prostate, and ovarian cancers, and for non-Hodgkin's lymphoma. The authors note that the primary limitation to their study was the use of other cancer cases as control subjects for comparative analyses. In addition, although the study included an area with a population estimate of over 500,000 persons, precision of OR estimates was limited due to small numbers of cases for specific cancer types following categorization to exposure groups or assignment to specific water districts with varying levels of PFOA exposure.

A second C8 Science Panel study involved a retrospective cohort design that included 32,254 participants living in the mid-Ohio River Valley in one of the six

water districts near the DuPont Washington Works plant (Barry et al. 2013). Of this cohort, 3,713 had ever worked at the DuPont Washington Works facility. Among these community residents and plant workers, 2,507 validated cancer cases comprising 21 different diagnostic types were observed. Cancer risk was analyzed based on cumulative PFOA exposure estimated from residential history as described by Shin et al. (2011) combined with additional occupational exposure estimates for workers (Woskie et al. 2012). The authors fit a proportional hazards regression model for each cancer type as the outcome and age at either diagnosis or last follow-up as the time scale. HRs were estimated for time-varying cumulative exposure to PFOA calculated as the sum of yearly drinking water concentrations. PH models were adjusted for sex, 5-year birth period, educational attainment, and time-dependent measures of smoking and alcohol consumption.

In the combined cohort, positive associations were noted for testicular cancer (HR = 1.34, 95 % CI: 1.00, 1.79), kidney cancer (HR = 1.10, 95 % CI: 0.98, 1.24), and thyroid cancer (HR = 1.10, 95 % CI: 0.95, 1.26). When analyzed by cumulative exposure quartile, the HRs for 17 testicular cancer cases distributed by increasing exposure quartiles were 1.0 (referent), 1.04 (95 % CI: 0.26, 4.22), 1.91 (95 % CI: 0.47, 7.75), and 3.17 (95 % CI: 0.75, 13.45), with significant trend tests reported ($p < 0.05$ for both trend tests). For the 105 kidney cancer cases, the HRs were 1.0 (referent), 1.23 (95 % CI: 0.70, 2.17), 1.48 (95 % CI: 0.84, 2.60), and 1.58 (95 % CI: 0.88, 2.84). For 86 thyroid cancer cases, the HRs were 1.0 (referent), 1.54 (95 % CI: 0.77, 3.12), 1.48 (95 % CI: 0.74, 2.93), and 1.73 (95 % CI: 0.85, 3.54). However, trend tests across quartiles based on increasing serum PFOA concentrations were not significant for either kidney or thyroid cancers ($p > 0.10$ for all tests).

Further, HRs for the 21 cancer types were stratified for community and occupational exposure groups (Barry et al. 2013 supplement). The numbers of cases and HRs among 28,541 community members with no occupational exposure are shown in Table 12.9 with the number of cases and HRs for the occupationally exposed group listed in Table 12.10. Among community residents only, the HR for testicular cancer was significantly increased (HR = 1.73, 95 % CI: 1.24, 2.40, 15 cases) while the HR for lung cancer was significantly decreased (HR = 0.85, 95 % CI: 0.73, 1.00, 95 cases) for increasing cumulative PFOA exposure. Among those with occupational exposure to PFOA, thyroid cancer was significantly increased (HR = 1.93, 95 % CI: 1.00, 3.71, 8 cases) and bladder cancer was significantly decreased (HR = 0.65, 95 % CI: 0.44, 0.95, 29 cases) for increasing cumulative exposure.

In a separate study of persons residing in six water districts in the mid-Ohio River Valley with PFOA contamination, a health survey of 47,359 adults with one-time serum PFOA and PFOS measures taken from blood samples collected in 2005 and 2006 was conducted (Innes et al. 2014). There were 292 colorectal cancer cases reported for this group, and the authors were able to confirm 208 cases by medical record validation. The median serum PFOA concentration among all adults was 27.9 ppb considered to be elevated compared to general population levels due primarily to exposure to contaminated drinking water. Meanwhile, the median serum concentration of PFOS was 20.2 ppb which was considered similar to the general U.S. population level at the time of serum sampling. The distribution of cases across

Table 12.9 Effect of estimated cumulative PFOA serum concentration on cancer risk in the community group (n = 28,541)

Cancer	# Cases	HR (95 % CI)
Bladder	76	0.96 (0.81, 1.14)
Brain	13	1.14 (0.78, 1.65)
Breast	546	0.96 (0.90, 1.02)
Cervical	21	0.94 (0.67, 1.32)
Colorectal	223	0.98 (0.89, 1.08)
Esophagus	12	1.00 (0.66, 1.51)
Kidney	87	1.14 (0.99, 1.32)
Leukemia	53	0.92 (0.76, 1.13)
Liver	8	0.62 (0.29, 1.29)
Lung	95	0.85 (0.73, 1.00)*
Lymphoma	121	1.05 (0.92, 1.19)
Melanoma	200	0.99 (0.89, 1.10)
Oral	17	0.96 (0.65, 1.40)
Ovarian	43	1.00 (0.79,1.25)
Pancreatic	21	1.06 (0.79, 1.43)
Prostate	317	0.97 (0.90, 1.05)
Soft tissue	13	0.68 (0.40, 1.14)
Stomach	11	0.70 (0.40, 1.23)
Testicular	15	1.73 (1.24, 2.40)*
Thyroid	78	1.04 (0.89, 1.23)
Uterine	96	1.02 (0.88, 1.18)

From Barry et al. (2013) supplement
*Statistically significant ($p \leq 0.05$)

quartiles of serum PFOA and PFOS concentration was evaluated. An inverse relationship was observed such that there were fewer cases of colorectal cancer reported among those persons categorized to higher quartiles of serum PFOA and PFOS concentrations. The fully adjusted ORs for PFOA serum concentration by quartile were 1.0 (referent), 0.48 (95 % CI: 0.31, 0.75), 0.51 (95 % CI: 0.34, 0.77), and 0.64 (95 % CI: 0.44, 0.94) with a significant trend test ($p = 0.002$), although the trend was not significant when PFOA serum concentration was evaluated as a linear, continuous variable ($p = 0.46$). Moreover, a similar inverse relationship was observed for higher serum PFOS concentrations. The fully adjusted ORs by quartile were 1.0 (referent), 0.38 (95 % CI: 0.25, 0.59), 0.27 (95 % CI: 0.17, 0.42), and 0.24 (95 % CI: 0.16, 0.37) with significant trend test values for both categorical and linear tests ($p < 0.00001$). Among several limitations to this study, the authors note that the study analyzed cross-sectional data that comprised measured PFOA concentrations collected for prevalent colorectal cancer cases. This limits the ability to assess causality due to the absence of a temporal relationship between PFOA exposure and colorectal cancer as both are determined simultaneously.

Table 12.10 Effect of estimated cumulative PFOA serum concentration on cancer risk in the occupational group (n=3,713)

Cancer	# Cases	HR (95 % CI)
Bladder	29	0.65 (0.44, 0.95)*
Brain	4	0.82 (0.26, 2.59)
Breast	13	1.01 (0.59, 1.74)
Cervical	1	–
Colorectal	41	1.12 (0.81, 1.54)
Esophagus	3	1.42 (0.21, 9.74)
Kidney	18	0.95 (0.59, 1.52)
Leukemia	13	1.30 (0.73, 2.33)
Liver	1	–
Lung	13	0.87 (0.51, 1.47)
Lymphoma	15	1.24 (0.72, 2.14)
Melanoma	41	0.80 (0.59, 1.08)
Oral	1	–
Ovarian	0	–
Pancreatic	3	0.98 (0.21, 4.65)
Prostate	129	0.94 (0.77, 1.17)
Soft tissue	2	1.20 (0.30, 4.76)
Stomach	1	–
Testicular	2	0.85 (0.04, 19.7)
Thyroid	8	1.93 (1.00, 3.71)*
Uterine	7	1.05 (0.56, 1.97)

From Barry et al. (2013) supplement
*Statistically significant ($p \leq 0.05$)

12.2.5 General Population Studies

A number of studies have reported on cancer outcomes in general populations and related the finding to either PFOA or PFOS. In the blood of a representative sampling of individuals in the general population of the United States, four polyfluoroalkyl substances (PFOA, PFOS, perfluorohexane sulfonate-PFHxS, and perfluorononanoate-PFNA) have been found in more than 95 % of those sampled in the NHANES survey (Kato et al. 2011). The geometric mean serum concentrations of each of these four chemicals are presented in Table 12.11. Attributing an association between a cancer outcome and any one of these chemicals (or any of the other chemicals contained in these blood samples) must be done carefully as it is obvious from this data that multi-chemical exposures are occurring.

The association between plasma (serum) concentrations of PFOA and PFOS with cancer risk was determined for a prospective Danish cohort of 57,053 participants with no previous cancer diagnosis at enrollment (Eriksen et al. 2009). From 1997 through 2006, 1,240 incident cancer cases were ascertained through the Danish Cancer Registry. The study included 713 prostate cancer cases, 332 bladder cancer cases, 128 pancreatic cancer cases, and 67 liver cancer cases. The PFOA and

Table 12.11 Serum perfluorochemical concentrations in the general population (participants ≥12 years old)

	Geometric mean in ppb				
Sampling wave	1999–2000	2003–2004	2005–2006	2007–2008	2009–2010
Chemical					
PFOA	5.21	3.59	3.56	3.99	2.84
PFOS	30.40	19.43	15.61	13.19	8.76
PFNA	0.56	0.88	1.01	1.46	1.49
PFHxS	2.30	1.90	1.55	1.93	1.51

Individual serum measurements not available in 2001–2002
Estimated from NHANES data based on Kato et al. (2011)

PFOS concentrations for these cases were compared to concentrations for a representatively selected referent sub-cohort of 772 persons (668 men and 92 women) without a cancer diagnosis during the ascertainment period. The median PFOA concentrations ranged from 5.4 to 6.9 ppb for the cancer groups with a median concentration of 6.6 ppb for the comparison sub-cohort. For PFOS, median concentrations for the cancer groups ranged from 31.0 to 36.8 ppb and the comparison sub-cohort had a median concentration of 34.3 ppb.

For prostate cancer, the adjusted incident rate ratios (RR) for quartiles 1 through 4 for PFOA were 1.00 (referent), 1.09 (95 % CI: 0.78, 1.53), 0.94 (95 % CI: 0.67, 1.32), and 1.18 (95 % CI: 0.84, 1.65), respectively. The same analyses for PFOS estimated incident RRs of 1.00 (referent), 1.35 (95 % CI: 0.97, 1.87), 1.31 (95 % CI: 0.94, 1.82), and 1.38 (95 % CI: 0.99, 1.93), respectively. The authors note that the lack of an increasing exposure-response trend suggests that the similar risk estimates at higher PFOS concentration levels are likely due to a chance finding of a lower incidence in the referent quartile rather than an increased risk with increasing PFOS concentrations. The authors conclude that plasma concentrations of PFOA and PFOS in the general Danish population do not appear to be associated with increased risk of prostate, bladder, pancreatic, or liver cancer (Eriksen et al. 2009).

In a study of persistent organic pollutants (POPs) and breast cancer in an Inuit population, 31 breast cancer cases were selected from a hospital registry in Greenland and 115 control subjects without a cancer diagnosis were sampled from an ongoing POPs monitoring study (Bonefeld-Jorgensen et al. 2011). Serum levels of PFOA as well other perfluorinated carboxylates and sulfonates were reported at higher concentrations for those with breast cancer relative to control subjects. The median concentrations of PFOA were 2.5 ppb for breast cancer cases and 1.6 ppb for control subjects. For PFOS, the median concentrations were 45.6 ppb among breast cancer cases and 21.9 ppb for control subjects.

No significant association with breast cancer case status was observed for increasing PFOA exposure while a significant association was reported for increasing PFOS exposure. The raw (crude) OR for a 1 ppb increase in PFOA was 1.07 (95 % CI: 0.88, 1.31, 31 cases) while the raw OR for a 1 ppb increase in PFOS was

1.01 (95 % CI: 1.00, 1.02). Fewer cases and controls were included in the adjusted OR model due to missing data for the variables including age, body mass index, pregnancy and breastfeeding history, serum cotinine and menopausal status. For 1 ppb increase in PFOA, the adjusted OR was 1.20 (95 % CI: 0.77, 1.88, 7 cases). For a 1 ppb increase in PFOS, the adjusted OR was 1.03 (95 % CI: 1.00, 1.07). The authors suggest that serum persistent organic pollutants including perfluorinated compounds might be a risk factor for the development of breast cancer in this population; however, the small number of cases and the high correlation between serum PFAA levels limited the study.

In a case-control study of breast cancer among mothers enrolled in the Danish National Birth Cohort from 1996 through 2002, 250 breast cancer cases that occurred through 2010 were matched by age and parity to 233 control subjects without a cancer diagnosis (Bonefeld-Jorgensen et al. 2014). Serum levels of 16 perfluoroalkylated substances (PFAS) including 10 carboxylates and 5 sulfonates were measured for blood samples taken between the 6th and 14th week of pregnancy during enrollment. PFOA and PFOS concentrations were measured in all study subjects and found at relatively higher concentrations than all other PFASs. The mean serum levels reported for control subjects were 5.2 ppb for PFOA and 30.6 ppb for PFOS while serum concentrations for breast cancer cases are not reported. In addition, the authors noted high correlations among the PFASs with a significant correlation coefficient of 0.69 found between PFOA and PFOS concentrations. No significant associations were observed between breast cancer case status and PFOA and PFOS concentrations. Slightly fewer cases and controls were included in the adjusted OR models due to missing data for other variables including age at blood sampling, body mass index before pregnancy, gravidity, oral contraceptive use, age at menarche, alcohol intake and smoking, maternal education, and physical activity. The adjusted OR for a 1 ppb increase in PFOA was 1.00 (95 % CI: 0.90, 1.11, 221 cases) while the adjusted OR for a 1 ppb increase in PFOS was 0.99 (95 % CI: 0.98, 1.01, 221 cases). The authors also categorized the exposure distributions into quintiles and observed no pattern of increasing ORs for higher levels of PFOA and PFOS when compared to the lowest quintile assigned as the referent group. The adjusted OR for PFOA among women in the fifth quintile (PFOA concentration greater than 6.53 ppb) was 0.94 (95 % CI: 0.51, 1.76, 40 cases) while the adjusted OR for PFOS in the highest quintile (PFOS concentration greater than 39.07 ppb) was 0.90 (95 % CI: 0.47, 1.70, 35 cases) with no significant ORs observed for other exposure quintiles. Moreover, the study subjects were stratified by age at breast cancer diagnosis with analyses conducted for cases and matched controls younger than 41 years of age at case diagnosis or older than 40 years of age at case diagnosis. Similar results consistent with the overall analyses were observed in both age strata for PFOA or PFOS. The authors conclude that the results of this study indicate that there is no association between breast cancer occurrence and PFAS concentrations taken during pregnancy.

A case control study in Sweden including 201 cases of prostate cancer compared to 186 population-based controls was reported (Hardell et al. 2014). Serum concentrations of six perfluorinated carboxylates and sulfonates were measured with no

significant differences reported between cases and controls for PFOA and PFOS. The median concentrations of PFOA were 2.0 ppb for prostate cancer cases and 1.9 ppb for control subjects. For PFOS, the median concentrations for prostate cancer cases were 9.0 ppb and 8.3 ppb for control subjects. There was no significant association between prostate cancer and increased exposure defined as having a concentration above the median for any PFAA reported in the study. The OR for having a PFOA concentration above the median was 1.1 (95 % CI: 0.7, 1.7), while for PFOS, the OR for exposure above the median was 1.0 (95 % CI: 0.6, 1.5).

The authors note that they expected heredity to be a risk factor for prostate cancer with cases more likely to report having a first degree relative with prostate cancer (OR = 1.8, 95 % CI: 1.0, 3.1). After stratifying cases and controls by heredity defined as having a first degree relative with prostate cancer and PFOA concentration above the median, the ORs were 1.1 (95 % CI: 0.5, 2.6) for those with heredity and PFOA less than the median, 1.0 (95 % CI: 0.6, 1.5) for those with no heredity and PFOA greater than the median, and 2.6 (95 % CI: 1.2, 6.0) for those with heredity and PFOA greater than the median, compared to a referent group of those without hereditary prostate cancer and PFOA concentration less than the median. A statistical test for interaction between heredity and PFOA concentration greater than the median was not significant ($p = 0.15$). For PFOS, the ORs were 1.2 (95 % CI: 0.6, 2.5) for those with heredity and PFOS less than the median, 0.9 (95 % CI: 0.5, 1.4) for those with no heredity and PFOS greater than the median, and 2.7 (95 % CI: 1.0, 6.8) for those with heredity and PFOS greater than the median, compared to a referent group of those without hereditary prostate cancer and PFOS concentration less than the median. Hardell and colleagues conclude that higher concentrations of PFOA and PFOS without hereditary prostate cancer did not increase the risk of prostate cancer. They suggest that there is an interaction between genes and PFAA exposure based on the observed increased risk for those with hereditary prostate cancer; however, a possible mechanism for this interaction is unknown.

A cross-sectional study compared serum PFOA and PFOS concentrations in 40 cancer patients without a specific diagnostic type to two groups without cancer: 56 employees of a research center in urban Athens, Greece and 86 patients undergoing medical checkups in rural Argolida, Greece (Vassiliadou et al. 2010). The mean serum PFOA levels were 2.3 ppb in the cancer patients, 2.9 ppb in the Athens employees, and 1.9 ppb in the Argolida patients. For PFOS, the mean serum levels were 12.97 ppb in the cancer patients, 14.9 ppb in the urban employees, and 13.6 ppb in the rural patients. Although the results demonstrate that PFASs are detectable in the serum and liver samples from a series of patients with hepatocellular carcinoma (HCC) and hepatitis C viral infection (HCV) as well as liver donors without existing disease, the comparative results indicate no association between PFOA or PFOS and cancer status. The study was limited for a number of reasons including the small sample size, lack of specific cancer types, and no information on potential confounders, selection criteria or participation rates for the study population.

A study compared 66 diseased liver tissues removed prior to liver transplants to 25 healthy liver specimens in Melbourne, Australia (Yeung et al. 2014). Serum and liver concentrations of PFOA and 11 other PFASs were measured. Cases included

those who had undergone liver transplantation for a range of conditions including hepatocellular carcinoma (HCC), cirrhosis due to chronic hepatitis C viral infection (HCV), amyloidosis, and acute liver failure. Among those with HCC, serum concentrations from 24 samples of PFOA were somewhat higher than those for 25 liver donor control samples with mean serum concentration of 2.82 ppb in HCC patients compared to 2.38 ppb in controls; however, mean liver concentrations were 0.59 ppb (ng/g) and 0.62 ppb for these groups, respectively. For PFOS, the mean serum concentrations were 13.3 ppb for those with HCC and 8.48 ppb for controls. Mean liver concentrations of PFOS were 6.24 ppb for HCC samples and 5.22 ppb for controls. The authors suggest that some of the pathologic changes in diseased livers might alter the distribution of PFASs between liver and serum. Overall, the results do not suggest a relationship between PFASs and liver cancer. The study had numerous limitations including a small sample size and the measurement of PFOA and PFOS concentrations after liver specimen removal that preclude its ability to test for an association between HCC and PFOA and PFOS concentrations.

12.3 Reviews and Evaluations

There have been a series of reviews of the carcinogenicity of PFOA. An EPA Draft Risk Assessment (2005) reviewed both the animal and human evidence for a possible relationship between PFOA exposure and cancer risk. Overall, based on no adequate human studies and uncertain human relevance of the tumor triad from rat studies, PFOA was described as having "suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential" under the draft 1999 Guidelines for Carcinogenic Risk Assessment (U.S.EPA 1999). PFOA induces liver tumors, pancreatic acinar cell tumors, and Leydig cell tumors in male rats. There is sufficient evidence to indicate that PFOA is a PPARα agonist and that liver carcinogenicity and toxicity is mediated by binding to the PPARα receptor in the liver. A mode of action analysis demonstrated that the hepatic effects are due to PPARα agonism and that this mode of action is unlikely to occur in humans. There is not sufficient evidence to link the mode of action for both the pancreatic acinar cell tumors and the Leydig cell tumors to PPARα. However, due to the quantitative differences in the expressions of luteinizing hormone and cholecystokinin receptors and other toxicodynamic differences between the rat and the human, tumors induced in the rat by PFOA probably do not represent a significant cancer hazard for man.

A report from the Subcommittee on Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council (Health Council of the Netherlands 2013) concludes that the available data on PFOA (and its salts) are insufficient to evaluate the carcinogenic properties (Category 3 according to the system of the Health Council of the Netherlands 2010). In reviewing the human information, it was concluded that the available epidemiologic studies were of varying quality with several having significant weaknesses. Several studies report elevated risks for certain types of cancer but overall there was no cancer type that

appeared to be consistently elevated in all studies. The report pointed out that kidney cancer could be a concern as a slight elevation was reported in 2 of the 3 worker cohort studies. With regard to the animal information, the report notes that none of the three tumor types seen in the rodent studies were malignant tumors and that benign tumor development in rodents may be explained in large part by peroxisome proliferation. Thus, it was reported that these tumors appear to be rodent specific and are unlikely to have relevance for liver, pancreatic, and testicular cancers in humans.

In a critical review, Chang et al. (2014) conclude that, taken together, the epidemiologic evidence does not support the hypothesis of a causal association between PFOA or PFOS exposure and cancer risk in humans. The review included the human epidemiologic and animal toxicologic studies covered in this chapter. It is noted that the majority of the relative risk estimates in these papers for both PFOA and PFOS range between 0.5 and 2.0 with the confidence intervals including 1.0. Results suggesting a positive association are counterbalanced by negative associations, no apparent monotonic dose-response, and the lack of concurrence between the animal and human findings. The authors conclude that many of the positive associations reported for PFOA exposure in the community and general population studies were not supported by studies of occupational exposures. Since occupational exposures are often one to two orders of magnitude higher than environmental exposures, this indicates that the positive associations in the community and general populations studies are most likely due to chance, confounding, or bias.

On the basis of limited evidence in humans that PFOA causes testicular and renal cancer, and limited evidence for cancer causality in experimental animals, an IARC working group classified PFOA as possibly carcinogenic to humans (IARC group 2B). The IARC working group noted reports of increased risk of kidney cancer with a statistically significant exposure-response trend in workers in a fluoropolymer production plant in West Virginia, USA and in an exposed community near the plant (Steenland and Woskie 2012; Vieira et al. 2013). In addition, there was an increase of about threefold in the risk of testicular cancer reported in the most highly exposed residents in communities near the same plant (Vieira et al. 2013; Barry et al. 2013). However, the working group considered the evidence regarding mechanisms of PFOA-associated carcinogenesis to be limited due to the inability to exclude chance as an explanation for these findings (Benbrahim-Tallaa et al. 2014).

12.4 Conclusions

Overall, there have been a number of studies investigating cancer and exposure to PFAAs, particularly PFOA. Historically, PFOA and PFOS have been the most widely used members of this chemical class making these substances the subject of the largest proportion of reported studies. Most persons in developed countries have detectable serum concentrations of PFOA ranging from 1 to 10 ppb. PFOS has similar environmental exposure conditions and has reported serum concentrations in general populations that are somewhat higher than those for PFOA. Due to

contaminated drinking water supplies near a DuPont fluoropolymer production facility in West Virginia, residents of six neighboring water districts in West Virginia and Ohio have mean serum concentrations that range from 10 to 300 ppb. Additionally, occupationally exposed cohorts typically have serum concentrations of PFOA and PFOS with an upper range of 3,500 ppb reported in some studies.

The toxicologic evidence for carcinogenicity of PFAAs is limited to four studies evaluating the carcinogenic potential of PFOA (two studies), PFHxA (one study), and PFOS (one study) in rats. Each of these chemicals produced a different response. PFOA causes the tumor triad common to peroxisome-proliferating chemicals including adenomas of the liver, pancreas, and testes. Rats exposed to PFOS developed liver tumors, but a study of PFHxA reported no increase in tumors of any type. Considerable research has been done to elucidate a potential carcinogenic mechanism. There is evidence that the liver is the main target of PFOA exposure due to activation of PPARα. This mechanism contributes to the induction of liver tumors in rats. There is limited evidence that Leydig cell tumors may be induced by a hormonal mechanism mediated by PPARα activation. Thus, one needs to be careful when predicting the presence or absence of carcinogenic activity for other perfluorinated chemicals using the results from the available studies.

Epidemiologic studies have been reported for several levels of population exposure. Limited evidence for associations with kidney and testicular cancer has been reported by studies among community members exposed to drinking water contaminated by PFOA. These associations are not consistently reported such that random chance cannot be excluded as an explanation. Studies of workers exposed to relatively higher levels of PFOA and PFOS have not shown consistent evidence for an association with any specific cancer type. More recent incidence studies among workers from 3M (Raleigh et al. 2014) and DuPont (Barry et al. 2013 supplement) did not report similar or strong associations with specific cancer types including kidney or testicular cancers. Studies of specific tumor types among populations exposed to low levels of PFOA and PFOS have shown equivocal results for a variety of specific cancer outcomes with no consistent associations reported. Based on the evidence reported to date on PFOA and PFOS and considering the relatively low and decreasing exposures to these compounds, the prospect for developing carcinogenic outcomes is remote. For other perfluorinated chemicals, there is not sufficient evidence regarding their potential carcinogenicity, and human exposures are low and appear to be decreasing.

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ED_002330_00119699-00313

Chapter 13
Epidemiological Findings

Naila Khalil, Miryoung Lee, and Kyle Steenland

Abstract Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are man-made compounds which have been extensively used over the past 60 years. They are detectable globally in humans and animals. Among several PFAS compounds, perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorononanoic acid (PFNA) have been most examined in epidemiological studies. In the United States PFOS is found at the highest levels in human serum, followed by PFOA. Median human serum levels are dropping for most PFAS in the US since phase out of production, but they are still being used in manufacturing a variety of products. PFHxS has a much longer elimination half-life [geometric mean: GM (GM: 7.3 years)] than PFOS (GM: 4.8 years) or PFOA (GM: 3.5 years).

Serum PFOA concentration has been linked with increased serum lipids, and uric acid levels in occupational cohorts, a highly exposed community population, and general population studies. PFAS exposure has also been associated with adverse effects on thyroid homeostasis, liver enzymes, osteoarthritis, non-malignant kidney disease, and immunotoxicity, in some studies but the associations are inconsistent. Data are sparse but largely negative for Type 2 diabetes neurodegenerative disease,

N. Khalil, M.B.B.S., M.P.H., Ph.D. (✉)
Center for Global Health, Department of Community Health, Boonshoft School of Medicine, Wright State University, 3123 Research Blvd., Suite #200, Dayton, OH 45420-4006, USA
e-mail: naila.khalil@wright.edu

M. Lee, Ph.D.
Division of Epidemiology, Lifespan Health Research Center, Department of Community, Boonshoft School of Medicine, Wright State University,
3171 Research Blvd., Dayton, OH 45420-4006, USA
e-mail: miryoung.lee@wright.edu

K. Steenland, Ph.D.
Department of Environmental and Occupational Health, Rollins School of Public Health, Emory University, 1518 Clifton Road NE, Claudia Nance Rollins Bldg., 2005,
Atlanta, GA 30322, USA
e-mail: nsteenl@emory.edu

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_13

ED_002330_00119699-00314

children's cognition, adult CVD and stroke, immune function, liver disease, and obesity. Despite a large body of literature, and some positive findings regarding low birth weight, the data are overall inconsistent regarding reproductive/developmental outcomes in relation to PFAS.

In conclusion current epidemiologic evidence suggests that there is an association between PFOA and six health outcomes: high cholesterol, ulcerative colitis, thyroid disease, testicular cancer, kidney cancer, and pregnancy-induced hypertension, although some of the findings come from only one large longitudinal study of a high exposed population, and have not been replicated elsewhere. Data remains limited for health effects of other PFAS. Longitudinal studies in populations with exposure above general background levels are needed to corroborate these results and increase our understanding of PFAS exposure and health outcomes.

Keywords Perfluoroalkyl • Polyfluoroalkyl substances (PFAS) • Perfluorooctanoic acid (PFOA) • Perfluorooctane sulfonic acid (PFOS) • Perfluorohexane sulfonate (PFHxS) • Perfluorononanoic acid (PFNA) • Epidemiology

13.1 Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are man-made chemicals. PFAS were widely used over the past 60 years because of their heat stable, non-flammable properties. They are detectable globally in human, animal and aquatic environments. PFAS can bioaccumulate and biomagnify through food chain. Among several of the PFAS compounds, perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorononanoic acid (PFNA) have been addressed in epidemiological studies.

Over the past decade, the National Health and Nutrition Examination Survey (NHANES) data in the US have shown that PFOS was the dominant PFAS in human blood, followed by PFOA (Kato et al. 2011). PFHxS has a much longer elimination half-life [geometric mean: GM (GM: 7.3 years)] than PFOS (GM: 4.8 years) or PFOA (GM: 3.5 years) (Olsen et al. 2007). As described in NHANES 1999–2008 data PFOS, PFOA, PFNA, and PFHxS were detected in >95 % of participants. Since 1999–2000, PFOS concentration in US general population exhibited a significant decreasing trend due to discontinued PFOS industrial production, but PFNA concentration showed an upward trend. PFOA levels in 1999–2000 were significantly higher that other surveys, but stabilized during 2003–2008. PFHxS concentrations exhibited a downward trend from 1999 to 2006, but increased in 2007–2008 (Kato et al. 2011).

Epidemiologic studies examining PFAS exposure on human health have been conducted at three 'exposure levels': (1) occupational cohorts such as workers who were

mostly exposed at the source of contamination, (2) highly exposed communities to PFAS through water, soil, and/or air contamination, and (3) general populations such as NHANES participants exposed to PFAS at background exposure level.

Occupational studies in workers employed at the PFAS chemical plants (Olsen et al. 2003; Lundin et al. 2009) comprised of medical surveillance cross sectional studies (reviewed in Costa et al. 2009) and a few longitudinal studies (Sakr et al. 2007b; Leonard et al. 2008; Shin et al. 2011). Costa et al. summarized health outcomes of 30 years (1978–2007) of medical surveillance in PFOA exposed workers. Workers aged 20–63 years had medical examination annually including blood tests for serum chemistry and serum PFOA (Costa et al. 2009). The monitoring data and its link with workers' mortality and health effect have been published in a number of epidemiological studies (Lundin et al. 2009; Costa et al. 2009; Sakr et al. 2007b; Leonard et al. 2008; Shin et al. 2011).

The most comprehensive epidemiological data linking PFOA exposure and health outcomes has been reported from Mid-Ohio Valley communities which is ongoing. The C8 Science Panel carried out exposure and health studies in residents potentially affected by the releases of PFOA (or C8) emitted since the 1950s from the Washington Works plant in Parkersburg, West Virginia. Exposures to the community started in 1951 and peaked in the early 1990s, due to contamination of drinking water by PFOA emissions (Winquist et al. 2013; Simpson et al. 2013). In 2005–2006, as part of the settlement of a class action lawsuit, a community survey called the C8 Health Project was conducted. In this survey, approximately 69,030 people who lived in one of six contaminated water districts in West Virginia and Ohio between 1951 and 2004 were surveyed and information regarding demographics, residential history, medical history, and health-related behaviors was collected. These participants were grouped into two cohorts based on their occupational exposure (worker cohort, n = 6,000) at the chemical plant or residential exposure (community cohort, n = 40, 000) to drinking water contaminated with PFOA. The summarized evidence and technical conclusion of these studies are publicly available in the form of non-peer reviewed Probable Link reports (www.c8sciencepanel.org).

In this chapter, we will review evidence between PFAS exposure and risk of adverse human health outcomes, each outcome has a separate section and epidemiological evidence is presented in separate studies segment as cross sectional or longitudinal studies (where available) and within each section occupational or population studies are discussed. Association between PFAS and cancer is not discussed as it is addressed in a separate chapter of this book.

13.2 Lipids

Epidemiologic studies have shown that environmental PFAS exposure may have an important role in elevating serum lipid (hypercholesterolemia), although findings have not been uniform (Frisbee et al. 2010; Sakr et al. 2007a, b; Lin et al.

2009, 2013; Olsen and Zobel 2007; Emmett et al. 2006; Steenland et al. 2009; Nelson et al. 2010).

Cross-sectional The inconsistent positive relationships between environmental PFAS exposure, in particular PFOA, and lipid and lipoproteins levels were reported in many cross-sectional studies even though the magnitudes of effect varied by studies. In occupational cohorts, positive associations have been observed between PFOA and total cholesterol (Sakr et al. 2007a, b), low-density lipoprotein cholesterol (LDL-C) (Lin et al. 2013) and triglycerides (Olsen and Zobel 2007). Two epidemiologic studies conducted on a highly exposed community yielded dissimilar results, with Emmett et al. reporting no association (n = 371) (Emmett et al. 2006), and Steenland et al. reporting a positive association between PFOA and total cholesterol, LDL-C and triglycerides (n = 46,294) (Steenland et al. 2009).

Studies on general populations report more positive associations between PFAS concentrations in blood and lipids than in studies on occupational cohorts or highly exposed communities. Using the data from NHANES 2003–2004, Nelson et al. reported positive associations between PFOA and PFOS exposure and total cholesterol and non high density lipoprotein cholesterol (HDL-C) in a general population sample of adults (Nelson et al. 2010). A Canadian study did not show significant evidence to support the association of cholesterol outcomes with PFOS and PFOA exposure. However, several significant positive associations with the PFHxS and cholesterol outcomes were noted (Fisher et al. 2013). In this study using cross-sectional data in adults from the Canadian Health Measures Survey (2007–2009), the associations between plasma levels of PFOA, PFOS and PFHxS and cholesterol were assessed. Evidence of significant positive associations between PFHxS, with total cholesterol, LDL-C, TC/HDL-C and non-HDL-C as well as elevated odds of hypercholesterolemia was noted (Fisher et al. 2013). A Danish cross-sectional study of middle aged adults described significant positive associations between both PFOS and PFOA and total cholesterol (Eriksen et al. 2013).

A previous study in children and adolescents had reported positive associations between PFAS and abnormal lipid levels in the C8 community cohort (Frisbee et al. 2010). In a study of adolescents (age <18 years) from NHANES 1999–2008, Geiger et al. (2014) identified positive relationships of exposure to PFOA and PFOS with high total cholesterol and LDL-C levels (Geiger et al. 2014). Compared to children in quartile 1 (reference), the adjusted odds ratios (ORs) and 95 % confidence interval (CI) for high total cholesterol among children in quartile 4 was 1.16 (1.05–2.12) for PFOA and 1.53 (1.11–1.64) for PFOS. PFOA and PFOS were not significantly associated with abnormal HDL-C and triglyceride levels.

Longitudinal Longitudinal studies on health effects PFAS on lipids are scarce. A C8 short term follow-up study on C8 community cohort (n = 560) without taking any lipid lowering medications showed interesting results between relationships between changes in PFAS and change in lipids (Fitz-Simon et al. 2013). While large decrease (~50 %) in serum PFOA and PFOS levels over a 4.4 follow-up year was noted, mean increase in LDL-C (1.8 % increase) and other lipids was minimal. Interestingly,

authors found that greater decreases in PFOA and in PFOS were associated with greater decreases in LDL-C levels, 3.6 % (1.5–5.7 %) for PFOA reduction in 50 % and 5.0 % (2.5–7.4 %) for PFOS reduction in 50 %, respectively. This tendency was significant but less prominent in the relationship between the decrease in total cholesterol and PFOA (e.g., predicted 1.7 % decrease per halving PFOA). This result renders some support that PFOA and PFOS may cause the reversible elevation of lipids, especially LDL-C.

In longitudinal analyses of worker and community cohorts (n = 32,254), the C8 Science Panel found a 'probable link' between incidence of hypercholesterolemia (n = 9,909) and PFOA (Winquist and Steenland 2014a). In a principal retrospective analysis, hazard ratios (HR) of hypercholesterolemia, defined as subjects taking medications, were significantly higher in estimated cumulative PFOA exposure quintiles from 2 to 5 compared to quintile 1. Participants in C8 Health Project in upper quintile groups of cumulative exposure had between 1.17 and 1.24 times higher hazards of having hypercholesterolemia compared with participants in the lowest quintile (Winquist and Steenland 2014a). However, a short-term follow-up (164 days) study of workers (n = 179) who involved in the demolition projects of 3M plants in Minnesota (medical surveillance study) showed no association between changes in serum PFOA concentrations and changes in TC levels (Olsen et al. 2012). Only significant positive associations noted in this study were between changes in PFOA and changes in TC/HDL-C in a sub-sample with low baseline PFOA levels (<15 ng/ml) (Olsen et al. 2012).

Conclusion Many epidemiologic studies on health effects of PFAS were cross-sectional in nature, which preclude conclusions about causality. Reverse causality is also possible: higher lipid levels may cause the increase in PFAS levels measured in blood samples. Alternatively, possible unknown confounders cause changes in both PFAS levels and lipid levels. Further, caution must be taken to interpret results for studies on general population with relatively low homogenous exposure, which may not correspond with those from occupational cohorts and from highly-exposed communities. However, despite these caveats, epidemiologic evidence, in particular from the longitudinal analysis of C8 Health Project, suggests that there is an association between PFOA and PFOS and adverse lipid profiles, and further that high PFOA exposure may increase the risk of incident hypercholesterolemia in adults.

13.3 Uric Acid

Uric acid is a metabolite of purine breakdown and is a renal function biomarker. Elevated uric acid is associated with risk of hypertension, diabetes mellitus (Bandaru and Shankar 2011), cardiovascular disease, and kidney disease (Cain et al. 2010) (reviewed in (Geiger et al. 2013)).

Cross Sectional In a cross sectional analysis of the same occupational cohort Costa et al. (2009) described mean uric acid levels of 6.29 μg/mL for 34 currently exposed

workers, versus 5.73 μg/mL for 34 matched non-exposed workers (p = 0.04) (reviewed in (Steenland et al. 2010a)). In a cross-sectional community study of C8 adult population Steenland et al. (2010b) reported a positive association between PFOA, PFOS and uric acid among 54,951 highly-exposed community residents (Steenland et al. 2010b). For PFOA, the OR of hyperuricemia increased modestly with increasing serum concentration of PFOA. A less steep trend for PFOS was observed (Steenland et al. 2010b). When PFOS and PFOA were included in the model together PFOA was a more significant predictor than PFOS. However cross sectional study design and possibility of reverse causality, prohibit inference of cause effect relationship.

In a cross sectional NHAAES survey 1999–2006 of the US general population (n = 3,883) a positive relationship between serum levels of PFOS and PFOA and serum uric acid was documented. This demonstrates that even at low PFAS exposure levels observed in the US general population, PFAS are associated with hyperuricemia (Shankar et al. 2011b). Additionally, evidence regarding a positive association between PFAS and hyperuricemia in children is emerging. As described in a cross-sectional NHNAES 1999–2008 survey a positive association between serum PFOA and PFOS levels and hyperuricemia (≥6 mg/dL) was seen in 1, 772 US children (Geiger et al. 2013).

Longitudinal In a longitudinal study of occupationally exposed workers (n = 56), Costa et al. (2009) found a positive association between uric acid and PFOA by using repeated measures of both PFOA and uric acid over a 7-year follow up.

Conclusion Limited evidence supports an association between hyperuricemia and PFAS, although the only data are available come from cross-sectional studies, which cannot provide evidence of causality.

13.4 Kidney Disease

Kidneys are an important target organ for PFAS; PFAS are stored and excreted there.

Cross-sectional Previous studies of occupational cohorts or communities highly exposed to PFOA did not find an association between serum PFOA concentrations and blood urea nitrogen or serum creatinine, markers of kidney damage, (Costa et al. 2009; Emmett et al. 2006). Cross-sectional NHANES 1999-2008 data showed positive relationship between serum levels of PFOS and PFOA and chronic kidney disease (CKD) in 4,587 adults (Shankar et al. 2011a). Because of the cross-sectional nature of NHANES survey authors could not conclude if high levels of PFOA and PFOS in serum preceded CKD or *vice versa*. In another cross sectional study association between estimated renal glomerular filtration rate (eGFR), a marker of kidney function, and serum PFASs in 9,660 children 1 to <18 years of age was studied (Watkins et al. 2013). The concurrent and historical serum PFOA concentrations were predicted

using an environmental exposure, and pharmacokinetic model utilizing residential history. It was hypothesized that predicted serum PFOA levels would be less prone to reverse causation than measured levels. Measured serum levels of PFOA, PFOS, PFNA, and PFHxS were associated with decreased eGFR (Watkins et al. 2013). However modeled serum PFOA was not associated with decreased eGFR.

Longitudinal In longitudinal unpublished analyses of the highly exposed mid-Ohio valley population, the C8 Science Panel found no 'probable link' between medically confirmed kidney disease (n = 50, 308) and PFOA (C8 Science Panel 2012c). In longitudinal mortality study of 6,027 highly exposed workers, Steenland and Woskie (2012) reported a higher kidney disease related mortality in PFOA workers compared to other workers in the plant; exposure response trend was also significant (Woskie et al. 2012).

Conclusion Evidence linking chronic kidney disease to PFAS exposure is limited and inconsistent. The observed cross-sectional positive association between eGFR and serum PFOA in C8 populations may be a consequence rather than a cause of decreased renal function. Longitudinal are sparse and inconsistent.

13.5 Heart Disease and Hypertension

Experimental studies have revealed that PFAS exposure is related to oxidative stress (Liu et al. 2007) and endothelial dysfunction (Qian et al. 2010), which are regarded subclinical antecedents to cardiovascular pathology. Although health effects of PFAS were inconsistent at different exposure levels, PFOA, PFOS and/or PFNA have been positively linked to total cholesterol and LDL-C levels (Starling et al. 2014; Winquist and Steenland 2014a), hyperuricemia (Steenland et al. 2010b; Shankar et al. 2011b; Geiger et al. 2013), altered glucose homeostasis (Lin et al. 2009; Lind et al. 2014), which are putative CVD risk factors. These results provide some epidemiologic evidence that PFAS exposure may play a role in the development of coronary heart disease (CHD).

Cross-sectional There are two conflicting reports on PFOA exposure and CHD prevalence on U.S. general population (Melzer et al. 2010; Shankar et al. 2012). Among 3,974 adults aged over 20 years in the NHANES 1999–2000 and 2003–2006, weighted CHD prevalence was 5.8 % (n = 321, unweighted). Across quartile groups using sex-specific cutoff s for PFOA and PFOS concentration, there were no significant increases in odds of reporting CHD in this study. Among 1,216 adults aged over 40 years (NHANES 1999–2003), however, Shankar et al. (2012) reported that the exposure to PFOA was positively associated with risk of self-reported CVD including CHD, heart attack, or stroke, and objectively measured peripheral artery disease (PAD). Reported weighted prevalence of CVD and PAD was 13.0 % and 4.5 %, respectively. Compared with participants in quartile 1 in serum PFOA levels (<2.9 ng/ml for women and <3.0 ng/ml for men), participants

in quartile 4 (>5.6 ng/ml for women and >6.1 ng/ml for men) had 2.01 times (95 % CI; 1.12–3.60) higher odds of reporting CVD and 1.78 (1.03–3.08) time higher odds of having PAD. Authors also reported that the adjusted OR in quartile 4 was 2.24 (1.02–4.94) for reporting specifically CHD compared to the quartile 1 (p-trend = 0.007) (Shankar et al. 2012).

There were two cross-sectional studies on general population examining the PFOA on hypertension or blood pressure levels (Geiger et al. 2014; Min et al. 2012). A cross-sectional study reported an association between PFOA exposure and hypertension in 2,934 US adults from 2003–2004 and 2005–2006 NHANES (Min et al. 2012). In the adjusted analysis on participants in quartiles for PFOA levels, odds of having hypertension was significant increased (p for tend =0.001):1.21 (0.86–1.70) for quartile 2, 1.60 (1.15–2.22) for quartile 3, and 1.71 (1.23–2.36) for quartile 4. Using NHANES data from 1999–2000 and 2003–2008, Geiger et al. (2014) reported no association between PFOA and PFOS exposure and hypertension in 1,655 children aged 18 years old in general US population (Geiger et al. 2014). Weighted hypertension prevalence was 23.4 % in this study.

Longitudinal Mortality studies on occupational cohorts reported no significant positive associations between PFOA exposure and CHD (Leonard et al. 2008; Lundin et al. 2009; Sakr et al. 2009; Steenland and Woskie 2012). Based on an occupational cohort (n = 3,922) at 3M plant in Cottage Grove, Minnesota exposed to PFOA, Lundin et al. reported no significant SMR (standardized mortality ratio) trend across three groups according to job category for PFOA exposure levels. SMRs (95 % CI) for non-exposed workers (n = 1,792) was 0.7 (0.6–0.9, 92 deaths), for probably exposed workers (n = 1,688) was 0.8 (0.7–1.0, 93 deaths), and for definitely exposed (n = 512) workers was 0.8 (0.5–1.4, 16 deaths). The CHD SMRs were generally lower than that of the general population in MN. CHD specific mortality rate ratios by characterizing the workers by job classification or cumulative exposure years yielded no significant association (Lundin et al. 2009). Based on data collected on 6,027 workers at DuPont plant in West Virginia, Leonard et al. reported no significant increase in ischemic heart disease (IHD) mortality of workers in comparison with U.S. population, the West Virginia and 8-state DuPont employee population (Leonard et al. 2008). Again, Sakr et al. reported there was no dose-response relationship between the cumulative exposure of serum PFOA and CHD mortality based on the same cohort (n = 4,747) with 239 reported IHD deaths (Sakr et al. 2009). C8 Scientific Panel conducted another study on this occupational cohort (i.e., C8 worker cohort) at DuPont plant (Steenland and Woskie 2012). Among 5,791 workers, cumulative serum PFOA based on eight job category was estimated (Woskie et al. 2012) and cause-specific mortality was documented. There was no significant increase in SMRs (287 deaths) compared with that of US general population [SMR (95 % CI) 0.68, (0.60–0.77)] or other DuPont workers [0.97 (0.86–1.09)] in the analysis using a no-lag. The results were similar in the analysis using 10-year lag (Steenland and Woskie 2012).

In longitudinal analyses of worker and community cohorts (n = 32,254), a recent report found no association between medically confirmed CAD such as heart attack

or angina or self-reported medicated hypertension and PFOA (Winquist and Steenland 2014a). In the primary retrospective for hypertension (n = 11,798) and CAD (n = 2,468), there were no significant trends in increase in HRs for either for hypertension and CAD in higher quintiles of cumulative exposure serum PFOA compared to quintile 1. Reported HRs (95 % CI) for CAD by increasing quintile were 1.00 (referent), 1.26 (1.10–1.45), 1.17 (1.02–1.35), 0.99 (0.86–1.14), and 1.07 (0.93–1.23) (Winquist and Steenland 2014a). Prospective analyses on data collected between 2005/2006 and 2008/2011 also showed no significant positive association between CAD and PFOA exposure.

Conclusion Mortality studies on occupational cohorts and a longitudinal study on C8 cohorts did not provide significant evidence to associate PFOA exposure with CHD and hypertension while cross-sectional studies on general population demonstrated inconsistent results.

13.6 Cerebrovascular Disease

Given positive association between PFAS and blood pressure or uric acid, researchers have examined the relationship between particularly PFOA exposure and cerebrovascular disease.

Cross-sectional Previous studies reporting association between strokes and PFOA is limited to two mortality studies among occupational cohorts (Leonard et al. 2008; Lundin et al. 2009). Leonard et al. (2008) found a deficit of deaths from cerebrovascular disease in workers (35 deaths) at DuPont plant vs. the general US population (estimated deaths = 57.9). The SMR was 0.86 (95 % CI, 0.60–1.20). Based on data on 3,993 workers at 3M plant, Lundin et al. (2009) r that SMRs were 1.6 (95 % CI: 0.5–3.7, 5 deaths) in definitely exposed workers, 0.7 (95 % CI: 0.4–1.1; 17 deaths) in probably exposed workers, and 0.5 (95 % CI: 0.3–0.8; 13 deaths) in non-exposed workers. There was no significant hazard in cerebrovascular disease mortality across workers in PFOA exposure categorized by job classification or cumulative exposure years (Lundin et al. 2009). Stroke mortality in C8 worker cohort reported by Steenland and Woskie showed modestly lower stroke mortality than the US population or other DuPont employee (Steenland and Woskie 2012). Stroke mortality may not be the best endpoint for evaluating stroke because fatality attributed to stroke is low.

There is lack of information associating PFOA with cerebrovascular disease in highly exposed communities or in general population. Shankar et al. reported the positive association between prevalence of stroke and serum levels of PFOA among general adult population from NHANES 1999–2003. In a model adjusting for age, sex, race/ethnicity, education level, alcohol intake, BMI, hypertension, DM, and serum total cholesterol levels, participants in increasing quartiles showed higher odds of reporting stroke (p-trend = 0.02); 1.00 (reference), 4.39 (1.44–13.37), 3.94 (1.48–10.50), and 4.26 (1.84–9.89) (Shankar et al. 2012).

Longitudinal No significant association between PFOA and self-reported stroke incidence (n = 1,596) has been identified from a longitudinal study on combined C8 worker and community cohorts (n = 32,354) (Simpson et al. 2013). In retrospective analysis on medical-record validated incident stroke (n = 825), the HRs of stroke in the higher quintiles was 1.39 (95 % CI: 1.11–1.76), 1.36 (1.08–1.71), 1.45 (1.15–1.82), and 1.13 (0.90–1.44) compared with the lowest quintile of cumulative PFOA exposure. Linear relationship between cumulative PFOA exposures with incident stroke was not significant.

Conclusion There is lack of data suggesting any significant positive association between PFAS exposure, specifically PFOA exposure, and incident and prevalent stroke.

13.7 Diabetes

While experimental studies suggested that PFASs are not directly linked to the pathways associated with dysglycemia, some human studies have reported positive association between PFASs and diabetes, but not all. Diabetes related outcomes examined in epidemiologic studies are T2DM mortality, self-reported or medical record-verified T2DM and glucose homeostasis related biomarkers such as fasting insulin and glucose, and homeostasis model assessment of insulin resistance (HOMA-IR).

Cross-sectional In a study on highly exposed C8 community cohort (n = 54,468), C8 scientific panel reported of no significant association between PFOA and prevalence of T2DM (MacNeil et al. 2009). Authors reported that 7.8 % prevalence of DM in C8 communities, which was comparable to that of Ohio and West Virginia at the time of survey (2005). In multivariable analyses on medical record-validated T2DM cases (n = 3,539) or validated T2DM case (n = 1,055) who resided in communities more than 10 years prior to diagnosis, there were non-significant decreasing trends in odds of having T2DM across serum PFOA decile groups compared with the lowest decile group. In addition, authors found no difference or pattern in fasting glucose levels across PFOA deciles among non-diabetics (MacNeil et al. 2009).

However, studies on general populations reported mixed yet confusing results between PFAS and diabetes related outcomes. Serum PFHS, PFNA, PFOA, and PFOS were examined in relation to glucose homeostasis and indicators of metabolic syndrome from 474 adolescents and 969 adults in the general population analyzed from NHANES 1999–2004 (Lin et al. 2009). In adolescents, elevated serum PFNA concentrations were related with higher blood glucose concentrations, but were significantly associated with lower odds of having metabolic syndrome (OR, 0.37 (0.21–0.64)). In adults, elevated serum PFOA concentrations were significantly related with higher HOMA calculated beta-cell function, which suggests favorable beta-cell function. Serum PFOS concentrations showed positive association with insulin, HOMA-IR, and beta-cell function (Lin et al. 2009). A study on Swedish

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elderly men and women (n = 1,016, aged 70 years) reported the positive but non-linear association between plasma PFNA concentration and prevalent T2DM self-reported or defined by glucose levels >126 mg/dl (7 mmol/L) (Lind et al. 2014). The pattern of relationship was similar between PFOA and T2DM (P = 0.01) while the other five PFASs examined were not related to T2DM. Any of seven PFASs examined in this study was not related to HOMA-IR (Lind et al. 2014). In a Canadian Health Measures Survey (2007–2009) of the general population (n = 2,700) the association between plasma levels of PFOA, PFOS and PFHxS, metabolic syndrome, and glucose homeostasis was examined in adults. Regardless of PFASs examined, there was no significant relation between any PFAS and any glucose homeostasis biomarkers and metabolic syndrome (Fisher et al. 2013).

Longitudinal In an occupational cohort (n = 3,993) at a 3M plant in Cottage Grove, MN, Lundin et al. reported that DM -specific SMRs was 2.0 (95 % CI: 1.2–3.2) among 'probably exposed' workers with 18 DM related death (Lundin et al. 2009). However, there was no reported death due to DM among 'definitely exposed' workers. Similar results were reported in studies on C8 worker cohort reported (Leonard et al. 2008; Steenland and Woskie 2012). C8 worker cohort (n = 5,791) had about a twofold (95 %: 1.35, 2.61, 38 deaths) excess of DM mortality compared to other non-exposed DuPont plant workers (Steenland and Woskie 2012). However, in comparison with US referent group, DM mortality rate in C8 workers was not significantly elevated (SMR, 1.06, 95 % CI, 0.75–1.46).

C8 Health Project reports are only available longitudinal study on the incidence of T2DM in adults (Karnes et al. 2014; MacNeil et al. 2009). In an analysis of combined C8 worker and community cohorts (n = 32,254), estimated cumulative retrospective PFOA exposure between 1951 or birth years of participants and 2011 was not associated with incidence of T2DM (n = 4,129). The adjusted HRs (95 % CI) by quintile of cumulative PFOA were 1.0 (reference), 0.91 (0.76–1.08), 1.18 (0.99–1.40), 0.96 (0.81–1.15), 1.04 (0.87–1.24), 1.11 (0.93–1.32), 1.06 (0.89–1.26), 1.00 (0.85–1.19), 1.03 (0.86–1.23), and 1.01 (0.84–1.20) (Karnes et al. 2014).

Conclusion There is little evidence of a significant positive relationship between PFOA and T2DM incidence or prevalence among highly exposed community sample or in the general population (Karnes et al. 2014; MacNeil et al. 2009; Fisher et al. 2013; Nelson et al. 2010; Lin et al. 2011) despite findings of increased diabetes mortality risk in occupational settings (Lundin et al. 2009; Steenland and Woskie 2012; Leonard et al. 2008). Considering that DM mortality examined may not be a good outcome because adult onset T2DM itself is not likely a fatal disease, it appears unlikely that there is an association between PFAS, in particular PFOA, and T2DM.

13.8 Liver Function and Liver Disease

PFOS and PFOA are known to be hepatotoxic in rodents (Lau et al. 2007). Most epidemiologic studies have examined the effects of PFASs on blood liver function enzymes; direct bilirubin, γ-glutamyl transpeptidase (GGT), aspartate

aminotransferase (AST), alanine aminotransferase (ALT) (Costa et al. 2009; Emmett et al. 2006; Gallo et al. 2012; Lin et al. 2010; Fei et al. 2012; Olsen and Zobel 2007; Sakr et al. 2007b; Yamaguchi et al. 2013).

Cross-sectional Increased GGT, AST, and ALT levels associated with increase in PFOA exposure were reported in several occupational cohort studies (Olsen and Zobel 2007; Sakr et al. 2007a) but not in all (Costa et al. 2009; Olsen et al. 2003). In a cross-sectional study on workers (n = 1,025), AST, ALT, and total bilirubin levels were not significantly associated with the level of PFOA, but GGT was positively associated with PFOA ($p = 0.02$) (Sakr et al. 2007a).

An early study on highly exposed community reported no significant relationship between PFOA and various biomarkers for liver function or history of liver disease (Emmett et al. 2006). This study was conducted on a selected group of volunteers (n = 371) residing in Little Hocking Water Association District nearby DuPont plant. Based on C8 community cohort (n = 47,092), however, Gallo et al. reported significantly positive cross-sectional associations between serum PFOA and PFOS concentrations and markers of liver function (ALT, GGT and direct bilirubin). The PFOS and PFOA exposures were related to increased ALT. One unit increase in log-transformed PFOA and PFOS concentrations was positively associated with significant increase in log-transformed ALT [β (95 % CI): 0.022 (0.018–0.025) for PFOA, 0.020 (0.014–0.026) for PFOS] in multiple regression analyses. However, no consistent association between PFOA and GGT or total bilirubin was noted (Gallo et al. 2012).

In a study of 2,216 adults enrolled in the NHANES 1999–2004, significant trends ($p < 0.05$) for increasing ALT and log-transformed GGT concentrations across quartiles of PFOA were described (Lin et al. 2010). There were significant differences in total bilirubin levels across PFNA quartiles ($p = 0.014$). In adjusted analyses, one unit increase in log-transformed PFOA levels was positively associated with increases in ALT [β (95 % CI), 1.86 (0.62), $p = 0.006$] and log-GGT [β (95 % CI), 0.08 (0.03), $p = 0.019$] concentrations. Borderline positive association was also observed between serum PFNA and total bilirubin levels [0.48 (0.25), $p = 0.053$] (Lin et al. 2010). A recent Japanese study in 307 men and 301 women (aged 16–76 years) reported cross sectional association between serum PFOS and PFOA and hepatic enzymes (GGT, ALT, AST) and omega-3 polyunsaturated fatty acids (DHA and EPA). Serum levels of ALT, AST, DHA and EPA showed significant positive correlations with PFOS and PFOA in blood (Yamaguchi et al. 2013).

Longitudinal In longitudinal unpublished analyses of the highly exposed C8 community cohort, the C8 Science Panel found no 'probable link' between estimated cumulative PFOA levels and medically confirmed non-malignant liver disease (n = 647) in 2008–2010 survey (C8 Science Panel 2012c). C8 Science Panel reported that HRs of liver disease was 1.0, 1.19, 1.08, 1.04, and 0.95 by increasing estimated cumulative PFOA exposure quintiles (p-trend = 0.32).

Conclusion A number of epidemiological studies in occupational settings and the general population reported positive associations of serum levels of PFOA with

biomarkers for liver function, although results are inconsistent. However, studies on the prevalence or incidence of liver diseases including hepatitis or non-alcoholic or alcoholic fatty liver disease have been negative, although they are few in number (C8 Science Panel 2012a).

13.9 Immune Function

Certain PFAS are considered immunotoxic as documented in some epidemiological studies (Grandjean et al. 2012; Okada et al. 2012; Looker et al. 2014) and numerous animal studies (Smits and Nain 2013; Taylor et al. 2005; Dewitt et al. 2008, 2009).

Cross-sectional In a recent cross sectional study of 411 C8 Health Panel participants elevated PFOA serum concentrations were associated with a weakened antibody response (Looker et al. 2014). However, no evidence for an association between self-reported colds or influenza and PFOA, and PFOS serum concentrations was noted. In a cross-sectional analysis in Faroese children aged 5 and 7, elevated exposures to PFAS were related to reduced humoral immunity after routine childhood immunizations (Grandjean et al. 2012).

Asthma is an immune response in the bronchial airways, and hence might be affected by PFAS induced alterations in immune function, although few epidemiologic studies have explored the link. Therefore evidence is inconsistent, as yet, and prospective studies are needed. A recent cross-sectional Taiwanese study described positive associations between PFAS serum concentrations with asthma, asthma severity, and immunological markers in children (Dong et al. 2013). A total of 231 asthmatic children and 225 non-asthmatic controls were recruited. Structured questionnaires and interviews were conducted. Serum concentrations of 11 PFAS and immunological markers were measured. Adjusted odds ratios for asthma among those with the highest versus lowest quartile of PFAS exposure ranged from 1.81 for the perfluorododecanoic acid (PFDoA) to 4.05 for PFOA. PFOS, PFOA, and subsets of the other PFCs were positively associated with immunological markers, and asthma severity scores among asthmatics. This study suggests an association between PFC exposure and juvenile asthma. Another cross-sectional analysis in participants 12–19 years of age from NHANES 1999–2000 and 2003–2008 documented positive relationship between PFOA and with self-reported lifetime asthma, recent wheezing, and current asthma. PFOS exposure had an inverse relationship with both asthma and wheezing. PFNA and PFHxS were unrelated to any outcome (Humblet et al. 2014).

Longitudinal In a Japanese prospective cohort study, Okada et al. (2012) investigated association between PFOS and PFOA maternal exposure from 2002 to 2005 and infant allergies and infectious diseases during the first 18 months of life (Okada et al. 2012). Concentrations of PFOS and PFOA in maternal serum and concentrations of IgE in umbilical cord serum at birth were measured. Development of infant allergies and infectious diseases was determined from self-administered questionnaires at

18 months of age. Although cord blood IgE level decreased significantly with high maternal PFOA levels among female infants, no relationship was found between maternal PFOS and PFOA levels, and allergies and infections at 18 months age.

In a prospective Norwegian study pre-natal exposure to PFAS was associated with immunosuppression in early childhood. In this analysis pregnant women were recruited during 2007–2008. Three annual questionnaire-based follow-ups were performed. Blood samples were collected from the mothers at the time of delivery and from the children at the age of 3 years. Pre-natal exposure to PFOA PFOS, PFNA, and PFHxS were determined in maternal blood in a subgroup. Main outcome measures were anti-vaccine antibody levels, common infectious diseases and allergy- and asthma-related health outcomes in the children up to the age of 3 years. In the children at age 3 years, an inverse association was noted between the level of anti-rubella antibodies and four PFAS concentration in serum. Furthermore, there was a positive association between the maternal concentrations of PFOA and PFNA and the incidence of common cold in children, and between PFOA and PFHxS and the number of episodes of gastroenteritis. No associations were found between maternal PFAS concentrations and the allergy- and asthma-related health outcomes investigated (Granum et al. 2013).

Conclusion Based on the current epidemiologic studies there is no evidence of any increased risk of non-infectious lung disease (Asthma and Chronic Obstructive Pulmonary Disease – COPD).

13.10 Autoimmune Disease

Longitudinal Steenland et al. (2013) reported that PFOA was linked with ulcerative colitis, an autoimmune disease in C8 cohort. In 2008–2011 past disease history was obtained in 32,254 C8 participants from 1952 onwards (or at birth if born after 1952). Any self-reported autoimmune disease (ulcerative colitis, lupus, juvenile diabetes, rheumatoid arthritis, multiple sclerosis, Crohn's disease) was validated through medical records. Cumulative exposure to PFOA was estimated from plant emissions, residential and work history, and exposure modeling during follow-up. The incidence of ulcerative colitis was significantly increased in higher quartiles of PFOA exposure (p-trend < 0.0001). Additional analysis of 29 prospective ulcerative colitis cases diagnosed after the 2005–2006 baseline survey suggested a positive non-monotonic association with PFOA exposure and incidence of ulcerative colitis. However, no association with any other autoimmune diseases was observed.

Conclusion On the basis of existing epidemiological data a probable link between exposure to PFOA and ulcerative colitis may exist. No probable link between PFOA and any of the other autoimmune diseases (rheumatoid arthritis, lupus, type1 diabetes, Crohn's disease, or multiple sclerosis) has been found based on existing data (C8 Science Panel 2012b).

13.11 Osteoarthritis and Bone Mineral Density

Cross-sectional In a large cross sectional study (2005–2006) Innes et al. assessed association between PFOA, PFOS and self-reported physician diagnosed osteoarthritis (OA) in 49,432 adult C8 Health Project participants (Innes et al. 2011). In adjusted analysis, a significant positive association between OA and PFOA serum levels was observed. In contrast, a significant inverse association with PFOS and OA was seen. Because of the cross sectional nature of the study it could not be ascertained whether PFAS exposure preceded OA.

In another cross sectional study Uhl et al. (2013) investigated the association between PFOA and PFOS exposures and self-reported OA in US general population aged 20–84 years using NHANES 2003–2008 data (Uhl et al. 2013). Compared to participants in the lowest quartile, those in the highest exposure quartile had elevated odds of OA. In gender stratified analysis, OA was associated with PFOA only in women. Further, a borderline association between OA and PFOS was noted only in women (Uhl et al. 2013). In a separate cross sectional analysis of NHANES data from 2005 to 2008, serum PFOS concentration was associated with decreased total lumbar spine bone mineral density in pre-menopausal women (Lin et al. 2014). However no association among PFOA and PFOS concentration and self-reported fracture was noted.

Longitudinal In longitudinal unpublished analyses of the C8 Health Project no 'probable link' was reported between PFOA, and either self-reported OA (n = 6,641), or the subset of cases reporting taking OA medication (n = 2,268) (C8 Science Panel 2012d).

Conclusion Data are limited to three studies, which are inconsistent. Two cross-sectional studies reported a positive association between PFOA and osteoarthritis, while the single large longitudinal study did not.

13.12 Thyroid Function

Numerous occupational and population cross-sectional studies report inconsistent relationships between PFAS exposure and thyroid function ranging from null (Bloom et al. 2010; Emmett et al. 2006) to modest changes (Olsen et al. 2003; Olsen and Zobel 2007). There are no longitudinal studies of thyroid function.

Cross Sectional In occupational settings 506 male workers who manufactured or used PFOA at three facilities, no statistically significant associations between PFOA and thyroid stimulating hormone (TSH) or thyroxine (T4) was reported. A negative association was observed for free T4 and a positive association for triiodothyronine (T3) (Olsen and Zobel 2007). In another cross sectional occupational study, PFOA or PFOS exposure assessed in quartiles showed no significant association with T3,

T4, or TSH (Olsen et al. 2003). However linear regression analysis showed positive association between PFOA concentration and T3 levels. In a longitudinal follow-up of a subset of participants, no association was observed between PFOA and thyroid hormones (Olsen et al. 2003).

A cross sectional analysis of C8 Health Project showed that PFOA and PFOS were associated with significant elevations in serum T4 and a significant reduction in T3 uptake (Knox et al. 2011a). In the same community, positive associations between PFOS, PFNA exposure with total T4, and of PFOA with hypothyroidism were observed in children (Lopez-Espinosa et al. 2012). In another cross sectional Taiwanese study in adolescents, and young adults that assessed 12 PFASs, serum PFNA was positively associated with serum free T4. The association was more significant in male participants aged between 20 and 30, active smokers or in those with higher BMI (Lin et al. 2013).

A cross-sectional study of U.S. general population using NHANES data from 2007 to 2010 examined the association between serum PFASs and thyroid function in 1,181 participants (aged >20 years). Elevated serum concentrations of PFOA and PFHxS were associated with total T3, total T4, and free T4 (Wen et al. 2013). In another cross sectional analysis of NHANES data from 2007 to 2008, Jain (2013) described association of six PFAS compounds including PFOS, PFOA, perfluorodecanoic acid, PFHxS, 2-(N-methyl-perfluorooctane sulfonamide acetic acid), and PFNA with thyroid hormones and thyroglobulin (Jain 2013). Elevated serum PFOA was associated with raised TSH, total T3. A positive association between PFHxS and total T4 was noted. No association between any of the six PFAS and thyroid hormones was observed.

PFAS exposure during pregnancy and effect on thyroid hormone homeostasis in pregnant women and fetuses has been addressed in a few cross sectional studies. In a cross sectional population based Norwegian study PFAS exposure during pregnancy and its effect on maternal thyroid function and their offspring was described. A total of 903 pregnant women were enrolled from 2003 to 2004. During the 18th week of gestation serum concentrations of 13 PFASs and TSH were measured. Pregnant women with higher PFOS had higher TSH levels. After adjustment, with each 1 ng/mL increase in PFOS concentration, there was a small rise in TSH. The odds ratio of having an abnormally high TSH, however, was not increased, and other PFASs were unrelated to TSH. It was concluded that PFOS was associated with slight increase in TSH in pregnant women that may be of no clinical significance (Wang et al. 2013).

In a separate cross sectional Taiwanese study serum concentrations of nine PFASs and four thyroid hormones were measured in 285 pregnant women (third trimester) along with cord serum thyroid hormones in 116 neonates (Wang et al. 2014). PFHxS concentrations were positively associated with maternal TSH levels. Pregnant women with elevated PFNA, perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA) had lower free T4 and total T4 levels. Also, maternal PFNA, PFUnDA, and PFDoDA levels were associated with lower cord T3 and total T4 levels, and maternal perfluorodecanoic acid was associated with lower cord total T3 (Wang et al. 2014).

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Conclusion Data are mainly limited to cross-sectional studies, and results are mixed.

13.13 Thyroid Disease

Most of the epidemiological research evaluation PFAS and thyroid disease is cross sectional and primarily comprises of general population studies.

Cross-sectional In cross sectional NHANES 1999–2006 data among 3,974 adults from the U.S. general population, men and women with elevated PFOA were more likely to report current treated thyroid disease. Similar association with PFOS exposure was reported only in men (Melzer et al. 2010). A cross sectional study in Canadian pregnant women in community evaluated link between four FFAS and thyroid hormones and thyroid peroxidase antibody (TPOAb, a marker of autoimmune hypothyroidism (Hashimoto's disease)) during the second trimester (Webster et al. 2014). PFASs had positive association with TSH and weak negative relationship with free T4 in the subset of pregnant women with high TPOAb. A cross sectional study in 10,725 children from C8 communities identified a link between hypothyroidism and elevated PFOA and elevated thyroid hormones with increase in PFOS and PFNA exposures (Lopez-Espinosa et al. 2012).

Longitudinal In a cohort study among participants of C8 Health Project, Winquist and Steenland (2014b) found that elevated PFOA exposure was associated with thyroid disease. Participants provided health information during 2008–2011. Retrospective exposure to PFOA was estimated for each participant starting at birth or in 1952, whichever came later. A total of 2,109 cases of thyroid disease on thyroid medication were identified after medical record review. PFOA was associated with increased risk of hyperthyroidism and hypothyroidism in women, and with hypothyroidism in men (Winquist and Steenland 2014b).

Conclusion Cross-sectional studies of thyroid disease and PFAS have had mixed results. Current evidence in a single longitudinal study reports a positive association between PFOA and thyroid diseases.

13.14 Neurological and Neurodegenerative Disorders

Cross-sectional A C8 Health Project analysis assessed the cross-sectional association between PFOA, PFOS, PFHxS, PFNA and self-reported memory impairment in 21,024 adults aged 50 years and over. Results indicated that as serum PFOS and PFOA increased, memory impairment decreased. Modest associations between PFNA and PFHxS were seen (Gallo et al. 2013). A cross-sectional study on the general population also suggested that there may be a protective association between

exposure to PFAS and cognition in older adults aged 60–85 years, particularly in diabetics. Power et al. examined the association between four PFASs exposure (PFOS, PFOS, PFHxS, PFNA), and self-reported memory problems in the NHANES 1999–2000 and 2003–2008 surveys (Power et al. 2013). In adjusted analyses, a protective association between PFASs and self-reported cognitive impairment was seen, which was more marked in diabetic older adults (Power et al. 2013).

Longitudinal In longitudinal unpublished of combined C8 worker and community cohorts, the C8 Science Panel found no 'probable link' between medically validated Parkinson's disease (n = 138) and PFOA (C8 Science Panel 2012e). C8 Science Panel reported non-significant relationship between cumulative PFOA exposure and risk of Parkinson's disease (p-trend = 0.61). Compared with participants in the lowest quartile, reported HRs was 1.0, 0.8 and 1.0 in increasing quartiles.

Conclusion A few cross-sectional studies on highly exposed community and general population suggested that higher blood PFAS (PFOS and PFOA) concentrations were favorably linked to memory function. A single longitudinal study found no link between PFAS and Parkinson's disease. Overall, the data are too sparse to assess the association between PFAS and development of cognitive impairment or neurodegenerative diseases.

13.15 Cognitive and Behavioral Disorders in Children

Epidemiologic evidence on PFOA exposure and child development is limited with varied exposure timing and levels, and measures of outcomes. Health outcomes studied in relation to PFAS exposures included cognitive and behavioral development milestones, performance testing, and attention deficit hyperactivity disorder (ADHD).

Cross-sectional Data based on highly exposed C8 community cohort, Stein and Savitz (2011) reported positive association between PFHxS level and ADHD (Stein and Savitz 2011). However, there was no significant association between any of four PFAS examined (PFOA, PFOS, PFHxS and PFNA) and parental reports of children' learning problems. Analysis was completed on data from 10,546 non-Hispanic white children 5–18 years of age. The prevalence rate of ADHD was 12.4 % (n = 1,303), and learning problem was 12.1 % (n = 1,281). In age and sex adjusted analyses, an inverted J-shaped association between PFOA and ADHD was noted. The prevalence of ADHD plus medication (5.1 %) was increased across PFHxS quartiles, with adjusted ORs of 1.00 (reference), 1.44 (95 % CI: 1.09–1.90) for quartile 2, 1.55 (1.19–2.04) for quartile 3 and 1.59 (1.21–2.08) for quartile 4 (Stein and Savitz 2011).

In a study of 571 children 12–15 years of age using the data from NHANES 1999–2000 and 2003–2004 data, elevated PFOA concentration was related to

parent-reported ADHD (Hoffman et al. 2010). One unit (ug/l) increase in PFOA was associated with 1.12 times increase in odds of having parentally reported ADHD (95 % CI, 1.01–1.23). The magnitudes of relationship between PFOS and PFHxS and ADHD were similar to that of PFOA. In a small study (n = 83) of children at background exposure levels, higher levels of five PFAS were associated with impulsivity in children (Gump et al. 2011).

Longitudinal Another analysis on the C8 community cohort did not find a relationship between PFOA exposure in 320 children and their performance on neuropsychological tests (Stein et al. 2013). Children enrolled in 2005–2006 had serum measures of PFOA, estimated in utero PFOA exposure, and prospective evaluation of neuropsychological tests 3–4 years later at ages 6–12 years. These tests included Intelligence Quotient (IQ), reading and math skills, language, memory and learning, visual-spatial processing, and attention. In multivariate analysis, children in the highest quartile as compared with the referent quartile of estimated in utero PFOA showed improved Full Scale IQ (beta 4.6, 95 % CI: 0.7–8.5) and decreases in ADHD (Stein et al. 2013). A recent study on children from C8 community cohort (n = 321) supplements the current evidence by providing a prospective assessment of childhood PFOA levels and mother and teacher report of child behavior measured using Behavior Rating Inventory of Executive Function scale assessed 3–4 years later (Stein et al. 2014a). Stein et al. reported that PFOA exposure were associated with behavioral problems in sex-specific manner. Survey results from mother's reports (n = 313) on behavioral problems suggested inverse associations between PFOA exposure and behavioral problem among boys but positive associations among girls (Stein et al. 2014a). Compared with boys in the lowest quartile, for example, boys in the highest quartile of PFOA exposure levels had about 6.39 lower score (95 % CI; −11.14, −1.35). A study of general population at background exposure level, prenatal PFOA and PFOS levels were measured in the Danish National Birth Cohort, and those were examined in relation to mothers report on behavioral (n = 787) and motor coordination (n = 526) outcomes (Fei and Olsen 2011). In this study, either prenatal PFOS or PFOA levels were not related to child's developmental milestones through age 7 (Fei and Olsen 2011; Fei et al. 2008).

Conclusion There are limited data on the associations between PFAS and adverse childhood behavioral and cognitive development. The data which do exist to not suggest any marked negative effects.

13.16 Reproductive and Developmental Outcomes

There have been a large number of epidemiologic studies of reproductive and developmental outcomes. These outcomes include decreased sperm count, longer time to pregnancy, birth defects, miscarriage and still birth, lower birth weight and birth size of neonates.

Cross-sectional Previous population studies in men had indicated a negative association between PFAS exposure and semen quality (Joensen et al. 2009) as seen in a cross-sectional study of 105 Danish men (median age, 19 years) in 2003 (reviewed in Steenland et al. 2010a, b). Men with high serum PFOS and PFOA had lower sperm count and fewer morphologically normal sperm than men with low PFOS, PFOA. In a recent cross sectional Danish population study of 247 men conducted during 2008–2009 PFOS levels were negatively associated with testosterone (T), calculated free testosterone (FT), free androgen index (FAI) (Joensen et al. 2014). However other cross sectional studies from European populations (Specht et al. 2012) and in the US (Raymer et al. 2012) have reported no consistent evidence that PFAS exposure affects semen quality or reproductive hormones in men. PFAS exposure in C8 Health Project was associated with delayed puberty in boys (cross sectional) (Lopez-Espinosa et al. 2011).

In a cross-sectional study in European women, PFOA exposure was associated with longer menstrual cycles (Lyngso et al. 2014). A total of 1,623 pregnant women were enrolled during antenatal care visits (2002–2004). Serum PFOA, and PFOS were assessed and retrospective information on menstrual cycle characteristics was obtained. Women with elevated PFOA had statistically significant longer cycles; a weaker association was also seen with PFOS.

Longitudinal PFAS exposure has been associated with infertility and subfecundity in women (reviewed in Steenland et al. 2010a, b). Fei et al. (2009) assessed time to pregnancy in 1,240 women from the Danish National Birth Cohort recruited from 1996 to 2002 (Fei et al. 2009). Plasma levels of PFOS and PFOA were measured during pregnancy. Higher maternal serum PFOS and PFOA were associated with longer time to pregnancy. Elevated PFOA and PFOS exposure was associated with infertility. A later study by Whitworth et al. (2012) reported that elevated PFOA and PFOS serum concentration was linked with subfecundity (ascertained as time to conceive >12 months) in multiparous Norwegian pregnant women enrolled in 2003–2004, no association was seen in nulliparous women (Whitworth et al. 2012).

Elevated exposure to PFAS in women has been associated with later menarche and earlier menopause. In a cross sectional analysis, PFOS and PFOA were related with delayed puberty in 3,076 boys and 2,931 girls aged 8–18 years in 2005–2006 survey of C8 communities (Lopez-Espinosa et al. 2011). Emerging evidence from prospective epidemiological studies suggest that prenatal exposure to PFAS has long-term effects on female reproductive function. From a Danish population-based cohort established in 1988–1989, levels of PFASs in maternal serum from 30th gestational week were used as a measure of prenatal exposure collected in mothers. The daughters were enrolled 20 years later and their reproductive history regarding age of menarche, menstrual cycle length, and levels of reproductive hormones were obtained. Daughters with elevated PFOA in utero exposure had a 5 months later age of menarche (Kristensen et al. 2013).

In the US, two studies have reported earlier menopause with higher exposure to PFAS (Knox et al. 2011b; Taylor et al. 2014). In 25,957 women aged 18–65 years enrolled from the C8 Health Project, serum estradiol levels and onset of menopause

were assessed (Knox et al. 2011b). The odds of menopause were significantly elevated with increased PFOA and PFOS exposure. In a cross sectional study among the US general population using NHANES 1999–2010 data, a positive association between PFAS exposure and earlier menopause was reported (Strongest for PFHxS) (Taylor et al. 2014). In this study, women with elevated PFOA, PFNA, and PFHxS were 36 %, 47 %, and 70 % more likely to have experienced menopause, respectively. Premenopausal women had the lowest levels of all four PFCs, whereas women who had undergone hysterectomy had the highest levels. PFHxS was most strongly associated with rate of hysterectomy. Women with elevated PFHxS were 3.5 times more likely to have had a hysterectomy. PFOA, PFNA, and PFOS were also significantly associated with having had a hysterectomy. However due to cross-sectional nature of the study, it was difficult to distinguish if raised PFAS led to earlier menopause or vice versa (reverse causality).

Association between PFAS exposure and preterm births has been investigated (reviewed in Darrow et al. 2014). In previous studies (Stein et al. 2009; Savitz et al. 2012a) no association between retrospective PFOA exposure and miscarriage risk was found. In a recent prospective study only limited evidence of association with PFOS was noted (Darrow et al. 2014).

PFAS exposure and major birth defects have been described in epidemiological studies (reviewed by Stein et al. 2014a, b). An earlier study from the C8 Health Project reported increased odds of birth defects with higher PFOA exposure (Stein et al. 2009). Another report from the same area described weak association with cardiac birth defects (Savitz et al. 2012b). A third study did not find any significant association between PFOA and birth defects (Nolan et al. 2010). The most recent study reports modest association of PFOA exposure with brain birth defects based on 13 cases (Stein et al. 2014b).

An association between PFOA exposure and pregnancy-induced hypertension (or pre-eclampsia) has been reported in C8 communities in two studies (Darrow et al. 2013; Savitz et al. 2012a), but not in one other (Savitz et al. 2012b). Overall this evidence was such that the C8 Science Panel decided that PFOA was more probably than not linked to pregnancy-induced hypertension (C8 Science Panel 2011).

Lower birth weight indicates insufficient fetal growth during pregnancy and is associated with future health problems. Both PFOA and PFOS can cross human placental barrier (Midasch et al. 2007); toxicological studies have reported evidence of LBW in animals at higher exposure levels than noted in human populations with background exposure (Lau et al. 2007).

Existing epidemiologic studies examining PFAS exposure and birth weight measured on a continuous scale in the general population provide inconsistent evidence of associations (Apelberg et al. 2007; Fei et al. 2007; Hamm et al. 2010; Washino et al. 2009). Two studies reported clear evidence of decreased mean birth weight in relation to increased PFOA (Apelberg et al. 2007; Fei et al. 2007). Smaller decrements were reported in two other (Hamm et al. 2010; Washino et al. 2009). One study (Fei et al. 2007) reported a reduction of 10.6 g per ng/ml increase in PFOA. Three smaller studies reported no association between PFOA and birth

weight (So et al. 2006; Monroy et al. 2008; Inoue et al. 2004). A large study (17,000 pregnancies) in C8 population, using estimated past exposure based on a well validated model which correlated well with recent measured PFOA, found no evidence of an association of PFOA with birth weight (Savitz et al. 2012b). Similarly a smaller study in the same population (1,600 births) found no association (Nolan et al. 2009). On the other hand, a recent meta-analysis of nine low-exposure general population studies, found a significant reduction of birth weight with higher PFOA (Johnson et al. 2014). The findings of no association in a population which much greater exposure contrasts (Savitz et al. 2012b) vs. an association in the meta-analysis of very low exposed general population (Johnson et al. 2014), are clearly contradictory. There could be a number of explanations. Effects are sometimes seen with low exposure contrasts which are less apparent using high exposure contrasts. Alternatively, an artificial association could be seen in general population studies due to increased maternal blood volume with more fetal growth, and decreased serum PFOA with increased blood volume.

Three other studies that examined PFOA exposure (dichotomous) with odds of low birth weight (LBW) (<2,500 g) rather than birth weight on a continuous scale reported no evidence of an association. In a general population study of the Danish National Birth Cohort, the authors reported no consistent association of either PFOA or PFOA with LBW (Fei et al. 2007). Two large studies in C8 population, using estimated past exposure based on a well validated model, found no evidence of an association of PFOA with LBW (Savitz et al. 2012a, b).

Conclusion Human population studies have reported inconsistent associations between PFAS exposure and reproductive and developmental outcomes. These outcomes include decreased sperm count, longer time to pregnancy, birth defects, miscarriage and still birth, pre-term birth lower birth weight and birth size of neonates. None of these outcomes show consistent associations with any PFAS, and when positive associations have been reported, their magnitude has been small. Two out of three studies have reported a positive association between pregnancy-induced hypertension and PFOA, but data remain sparse. 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 LBW.

13.17 Overweight and Obesity in Offspring

Epidemiological findings support developmental (pre-natal or early life) effects of low-dose exposures to PFAS. In a recent Danish prospective study, low dose pre-natal exposure to PFOA had an obesogenic effect in female offspring at 20 years of age (Halldorsson et al. 2012). Pregnant women (n = 665) were enrolled in 1988–1989 and PFOA was measured in serum at 30 weeks of pregnancy. Body mass index

(BMI) and waist circumference in offspring were recorded at follow-up (n = 665), and biomarkers of adiposity were measured in 422 participants. Pre-natal PFOA exposure showed a positive relationship with BMI and waist circumference only in female offspring 20 years of age (Halldorsson et al. 2012). It has been hypothesized that early life PFAS exposure may alter weight controlling hormones, possibly by activating peroxisome proliferator-activated receptor alpha (PPAR-α). PPAR-α, is a hormone receptor that plays a role in energy homeostasis. Another mechanism hypothesized mechanism is that PFOA may stimulate steroid hormone production in ovaries and thereby women may be more susceptible to the effects than men (White et al. 2011).

Longitudinal In a retrospective study, Barry et al. (2014) reported no association between PFOA exposure during the first 3 years of age and BMI measured later in adulthood among C8 Health Project participants (Barry et al. 2014). Data for height and weight of 8,764 adults aged 20–40 years were collected between 2008 and 2011. Using exposure modeling, annual retrospective early life PFOA serum concentrations were estimated for each participant based on residential history, and proximity to plant emissions. Elevated PFOA exposure in early life (up to 3 years of age) was not associated with overweight and obesity risk in adult hood.

Conclusion Data are sparse and contradictory regarding whether early life exposure to PFAS is associated with later obesity.

13.18 Conclusion

Serum PFOA has been linked relatively consistently with increased serum lipids, and uric acid levels in occupational, a highly exposed community population and general population studies. The largest body of evidence is for serum lipids. Furthermore, a large cohort study found a positive association with hypercholesterolemia and PFOA. A positive association has been described between PFOS serum levels and total cholesterol, triglycerides, and uric acid in the general population. In contrast, occupational studies did not indicate consistent associations between PFOS and cholesterol and/or triglycerides in either cross-sectional surveys or in a longitudinal analysis.

A large cohort study found a strong positive relationship between cumulative PFOA exposure and ulcerative colitis, an auto-immune disease. There was no other association between PFOA and other auto-immune diseases. One large cohort study of a highly exposed population did find a positive association between PFOA and thyroid disease.

Although the relationship between PFAS and cancer has not been addressed in this chapter, current data are also sparse, and largely restricted to PFOA. Occupational mortality studies have found isolated increases in some cancers based on small numbers, including one study with a significant elevation of kidney cancer (Steenland and Woskie 2012; Vieira et al. 2013). One large population cohort study

found a non-significant elevation of prostate and pancreatic cancer (Eriksen et al. 2009). One large cohort study and one ecologic study in a highly PFOA-exposed population in the mid-Ohio valley found positive associations with testicular cancer and kidney cancer (Barry et al. 2013). Testicular tumors were of a prior interest because they have been found in animal studies (Biegel et al. 2001; Lau et al. 2007). Overall, an association between PFOA and testicular and kidney cancer seems plausible, with limited data.

PFAS exposure has also been associated in some studies with adverse effects on thyroid homeostasis, liver enzymes, osteoarthritis, non-malignant kidney disease, and immunotoxicity, but the data are inconsistent. Data are sparse but largely negative for T2DM, neurodegenerative disease, children's cognition, adult CVD and stroke, immune function, liver disease, and obesity. Despite a large body of literature, and some positive findings regarding low birth weight, the data are overall inconsistent regarding reproductive/developmental outcomes. Two of three longitudinal studies found a positive association between PFOA and pregnancy-induced hypertension, but data remain sparse.

Epidemiologic evidence available at present is derived from occupational cohorts, highly exposed community cohorts, or general population studies. There are a number of limitations to the evidence. Interpretation of results from general population studies with low exposures may not match with those from occupational cohorts and with those from highly-exposed communities.

PFAS studies are often cross-sectional in nature, and cannot be used to determine causality. Further, cross sectional studies may report subclinical endpoints (blood chemistry) which may not have clinical significance because of a small magnitude of effect. These studies may suffer from reverse causality where it cannot be distinguished, for example, if increase in PFAS decreases glomerular function, or *vice versa* (Watkins et al. 2013). Alternatively, a third parameter that changes overtime may change both PFAS and glomerular function. Furthermore, interpretation of cross-sectional studies of disease endpoints in the general population has often been hindered by the lack of validation of outcomes (i.e., self-reported stroke). There are only a limited number of prospective studies exploring PFAS and human health outcomes in selected populations, which limits generalizability.

In summary, epidemiologic evidence suggest that there is an association between PFOA and six health outcomes: diagnosed high cholesterol, ulcerative colitis, thyroid disease, testicular cancer, kidney cancer, and pregnancy-induced hypertension. Data remains limited for other PFAS. To validate these results and increase our understanding of PFAS exposure, longitudinal studies in populations with exposures above general population background levels are needed. However, there are limited numbers of such populations.

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Chapter 14
Dosimetric Anchoring of Toxicological Studies

John Wambaugh

Abstract Comparing the onset of effects between different toxicological studies is often confounded by pharmacokinetics (PK). Differences between studies can be biological in origin (e.g. species, gender) as well as due to dose regimen (e.g. spacing, magnitude, duration, and route of administration). However, if the pharmacodynamic mechanism underlying the observed toxicological effect is conserved, and some measure of the tissue concentration (i.e., dosimetry) at the site of effect can be determined, then it is expected that this dosimetric anchor should also be conserved across studies. Careful consideration of the PK is required, and mathematical models for PK can address this need. It is relatively easy to extrapolate model predictions if there is a reasonable expectation of linear behavior and conserved PK between test conditions and those to be predicted. For perfluorinated compounds (PFCs), however, we expect PK extrapolation to be much more difficult. Aspects of the distribution, metabolism, and elimination of PFCs have unusual and non-linear features that must be considered. The PK of PFCs is especially unusual in that the half-lives of the longer chain PFCs vary by many orders of magnitude across species, dose regimen, and in some cases, across gender. The empirical saturable renal resorption hypothesis of the Andersen et al. (Toxicol 227(16978759):156–164, 2006) model provides the simplest available non-linear PK model that describes PFCs PK. However, despite the plausible biological mechanism, this model is still empirical, requiring that species-specific parameters are estimated using species-specific PK data. With this model, diverse toxicological studies of PFCs can be shown to be roughly consistent with respect to the internal, dosimetric anchors induced by their various study designs.

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J. Wambaugh, Ph.D. (✉)
National Center for Computational Toxicology, Office of Research and Development,
U.S. Environmental Protection Agency, Mail Drop B205-01 109 T.W. Alexander Dr.,
Research Triangle Park, NC 27711, USA
e-mail: Wambaugh.john@epa.gov

© Springer International Publishing Switzerland 2015

J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_14

ED_002330_00119699-00345

Keywords Pharmacokinetics • Dosimetric anchor • Mathematical models • Extrapolation • Dose regimen • Biological variability

14.1 Introduction

If a test compound activates a consistent mode of action across studies, then somewhere between the no observed effect level (NOEL) and the lowest observed effect level (LOEL), the tissue concentrations must be sufficient to perturb that mechanism in a statistically significant number of test animals (Allen et al. 1994). Although more sophisticated analyses (e.g., benchmark dose) are often possible for new studies that provide sufficient detail, meta-analyses comparing across historical literature often have to rely upon the NOEL and LOEL alone (Martin et al. 2009).

Comparing LOELs between studies is often confounded by differences in pharmacokinetics (PK), which can be not only biological in origin (e.g. species, gender) but also due to dose regimen (e.g. spacing, magnitude, duration, and route of administration). However, if the pharmacodynamic mechanism is conserved, and some measure of the tissue concentration (i.e., dosimetry) at the site of toxicological effect can be determined, then it is expected that this *dosimetric anchor* should also be conserved across studies (Rowlands et al. 2014). If the mode of action that is activated in the LOEL dose group is conserved in humans, then the chemical exposures that might cause these effects in humans may be inferred (Boobis 2010).

Careful consideration of the pharmacokinetics (PK) is therefore required in order to link the chemical exposures in the LOEL and NOEL dose groups to the onset of toxicity endpoints (Blaauboer 2010). PK models are needed to make predictions of the tissue concentrations that were caused by a given exposure. It is relatively easy to extrapolate predictions if there is a reasonable expectation of linear behavior and conserved PK between test conditions and those to be predicted. For perfluorinated compounds (PFCs), however, we expect PK extrapolation to be much more difficult. A list of PFCs mentioned in this chapter is provided in Table 14.1.

Table 14.1 The perfluorinated compounds (PFCs) discussed in this chapter

PFC abbreviation	Full name	Carbon chain length (linear form)
PFBS	Perfluorobutanesulfonate	4
PFHxS	Perfluorohexanesulfonate	6
PFHpA	Perfluoroheptanoic acid	7
PFOA	Perfluorooctanoic acid	8
PFOS	Perfluorooctane sulfonate	8
PFOSA	Perfluorooctanesulfonamide	8
PFNA	Perfluorononanoic acid	9
PFDA	Perfluorodecanoic acid	10

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If the study of PK can be considered to be concerned with the absorption, distribution, metabolism, and elimination of xenobiotic compounds by the body, then PFCs may be considered to be unusual with respect to three of those four elements. PK studies in animals have shown that many PFCs are well absorbed (Kudo and Kawashima 2003), but: the distribution of PFCs to tissue is unusual due to both fluorous chemistry (Dobbs and Kimberley 2002) and interactions with numerous transporters in multiple tissues (Kudo and Kawashima 2003); PFCs are extremely metabolically inert (Ylinen et al. 1990); and excretion of some PFCs is complicated by entero-hepatic circulation (Johnson et al. 1984) and potentially by active reabsorption in the kidneys (Andersen et al. 2006).

The PK of PFCs is even more extraordinary in that the half-lives of the longer chain PFCs vary by many orders of magnitude across species, and in some cases, across gender (Lau et al. 2007). Humans typically exhibit the longest half-lives (several years for PFOS and PFOA) (Bartell et al. 2010; Olsen et al. 2007), with monkeys, mice, male rats, and female rats having half-lives of months, weeks, days, and hours respectively (Lau et al. 2007). Such large differences in PK (e.g., female rats excrete PFOA more quickly than males) may result in vast differences in the external dose needed to achieve the same internal dose (Rodriguez et al. 2009; Wambaugh et al. 2013).

Administration of single doses of a PFC to laboratory animals typically produces serum concentration time course curves consistent with a two-compartment distribution (Andersen et al. 2008). However, PFOA is known to have dose-dependent (non-linear) pharmacokinetic properties: though repeated doses rapidly accumulate to a quasi-equilibrium blood concentration, a single dose results in a much longer half-life than would be consistent with the rapid approach to quasi-equilibrium (Andersen et al. 2006; Lou et al. 2009). Given its long half-life, using a linear PK model (e.g. the two-compartment model) to predict exposures resulting from multiple PFOA exposures results in large overestimates of reality (Lou et al. 2009).

The confidence in the PK predictions for PFCs that is needed to provide dosimetric anchoring of in vivo toxicity studies depends on how well one can answer the three big questions of PFC PK from the past 20 years:

1. Why are there huge discrepancies in half-lives of some PFCs between species?
2. Why do serum concentrations of PFOA and PFOS appear to rapidly approach steady-state after repeated dosing despite their long half-lives?
3. Why is there a gender difference in the excretion of PFOA by rats?

To date research have confidently answered the third question (hormone regulated transporter expression), and have reasonable hypotheses for the second (saturable resorption in the kidney proximal tubules), but an answer to the underlying mechanism driving the inter-species question remains elusive, forcing us to rely on empirical approaches that can explain the data we have, but offer little insight into the why or confidence for extrapolation (Andersen et al. 2008; Wambaugh et al. 2013). Crucial challenges remain in understanding the biological processes that drive the time and dose dependent PK phenomena of PFCs (Andersen et al. 2008).

The empirical saturable renal resorption hypothesis of the Andersen et al. (2006) model provides the simplest available PK model with non-linear kinetics. However, despite the plausible biological mechanism, this model is still empirical, requiring that species-specific parameters are estimated using species-specific PK data (Wambaugh et al. 2013). A physiologically-based PK (PBPK) model for PFCs might be preferable because it would allow extrapolation between species, provide better estimates of chemical-specific parameters, and allow estimation of chemical concentration in the specific tissues for which toxicity is observed. However, data for chemical-specific partitioning into most tissues exists only for PFOA. Given the limitations of the available data for estimating parameters, the simpler (Andersen et al. 2006) empirical PK model seems preferable.

14.2 Understanding the Non-linear PK of PFCs

The vast differences between species in the elimination half-life of PFCs is the most notable feature of PFCs PK (Lau et al. 2006). The carbon-chain length of PFCs appears to influence the excretion of PFCs, with shorter chain molecules tending to be eliminated more rapidly (Andersen et al. 2008; Ohmori et al. 2003). However, there are exceptions: e.g., the elimination half-life of PFHxS in humans is longer than that of PFOS (Lau et al. 2006). The predominance of excretion of the PFCs PFOA and PFOS is through urine, rather than feces (Cui et al. 2009; Wambaugh et al. 2008). However, administration of cholestyramine to rats increased excretion of both PFOS and PFOA nine times, indicating that there is considerable entero-hepatic circulation of these PFCs since cholestyramine is a drug which complexes with anions in the liver to promote biliary excretion (Johnson et al. 1984).

The gender difference in serum half-life of PFCs in rats depends on chain length with larger differences for the longer chain compounds (30 for males vs. 2.5 days for females for PFNA) (Andersen et al. 2008). Estradiol administration to both castrated and non-castrated male rats produced PFOA urine excretion at similar rates to female rats (Ylinen et al. 1990). Castration alone makes clearance in males similar to that of female rats (Kudo and Kawashima 2003). Treatment of castrated males with testosterone reduces clearance to normal male rat levels (Kudo and Kawashima 2003).

Renal clearances of PFOA are significantly smaller than passive elimination by glomerular filtration would predict, indicating a role for reabsorption by transporters in the proximal tubules of the kidney (Harada et al. 2004). The gender differences in the clearance of PFOA may be due to the actions of organic anion transporters in the kidney since several transporter proteins are expressed differentially in male and female adult rats (Buist et al. 2002; Buist and Klaassen 2004; Kudo et al. 2002; Lau et al. 2006). This "saturable resorption process" has been observed for other chemicals (Corley et al. 2005), albeit without gender differences. Both oatp1 and OAT3 mediate the resorption of PFOA in the proximal tubules of rat kidney (Katakura et al. 2007). Oatp1/OATP and OAT3 are both expressed abundantly

in rat, mouse, and human kidneys (Buist et al. 2002; Motohashi et al. 2002; Nakagawa et al. 2008), but their expression is enhanced by the presence of testosterone in rats (Ljubojević et al. 2004). Sex hormone regulated expression of transporters capable of resorbing PFCs in the proximal tubules of the kidney, from which they would otherwise be excreted, provides a plausible mechanism for explaining the gender differences in rat half-lives.

To date, the single biggest advance in the modeling of PFCs PK has been the non-linear model proposed by Andersen et al. (2006). In this model (shown in Fig. 14.2c) it is assumed that PFCs are passively excreted into the proximal tubules of the kidney by glomerular filtration, but that there is a counter process of active transport of the PFCs back from the proximal tubules. This might arise from transporters designed to prevent the excretion of endogenous fatty acids misidentifying PFCs for their non-perfluorinated fatty acid analogs (Andersen et al. 2006). In the event that these transporters are overwhelmed (i.e., saturated) by the concentration of PFC in the proximal tubule filtrate, the remaining PFC in the filtrate is rapidly excreted.

Both linear and branched PFCs have been used in the production of commercial products (Beesoon et al. 2011; Chu and Letcher 2009; Loveless et al. 2006). In rats given equivalent doses, branched, long-chain PFCs resulted in lower serum concentrations than those treated with linear, long-chain PFC (Loveless et al. 2006). Most PK studies focus on linear PFCs. Resorption of PFCs could explain why equivalent doses of linear and branched PFCs produce higher concentration for the linear molecules: if linear PFCs are more similar to endogenous fatty acids, then there may be differing affinities for organic anion transporters (Loveless et al. 2006).

However, if the saturable resorption hypothesis is true, then we must characterize the interactions of PFCs with the endogenous fatty acids that are competing for the same transporters (Andersen et al. 2008). Unfortunately, the difference between the PFOA half-lives in human beings and other animals is not likely to be attributable to differences in the affinities of PFOA for or expression levels of Oatp1/OATP and OAT3 transporters (Nakagawa et al. 2008). However, human Organic Anion Transporter (OAT4) is a transporter of PFOA (Nakagawa et al. 2009), that is only expressed in humans, is an apical type isoform in proximal tubules, and mediates the re-absorption of organic anions (Ekaratanawong et al. 2004; Nakagawa et al. 2009). The uptake of PFOA by OAT4 was greater than that by hOAT1 (Nakagawa et al. 2009). hOAT4 mRNA is abundantly expressed in the placenta as well as in the kidney (Cha et al. 2000; Nakagawa et al. 2009). Thus, OAT4 provides a plausible mechanism of inter-species half-differences in need of further study, but correlation between interspecies expression of OAT4 and half-life of PFCs has not yet been demonstrated.

Although the saturable resorption model of Andersen et al. (2006), does explain the non-linear PK of PFOA and PFOS, there are many other potential non-linearities at play for PFCs PK:

The acid dissociation constants (pKa) of PFOS and PFOA are <1, and for other PFCs they are predicted to be between 0 and 1.5, so it is reasonable to expect that most PFCs are ionized in tissue (Goss 2008; Johnson et al. 1984). The passive

(i.e., not transporter-facilitated) distribution of an arbitrary xenobiotic organic compound into tissue is often understood by studying the partitioning of the ionized and molecular forms of the compound into the aqueous and variously charged lipid phases of the tissue (Peyret and Krishnan 2011; Schmitt 2008). Unfortunately, PFCs confound this approach due to the high self-affinity of perfluorinated chemicals for each other, leading to a "fluorous phase" in addition to the typical aqueous and lipid phases (Dobbs and Kimberley 2002).

Further, at high concentrations, PFCs may even aggregate, further sequestering them from traditional PK interactions. Molecular aggregates (e.g., dimers and trimers) of PFCs have been reported at concentrations as low as the pM range (López-Fontán et al. 2005; Rayne and Forest 2009a). In the mM concentrations range, PFCs can even form large micelles (Rayne and Forest 2009a).

The predicted hydrophobicity (ratio of concentration of in octanol to that in water, or log P) increases with chain length for perfluorinated carboxylic acids: 2.91 (for PFBA), 3.69 (PFPA), 4.50 (PFHxA), 5.36 (PFHpA), 6.26 (PFOA), 7.23 (PFNA), and 8.26 (PFDA) (Rayne and Forest 2009b). The log P for PFOS is 4.67 (Rayne and Forest 2009b). The log P for PFOS or PFOA is roughly two orders of magnitude higher than their non-perfluorinated alkyl counterparts (Jing et al. 2009). These relatively high log P's present something of a paradox, since perfluroalkyl groups on molecules tend to make compounds oleophobic (Jing et al. 2009). However perfluroalkyl groups on molecules also tend to make compounds hydrophobic (Jing et al. 2009), so we can presume that the log P to some extent represents the competition between the fluorous phases and avoidance of both aqueous and lipid phases.

PFCs are highly bound to plasma protein; for example, albumin in plasma has a large capacity for binding PFOA (6–9 binding sites per molecule and mM concentration in plasma) (Han et al. 2003). PFHxS, PFOS, and PFOA are highly bound to human plasma albumin (>99.9 %, 99.8 %, and 99.7 % bound, respectively) (Kerstner-Wood et al. 2003). Plasma protein binding, estimated in vitro, was over 98 % for four PFCs tested in rat (Ohmori et al. 2003). Serum to plasma ratios for PFHxS, PFOS, and PFOA were 1:1 (Ehresman et al. 2007). Whole blood to plasma ratios were roughly 1:2 (Ehresman et al. 2007). PFOA is similarly bound by the serum of female and male rats, indicate that this is not a reason for gender-dependent differences in half-life (Ylinen et al. 1990).

In animals, the liver is a primary organ for distribution (Kemper 2003; Loccisano et al. 2012), at least at low doses (Kudo et al. 2007). PFOS and PFOA liver concentrations are several times higher than serum concentrations, with lesser distribution to the kidneys (Hundley et al. 2006; Johnson and Ober 1980; Lau et al. 2006; Seacat et al. 2002, 2003), however partitioning to liver may be less pronounced in humans (Fàbrega et al. 2014; Pérez et al. 2013). The Kemper (2003) data set for PFOA is perhaps the greatest source of partitioning information, with multiple rat tissues at multiple time points, but unfortunately these studies focused on single doses in the linear PK regime (Wambaugh et al. 2008), and therefore they do not illuminate the non-linearities (Andersen et al. 2006; Lou et al. 2009) or time-dependencies (Harris and Barton 2008; Tan et al. 2008) of PFC PK.

Distribution of PFOA to the liver decreases with increased dose in rat (Kudo et al. 2007), which possibly indicates the saturation of transporters. However, in the liver at least, passive diffusion has been shown to contribute significantly to the overall hepatic uptake (Han et al. 2008). In one analysis of human cadaver livers, the mean liver to serum ratio of PFOS was 1.3:1 (Olsen et al. 2003) which is comparable cynomolgus monkeys (Butenhoff et al. 2004b) but lower than in rat (Fàbrega et al. 2014; Kemper 2003). In the same study, the concentration of PFOSA, PFOA, and PFHxS were below the limit of quantitation in most of the individual liver samples as well as many serum analyses (Olsen et al. 2003). Based on an analysis of multiple human cadaver tissues, there is some evidence that most PFCs in general are found at higher concentrations in human lung tissues than elsewhere in the body, however even in that study PFOS was most concentrated in the liver and PFOA was found to be highest in bone (Pérez et al. 2013).

In summary, although the Andersen et al. (2006) model, derived from saturable resorption hypothesis, provides a good description of the non-linear PK of the PFCs PFOS and PFOA, the non-linear process described by that model could also in part be due to fluorous phase chemistry, plasma protein binding, or hepatic accumulation.

14.3 Selecting an Appropriate PK Model for PFCs

Due to the gender and pronounced species differences in elimination of PFCs, comparisons of toxicological effects must use a measure of internal, tissue dose rather than frank administered dose (Lau et al. 2006; Rodriguez et al. 2009; Wambaugh et al. 2013). PK models make predictions of internal, tissue doses; these predictions can be useful for interpolation – e.g., inferring what will happen for a dose between two tested doses – but the primary draw of PK modeling is often extrapolation beyond measured data.

For example, physiologically-based PK (PBPK) models allow extrapolation across physiologies, and therefore species, by separating physiologic PK factors (e.g., cardiac output) from chemical-specific factors that are believed to be independent of physiology (e.g., ratio of tissue concentration to plasma concentration at steady state). Simpler PK models tend to be phenomenological, and are therefore better suited to interpolation, while more complicated models can include biological processes that are understood to be conserved (e.g., between species) and so are suited to extrapolation.

The PK modeling literature for PFCs ranges from empirical one compartment models to PBPK models coupled to empirical excretion models. The general progression of these models is illustrated in Fig. 14.2.

The PFCs studied include PFBA (Chang et al. 2008a), PFBS (Olsen et al. 2009), PFNA (Tatum-Gibbs et al. 2011), PFHxS (Sundström et al. 2012) with most of the literature focusing on PFOS (Andersen et al. 2006; Chang et al. 2012; Harris and Barton 2008; Loccisano et al. 2011, 2012, 2013; Luebker et al. 2005b; Thompson et al. 2010; Trudel et al. 2008) especially PFOA (Andersen et al. 2006; Butenhoff et al. 2004b; Cui et al. 2010; Hinderliter et al. 2005; Hundley et al. 2006; Judson

et al. 2008; Kemper 2003; Lau et al. 2006; Loccisano et al. 2011, 2012, 2013; Lorber and Egeghy 2011; Rodriguez et al. 2009; Tan et al. 2008; Thompson et al. 2010; Trudel et al. 2008; Wambaugh et al. 2008).

The species studied typically include mouse, rat, monkey, and in some observational cases, humans. At this point, no single model yet exists that sufficiently explains the PK of any PFC such that cross-species extrapolation is a matter of simply changing physiological parameters, i.e. even the most elaborate PBPK model for PFOA still requires empirical calibration of the non-linear term in order to describe different species.

Describing the non-linear PK of PFOA and PFOS has been a key focus of many PFC PK efforts. Empirical models, such as the one compartment model in Fig. 14.2a typically allow a crude explanation of the kinetics from a single, low dose, via a constant elimination rate and a volume of distribution describing the empirical relationship between the concentration in a tissue that has been collected experimentally (typically, serum) and the concentration of chemical in the rest of the body. No insight into where in the body the remaining chemical is concentrated is allowed. One compartment models can include an absorption phase during which the concentration of chemical increases, but once the maximum concentration is reached (C_{max}) the elimination phase occurs at a fixed clearance flow (CL in L) rate (i.e., CL x V_d).

The elimination PK of PFOA and PFNA from serum following a single dose has been shown, however, to have at least two phases – at long times the elimination slows (Kemper 2003; Tatum-Gibbs et al. 2011; Wambaugh et al. 2008). The empirical two compartment model, shown in Fig. 14.2b, predicts this sort of biphasic elimination as the result of exchange between the plasma (or other tissue described by the primary concentration "compartment" C_1) and a tissue reservoir of the chemical (the second or deep tissue compartment C_2). The two compartment model is still linear; for example, linear models predict that the concentrations from twice the dose will be exactly twice as high at all times.

Figure 14.1 shows results from Lou et al. (2009) in which the PK of PFOA in mice are compared for single doses of 1, 10, and 60 mg/kg, and 2-week regimen of repeated daily 20 mg/kg. The two compartment model predictions (dashed line in Fig. 14.1) demonstrate how a linear model that describes the concentration time-course resulting from the lower, 1 and 10 mg/kg doses does not correctly describe the higher, 60 mg/kg dose. This is because the PK of PFOA become non-linear at higher doses. Further, even a two compartment model calibrated to describe the highest single dose (60 mg/kg, predictions shown by a dotted line in Fig. 14.1), does not correctly extrapolate to predict what happens for repeated doses. This is because at high doses PFOA reaches a steady state much faster than its long half-life would imply. Under linear PK, repeated doses of a chemical with a long half-life (i.e., slow clearance) would take a long time to reach steady state as the concentration gradually builds to a relatively high value (dashed line in Fig. 14.1). As shown by the data points in Fig. 14.1, repeated doses of PFOA rapidly (~2 days) results in a lower, steady state despite a single dose half-life of 3 weeks in mice.

The predictions of the Andersen et al. (2006) saturable resorption model in Fig. 14.1 are shown by a solid line. With this single model, the plasma concentrations resulting

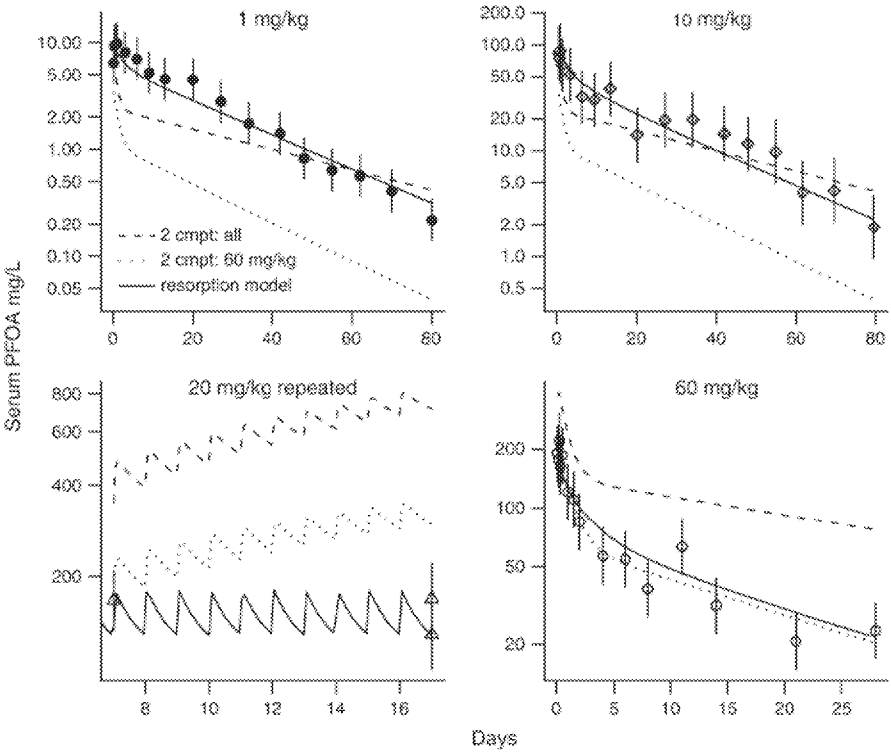

Fig. 14.1 Comparing predictions for the two-compartment model when fit to all the available data (*dashed line*) with a fit to just the 60 mg/kg data (*dotted line*). Neither model does a good job of describing all of the data, whereas the saturable resorption model (*solid line*) is more consistent between doses (Lou et al. (2009), by permission of Oxford University Press)

from low and high single doses, and from repeated doses, can all be reconciled. Although this model is biologically motivated (i.e., saturable resorption in the proximal tubules is a plausible process) it is still empirical. To date we cannot simply change the parameters describing the filtrate and the transporters involved in order to reconcile the differences in half-lives between species. We can, however, empirically estimate the values of those parameters to make the saturable resorption model fit multiple species (Wambaugh et al. 2013). Because we are making empirical adjustments rather than changing parameters to describe a biological process that is known to be conserved between species, it is prudent to think of the Andersen et al. (2006) model as a model that is generically non-linear, with saturable resorption being the most likely explanation. However, one could hypothesize other non-linear processes that might produce similar results, including saturable plasma protein or other binding, saturable sequestration in the liver, and saturable entero-hepatic recirculation.

The final class of PK models for PFCs, shown in Fig. 14.2d, is a PBPK model. Developed by Loccisano et al. (2011, 2012, 2013), this PBPK model combines partition coefficients estimated from single dose PK studies (Kemper 2003) with the

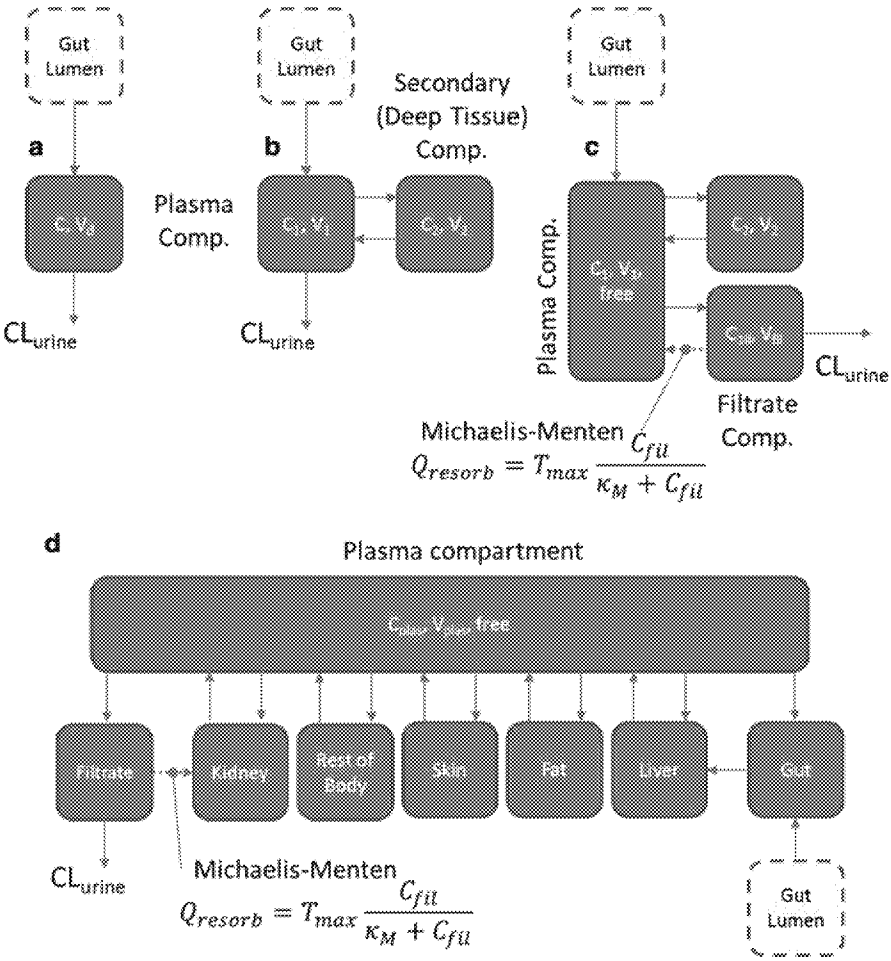

Fig. 14.2 PK models for PFCs have progressed incrementally from the empirical, linear one compartment (**a**) and two compartment models (**b**) to the empirical, non-linear "saturable resorption" model of Andersen et al. (2006) (**c**) Loccisano et al. 2011, 2012) incorporated the saturable resorption model into a PBPK model (**d**) although the non-linear excretion remained empirical (i.e., must be calibrated to data)

saturable resorption excretion process from the Andersen et al. (2006) model. This PBPK model allows prediction of tissue-specific concentrations, (e.g., liver, fat) for PFOA and PFOS. This PBPK model was extended to a pregnant maternal scenario by Loccisano et al. (2013). Fàbrega et al. (2014) updated this model with partition coefficients derived from human cadavers.

Both the original Andersen et al. (2006) and PBPK models assume a "highly bound" free fraction of 2 % free for both PFOS and PFOA; however, it should be

noted that the measured values of 0.2 % and 0.3 % indicate roughly ten times less PFC available for excretion via glomerular filtration (Kerstner-Wood et al. 2003).

It is important to note the progression of models from Fig. 14.2a–d; even the PBPK model still includes an empirical component with respect to the saturable resorption process. Unfortunately, the parameters describing the saturable resorption process are currently not independent of physiology, cannot be extrapolated, and therefore must be empirically estimated for each new species or physiology. This need for empirical calibration reflects that the current understanding of the non-linear kinetics of PFCs is not yet complete. Any extrapolation with empirical grounding may be fraught with uncertainty.

14.4 Dosimetric Anchoring of Animal Studies

Given the dose regimen of a toxicological study, different dose metrics can be predicted using an appropriately parameterized PK models: the time-integrated serum concentration (area under the curve or AUC), average serum concentration, and maximum serum concentration can be predicted for each *in vivo* study. These dosimetric anchors allow comparison across multiple in vivo studies in different species, despite the unusual PK of PFCs. For example, (Rodriguez et al. 2009) determined that, while the administered dose for two PFOA in vivo toxicological studies with similar toxicity endpoints in rats and mice differed by 30-fold (3 mg/kg/day and 0.1 mg/kg/day, respectively) the time-integrated serum concentrations (AUC) values were in fact similar.

There is an abundance of PK data allowing the use of empirically calibrated models for predicting tissue concentrations as the result of exposure to PFOS and PFOA. Wambaugh et al. (2013) collected the results of in vivo toxicity experiments on these PFCs with Tables 14.2 and 14.3 summarizing the study design, LOELs, and where available NOELs, from 10 PFOA studies and 13 PFOS studies. Toxicity endpoints were categorized as liver, thyroid, developmental, reproductive, or immunological (Wambaugh et al. 2013). Dosimetric anchoring via PK modeling demonstrated consistency between these in vivo studies.

In order to facilitate dosimetric anchoring, Wambaugh et al. (2013) used a Bayesian framework to incorporate uneven amounts of PK data from eight in vivo studies that used varying animals and dosing regimens. Model parameter distributions for a consistent PK model were estimated such that a 95 % credible interval for each dose metric could be predicted. The breadth of the credible interval of the predicted dose metrics reflects the uncertainty corresponding to the appropriateness of the PK model used and the available in vivo PK data sets for each species, strain/stock, and gender. Model predictions were assessed by comparing the predicted final serum concentration for each treatment with any measured final serum concentration in the in vivo toxicity experiments, and the predictions were generally similar to the measurements (within a factor of 2) (Wambaugh et al. 2013).

Table 14.2 PFOA in vivo toxicity studies

Study	Subject	Dose mg/kg/day	Exposure	NOEL mg/kg/day	LOEL mg/kg/day	Critical effect
						Liver
Butenhoff et al. (2002), (2004b)	Monkey (M)	3, 10, 30/20	26 weeks	NA	3	Increased liver weight
	Cynomolgus		Oral capsule			
Perkins et al. (2004)	Rat (M)	0.06, 0.64, 1.94, 6.50	13 weeks	0.06	0.64	Increased absolute and relative liver weight, hepatic hypertrophy- reversible following 8 week recovery period
	ChR-CD		Diet			
Butenhoff et al. (2004a) and York et al. (2010)	Rat (M)	1, 3, 10, 30	6 week pre mating- mating	NA	1	Increased absolute and relative liver weight
	Sprague-Dawley		Oral gavage			
White et al. (2009) and Wolf et al. (2007)	Mouse (F)	5, 20	GD7-17	Maternal: NA	Maternal:5	Maternal all groups except
	CD-1		GD10-17			5(15–17): increased relative liver weight
			GD13-17			
			GD15-17			
			Oral gavage			
White et al. (2009) and Wolf et al. (2007)	Mouse (F)	3, 5	GD1-17	Maternal: NA	Maternal: 3	Maternal: increased absolute and relative liver weight
	CD-1		Oral gavage			
DeWitt et al. (2008)	Mouse (F)	3.75, 7.5, 15, 30	15 days	NA	3.75	Increased relative liver weight
	C57BL/6 N		Drinking water			
						Developmental
Lau et al. (2006)	Mouse (F)	1, 3, 5, 10, 20, 40	GD1-17	Maternal: NA	Maternal: 1	Maternal-increased liver weight
	CD-1		Oral gavage	Developmental: NA	Developmental: 1	Developmental-accelerated sexual maturity in males

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White et al. (2009) and Wolf et al. (2007)	Mouse (F)	5 (all but GD15-17 group), 20 (GD15-17 group only)	GD7-17	Maternal: NA	Maternal: 5	Maternal all groups except
	CD-1		GD10-17	Developmental: NA	Developmental: 5	5 (15–17): increased relative liver weight
			GD13-17			20 GD15-17: decreased pup survival
			GD15-17			Developmental all groups: increased relative liver weight, delayed mammary gland development at PND29 and PND32
			Oral gavage			5(GD7-17, 10–17): delayed eye opening and body hair growth
White et al. (2009) and Wolf et al. (2007)	Mouse (F)	3, 5	GD1-17	Maternal: NA	Maternal: 3	Maternal: increased absolute and relative liver weight
	CD-1		Oral gavage	Developmental: NA	Developmental: 3	Developmental 3U + L, 5U, 5U + L: delayed eye opening and hair growth
			Cross-foster at birth			PND 22-all groups: increased relative liver weight
						PND22-all except 3 L: delayed mammary gland development
						PND42 all except 3U + L: delayed mammary gland development
						PND 63 all groups: delayed mammary gland development

(continued)

Table 14.2 (continued)

Study	Subject	Dose mg/kg/day	Exposure	NOEL mg/kg/day	LOEL mg/kg/day	Critical effect
Macon et al. (2011)	Mouse (F)	0.3, 1, 3	GD1-17 Oral gavage	NA	0.3	All groups: increased offspring relative liver weights
	CD-1	0.01,0.1, 1.0	GD10-17 Oral gavage	NA	0.01	All groups: stunted mammary epithelial growth 1 mg/kg group only: increased offspring relative liver weights
						Immunological
DeWitt et al. (2008)	Mouse (F) C57BL/6 N	0, 3.75, 7.5, 15, 30	15 days Drinking water	NA	3.75	Reduced SRBC-specific IgM antibody titers

Wambaugh et al. (2013) by permission of Oxford University Press

NA not applicable/could not be determined, *M* male, *F* female, *GD* gestation day, *LD* lactation day, *PND* post natal day, *U* in utero exposure, *L* lactational exposure, *U+L in utero* and lactational exposure, *SRBC* sheep red blood cells, *IgM* immunoglobulin M

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Table 14.3 PFOS in vivo toxicity studies

Study	Subject	Dose mg/kg/day	Exposure	NOEL mg/kg/day	LOEL mg/kg/day	Critical effect
						Liver
Curran et al. (2008)	Rat (M) Sprague-Dawley 15/group	0.14, 1.33, 3.21, 6.34	28 days, feed	0.14	1.33	Increased final relative (to BW) liver weight; decreased serum total T4
Curran et al. (2008)	Rat (F) Sprague-Dawley 15/group	0.15, 1.43, 3.73, 7.58	28 days, feed	NA	0.15	Increased final relative (to BW) liver weight
Seacat et al. (2003)	Rat (M) Crl:CD(SD) IGS BR 5/group	0.035, 0.14, 0.35, 1.4	98 days, feed	0.14	0.35	Centrilobular hepatic hypertrophy (at 1.4 mg/kg/day increased absolute/relative liver wt and ALT)
Seacat et al. (2003)	Rat (F) Crl:CD(SD) IGS BR 5/group	0.038, 0.15, 0.38, 1.56	98 days, feed	0.38	1.56	Centrilobular hepatic hypertrophy and increased relative liver wt
Seacat et al. (2002)	Monkey (MF) cynomolgus 6/sex/group	0.03, 0.15, 0.75	182 days, oral capsule	0.15	0.75	Increased absolute and relative hepatic wt; centrilobular or diffuse hepatocellular hypertrophy
						Thyroid
Chang et al. (2008b)	Rat (F) Sprague-Dawley 5–15/group	15	Single oral dose	NA	15	Decreased total T4 at 2, 6 and 24 h Decreased total T3 and rT3 at 24 h Increased free T4 at 2 and 6 h; normal at 24 h

(continued)

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Table 14.3 (continued)

Study	Subject	Dose mg/kg/day	Exposure	NOEL mg/kg/day	LOEL mg/kg/day	Critical effect
Curran et al. (2008)	Rat (F) Sprague-Dawley 15/group	0.15, 1.43, 3.73, 7.58	28 days, feed	0.15	1.43	Decreased total T4
Curran et al. (2008)	Rat (M) Sprague-Dawley 15/group	0.14, 1.33, 3.21, 6.34	28 days, feed	0.14	1.33	Decreased total T4
						Developmental
Butenhoff et al. (2009) and Chang et al. (2009)	Rat (F) Sprague-Dawley 25/group	0, 0.1, 0.3, 1.0	GD 0- PND 20 (41 days), oral gavage	0.3	1.0	M offspring: decreased habituation response
Lau et al. (2003) and Thibodeaux et al. (2003)	Rat (F) Sprague-Dawley 16-25/group	1, 2, 3, 5, 10	GDs 2–20 (19 days), oral gavage	1	2	Decreased pup survival and developmental delays
Luebker et al. (2005b)	Rat (F) Crl:CD(SD) IGS BR VAF/+	0.1, 0.4, 1.6, 3.2	63–76 days (6 weeks prior to mating through gestation and lactation), 2 generations (only 0.1 and 0.4), oral gavage	0.1	0.4	Developmental delays (eye opening)
Lau et al. (2003)	Mouse (F) CD1	1, 5, 10, 15, 20	GD1-18, oral gavage	5	10	Decreased pup survival
						Reproductive
Luebker et al. (2005a)	Rat (F) Crl:CD(SD) IGS BR VAF/+	0.1, 0.4, 1.6, 3.2	63–76 days, oral gavage	0.4	1.6	Decreased F1 reproductive outcome

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Luebker et al. (2005a)	Rat (F) Crl:CD(SD) IGS BR VAF/+	0.4, 0.8, 1.0, 1.2, 1.6, 2.0	63–76 days, oral gavage	1.2	1.6	Decreased viability
Chen et al. (2012)	Rat (F) Sprague-Dawley 10/group	0.1, 2	GD1-2, oral gavage	0.1	2.0	Histopathological changes to lungs; increased mortality
						Immunological
Dong et al. (2009)	Mouse (M) B6C3F1	0.0083, 0.083, 0.42, 0.83, 2.08	60 days, oral gavage	0.008	0.083	Increased splenic natural killer cell activity
Peden-Adams et al. (2008)	Mouse (M) B6C3F1	0.00018, 0.0018 0.0036, 0.018, 0.036, 0.18	28 days, oral gavage	0.00018	0.0018	Suppressed SRBC plaque-forming cell response
Peden-Adams et al. (2008)	Mouse (F) B6C3F1	0.00018, 0.0018 0.0036, 0.018, 0.036, 0.18	28 days, oral gavage	0.0018	0.0036	Suppressed SRBC plaque-forming cell response

Wambaugh et al. (2013) by permission of Oxford University Press

BW body weight, *T*4 Thyroxine, *T*3 Triiodothyronine, *rT*3 reverse Triiodothyronine, *ALT* Alanine Aminotransferase, *F1* first filial generation, *SRBC* sheep red blood cells

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Wambaugh et al. (2013) predicted dose metrics for the LOEL dose group for each endpoint in each study and, where available, the NOEL dose group. For many of the PFOA studies a NOEL group was lacking (i.e. the lowest dose tested showed an effect). The mean and maximum serum concentrations were found to be consistent dose metrics across in vivo studies.

PFOA hepatic effects have the most in vivo studies (Table 14.2). For this combination of chemical and effect, there are ten different in vivo LOELs from six studies (note that Wolf et al. (2007) identified five different LOELs for dosing on different windows of gestational days, e.g. days 7 through 17). For PFOA hepatic effects the outliers with respect to total dose and AUC are from the 180 day monkey study (Butenhoff et al. 2002). Although that study had a LOEL of 3 mg/kg/day, which is superficially similar to the LOELs of the other studies, the total dose of 540 mg/kg is a clear outlier with respect to the other studies.

Figure 14.3 compares the predicted mean serum concentration dose metric corresponding to the LOEL treatment group for each PFOA in vivo study. Where available, the dose metric for the NOEL treatment groups is also shown. If there is no NOEL dose group, all we know is that the effect happened somewhere between zero and the dose metric for the LOEL dose group.

The (Macon et al. 2011) study, which identified developmental effects in the growth of mammary tissue, is the most sensitive PFOA toxicity study considered here, as the predicted average concentration for that gestational day 10–17 LOEL is

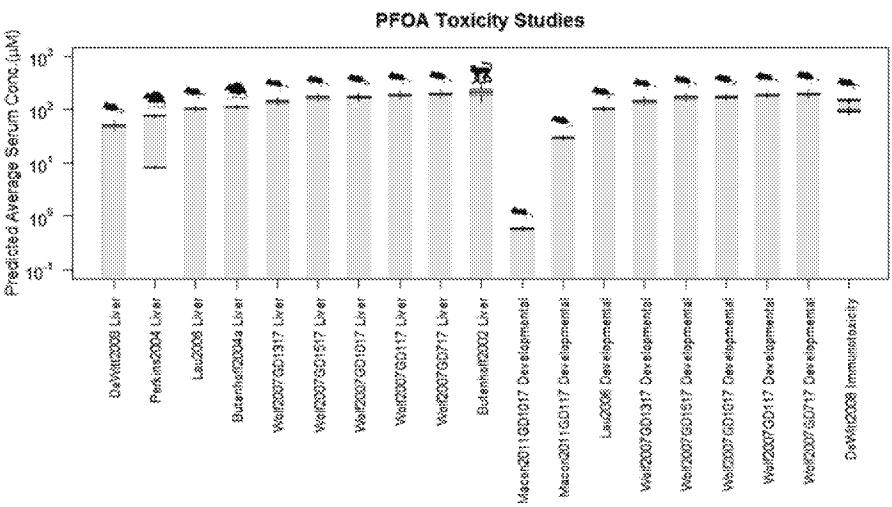

Fig. 14.3 Average serum concentration during PFOA in vivo toxicity studies (studies grouped by endpoint along y-axis). For each study the box and whisker plots indicate median, mean +- standard deviation, and 95 % credible intervals for LOEL and NOEL (lower of two points when NOEL was observed). Credible intervals are calculated using the distribution of PK model parameters for the Andersen et al. (2006) model, as determined by Wambaugh et al. (2013), for the animal and dose regimen used in each in vivo study

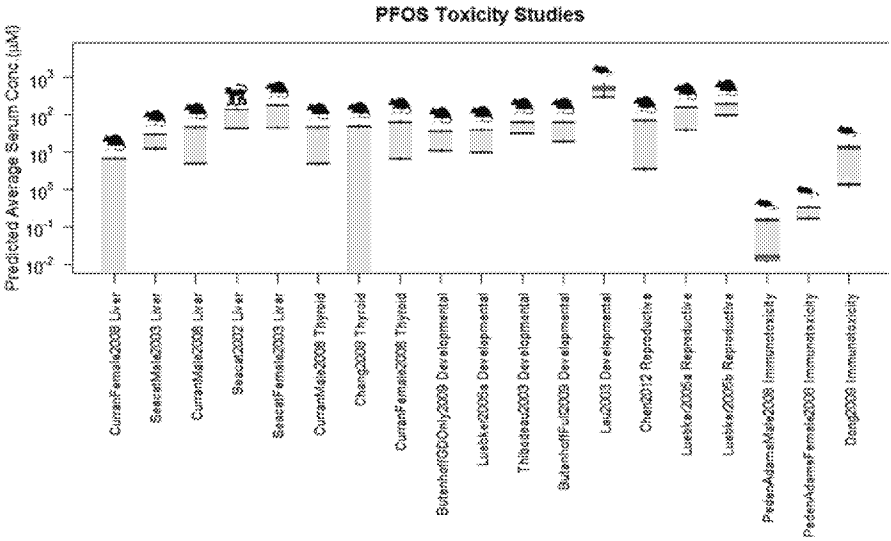

Fig. 14.4 Average serum concentration during PFOS in vivo toxicity studies (studies grouped by endpoint along y-axis). For each study the box and whisker plots indicate median, mean +- standard deviation, and 95 % credible intervals for LOEL and NOEL (lower of two points when NOEL was observed). Credible intervals are calculated using the distribution of PK model parameters for the Andersen et al. (2006) model, as determined by Wambaugh et al. (2013), for the animal and dose regimen used in each in vivo study

two orders of magnitude lower than most of the other studies, which regardless of species or endpoint, appear roughly consistent in Fig. 14.3a.

In Fig. 14.4 the PFOS in vivo effects have been compared using the predicted mean serum concentration dose metric across studies, species, and genders. Unlike with PFOA, the presence of NOELs for most PFOS studies allows clear argument that the dose metrics are generally consistent. The LOELs and NOELs for the three studies with thyroid effects are entirely consistent, but for each of liver, developmental, reproductive, and immunological effects there is one outlier study (e.g. a study with a NOEL predicted higher than LOELs of the other studies).

The LOELs and NOELs for liver effects are consistent for four studies, but the Curran et al. (2008) female rat LOEL is lower than the NOEL for the other four studies, including the Curran et al. (2008) male rat study. For developmental effects, the LOELs and NOELs are consistent for four studies, but the Lau et al. (2003) mouse study has a NOEL higher than the LOELs of the other studies (which were all rat studies). For the three studies showing reproductive effects, the Chen et al. (2012) LOEL is higher than the NOEL for the Luebker et al. (2005a) study.

Immunological effects for PFOS appear to be much more sensitive than the other endpoints observed. However, there is disagreement between the predicted dose metrics for the Dong et al. (2009) and the Peden-Adams et al. (2008) studies since the Peden-Adams et al. (2008) study identified a LOEL of 0.00018 mg/kg/day for suppressed sheep red blood cell plaque-forming cell response while the Dong et al. (2009) LOEL was 0.008 mg/kg/day for increased splenic natural killer cell activity.

14.5 Conclusion

Comparing the onset of in vivo effects across studies requires dosimetric anchoring to a measure of the tissue concentration at the site of toxicological effect (Rowlands et al. 2014). Predicting dose metrics requires PK models, which is challenging for PFCs because, as yet, there is not a model that allows extrapolation between species. Fortunately, there have been several cross species PK studies to collect the necessary data to allow empirical calibration of PK models to specific PFCs and species. For PFOS and PFOA, this sort of data has shown that, despite large differences in half-lives and the administered dose necessary for the onset of toxicological effects, no one species appears to be especially sensitive (Rodriguez et al. 2009; Wambaugh et al. 2013).

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Chapter 15
Human Health Risk Assessment of Perfluoroalkyl Acids

John L. Butenhoff and Joseph V. Rodricks

Abstract In this chapter, the major human health risk assessment activities that have been undertaken for human exposure to perfluoroalkyls, with emphasis on perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA), have been summarized. Margin of exposure risk assessments, risk assessments based on dietary exposure, and the establishment of regulatory levels for PFOS and PFOA concentration in drinking water are covered in detail. Although a large and robust database exists for PFOS and PFOA that covers multiple health endpoints, data are more limited for other perfluoroalkyls. A brief review of the chemical/physical properties and hazard profiles of PFOS and PFOA in the context of risk assessment and human relevance is given. It becomes apparent that the methods used to assess human health risk from exposure to perfluoroalkyls have been evolving and will likely continue to develop as new information and approaches are introduced. Perhaps the most important direction that risk assessment for perfluoroalkyls has taken has been in the use of internal dose metrics to bridge differences in pharmacokinetic elimination kinetics between species. There is a need to better inform epidemiological investigations with the understanding obtained from toxicological and pharmacokinetic investigations and principals. Translating our understanding from toxicological systems into a human context will improve our collective ability to understand whether environmental exposure to perfluoroalkyls affects human health risk.

Keywords Perfluoroalkyls • Risk assessment • Margin of exposure • Hazard determination • Drinking water

J.L. Butenhoff, Ph.D., DABT (✉)
SaluTox, L.L.C., Lake Elmo, MN, USA
(Formerly, Medical Department, 3M Company, St. Paul, MN, USA)
e-mail: SaluTox@gmail.com

J.V. Rodricks, Ph.D., DABT
ENVIRON International, Arlington, VA, USA

© Springer International Publishing Switzerland 2015

J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_15

ED_002330_00119699-00370

15.1 Introduction

This chapter will focus on the human health risk assessments for populations exposed to perfluoroalkyls, with particular emphasis on assessments for exposure to perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA). In addition, the development of major regulatory guidelines and standards for exposure is described. The principals, methods, and influencing factors related to the assessment of human health risk from perfluoroalkyls exposure will be presented. The risk assessments that have been conducted for PFOS and PFOA have either taken into account all sources of exposure by the use of PFOS and PFOA measurements from biomonitoring studies of human populations or have addressed a specific source of exposure, such as diet or drinking water.

The term "perfluoroalkyl acid" (or PFAA) describes a group of compounds that share the characteristic of being fully-fluorinated organic acids, with carboxyl, sulfonyl, or phosphonyl functional groups. These are typically strong organic acids with low pKa values compared to their hydrogenated analogs. Thus, under most common environmental and biological conditions, they are highly dissociated and exist principally in the anionic form. Salt forms of PFAAs typically have been used in commercial applications requiring high chemical stability and strong surface tension reducing properties. Examples include the use of ammonium salt of perfluorooctanoate (C8 or APFO) as an emulsifying agent in the production of polytetrafluoroethylene (PTFE) and of potassium salt of (PFOS) as a surfactant for fire-fighting foam and acid mist suppression in electroplating operations. While occupational and environmental exposures to PFAAs can occur via these direct surfactant applications, the generation of PFAAs through environmental and metabolic degradation of other fluorochemical substances used in commerce can also lead to exposure.

Small amounts of the element fluorine had been observed in human blood in the mid 1800s (Nicklès 1856). Nonetheless, in 1968, when Taves (1968) identified an organically-bound fluorine (organofluorine) in human blood, the source, natural or anthropogenic, was not apparent. Because organofluorine was observed in human blood and not in the blood of non-human animals, Taves suggested an industrial or commercial exposure source (Taves 1971). Several years thereafter, Guy et al. (1976) reported experimental data that suggested that the organofluorine in blood was consistent with fluorinated organic acids and was likely from an industrial source. They postulated that perfluoroalkyl carboxylates, in particular perfluorooctanoate (PFOA), may be a major component of this organofluorine. Following on these reports of the presence of organofluorine with the suggestion of PFOA as a principal component, Ubel et al. (1980) reported on the health of workers exposed to fluorochemicals. In their analysis, serum organofluorine measurements were made. In a small group of employees at the 3M Company's Cottage Grove, Minnesota location (see Chap. 4), serum was also analyzed specifically for PFOA, and it was found that approximately 90 % of the organofluorine measured was accounted for by PFOA in these workers. In the same year, Griffith and Long (1980)

reported on the basic toxicological hazard profile of APFO, the ammonium salt of PFOA. Ophaug and Singer (1980) found that a dose of approximately 8 mg PFOA (as acid)/kg body weight given by gavage to female rats (2 mg/rat) was rapidly absorbed, tightly bound to serum proteins, and fully excreted within 96 h, with 89.3 ± 2.6 % recovered in urine and 14.3 ± 4.1 % recovered in feces. They also noted that PFOA did not appear to be metabolized.

In reporting on the concentrations of ionic fluoride and organofluorine in eight serum samples from an area of rural China purported to have negligible exposure to industrial sources of organofluorine, Belisle (1981) found that the organofluorine concentrations in the samples were similar to concentrations in samples reported from more industrial areas, yet somewhat lower, and concluded that there was no compelling evidence that the organofluorine in human blood was largely from anthropogenic sources. These early studies were important in that they demonstrated the presence of organofluorine in human blood bound to serum proteins, suggested natural as well as anthropogenic sources, led to the medical monitoring of workers engaged in the production of fluorochemicals, and encouraged the development of more sensitive and specific bioanalytical methods.

As analytical methodology progressed, mass spectrometry became a tool that promised highly sensitive and specific identification of certain fluorochemicals. These analytical developments led to the identification by Hansen et al. (2001) of specific fluorochemicals in human serum using ion-pair extraction and high pressure liquid chromatography (HPLC) followed by negative ion electrospray tandem mass spectrometry (LC-MS/MS). They reported that the summed serum compound-specific concentrations agreed well with reported organofluorine concentrations in the early literature. In serum samples from 65 non-occupationally exposed volunteers, PFOS was detected in all samples above the lower limit of quantitation (LOQ = 5 ng/mL), with an average of 28.4 ± 13.6 (standard deviation) ng/mL (range 6.7–81.5 ng/mL). PFOA was also detected in all samples, and perfluorohexanesulfonate (PFHxS) was detected in all but one sample. In converting the mean ng/mL concentrations of PFOS, PFOA, and PFHxS to their non-specific organofluorine equivalent ng/mL concentrations (18.4, 4.4, and 4.1 ng/mL, respectively) and summing these organofluorine concentrations, Hansen et al. found an average of approximately 27 ng/mL organofluorine for the three analytes combined, consistent with the 26 ng/mL organofluorine reported by Guy et al. (1976).

The identification and quantitation of specific perfluoroalkyls (PFOS, PFOA, and PFHxS) in human sera, and the additional report of the widespread presence of PFOS in wildlife by Giesy and Kannan in the same year using similar methodology (Giesy and Kannan 2001), led to a renewed focus on the potential health implications of exposure, not only for fluorochemical workers, but also for the general population and populations that may have unique exposure from industrial sources. As the number of perfluoroalkyls and polyfluorinated alkyls substances detected has expanded, so has the number of investigators and government bodies that are actively enhancing our understanding of the potential for human health risk that may be associated with exposures to these materials.

15.2 Basic Principals Used in Risk Assessment of Perfluoroalkyls

Assessments of human health risk have been conducted for several perfluoroalkyls by a number of governmental authorities as well as independent assessments published in the scientific literature or formally submitted to government agencies. These assessments have varied in form and methodology. All of the assessments share the property of reviewing the human health hazard information, mostly in the form of toxicological studies, to identify a critical study or studies and critical endpoint(s). Dose or exposure levels from the critical study that are not expected to produce the critical effect are chosen as the point of departure (POD) for risk assessment. Although it is typical for POD values to be based on doses administered by a given route of exposure, typically oral, in the case of perfluoroalkyls, a number of PODs have been based on serum or liver perfluoroalkyl concentrations resulting from treatment as an indication of internal dose related to total body burden. Where human serum or liver perfluoroalkyl concentration data are available, uncertainties resulting from the large differences in elimination kinetics between humans and the animal models used in toxicological studies can be reduced significantly.

Typically, either the study no observed adverse effect level (NOAEL) or a modeled benchmark dose (BMD) derived from regression modeling of the study dose-response data are chosen as a POD. Modeled BMD values are preferable where possible based on established criteria for goodness of fit, as they use all of the study data and are less sensitive to the somewhat arbitrary spacing between experimental doses or concentrations. Occasionally in toxicological studies, a dose or exposure level producing no effect is not determined, in which case either a BMD value or the lowest study dose/exposure (LOAEL) are used. When the LOAEL is used, it is typically adjusted for uncertainty to account for the lack of a NOAEL.

In risk assessment, the dose/exposure corresponding to the POD is used either directly, as in some margin of exposure (MOE) analyses, as will be described later, or is reduced by various uncertainty factors to derive an acceptable level of exposure, often referred to as a reference dose (RfD) or reference concentration (RfC), tolerable daily intake (TDI), or derived no effect level (DNEL). Uncertainty adjustments do vary, but typically include adjustments for inter-individual (within species) as well as interspecies variability (across species) with respect to both pharmacodynamic response and pharmacokinetic handling. Other considerations of uncertainty related to the critical study and critical effect(s) as well as the strength of the overall hazard identification database may also be included. As will be described in the following sections, adjustments based on differences in clearance between humans and the animal models used in toxicological studies can be made to account for the differences in accumulation potential at a given dose. After deriving an acceptable dose/exposure level, it can then be used to compare with an observed or estimated exposure of a population for potential exceedance. Such risk assessments are used in determining the need for and degree of risk management.

Several risk assessments have been based on evaluating the MOE, which is the ratio of the POD to the observed or estimated population exposure. As an example, if the POD level of exposure is 100 units and the observed or estimated population level of exposure to a compound is 1 unit, the ratio (MOE) would be 100. Ascertainment of an acceptable MOE is a matter of judgment and policy; however, in general, the larger the margin of exposure, the less concern for health risk. An understanding of the mode of action involved in effecting biological responses in experimental models and the applicability or relevance of the mode of action for humans also aids in reducing uncertainty. MOE risk assessments that are based on serum or liver perfluoroalkyl concentration allow assessment based on all potential sources of exposure and reduce uncertainty relative to interspecies differences in elimination kinetics.

15.3 Evolution of Approaches to Human Health Risk Assessment for Perfluoroalkyl Exposure

15.3.1 Influence of the Pharmacokinetic Properties of Perfluoroalkyls on the Use of External Dose Versus Internal Dose Metrics in Risk Assessment

Most traditional human health risk assessments for exposure to chemical substances in the environment have been based on derivation of acceptable levels of exposure based on the administered dose-response profile of the chemical in question with appropriate adjustments for uncertainty (e.g., RfD, TDI, DNEL) and comparison of these levels of exposure to those estimated for human populations based on their intake of the chemical from various sources of exposure. This practice requires a fair understanding of the human exposure pathways and associated doses. This external dose risk assessment paradigm also works best if the pharmacokinetic and pharmacodynamic properties of the chemical are within the margins of uncertainty which are typically used, approximate half logs (i.e., factors of approximately 3) for each of pharmacokinetic and pharmacodynamic uncertainty, both within and between species.

While there is nothing inherently wrong with this traditional approach for many chemicals, it became apparent early in consideration of potential human health risk for PFOS and PFOA that a traditional approach had significant limitations. For example, the rather rapid elimination of PFOA in female rats, as first observed by Ophaug and Singer (1980), with an elimination half-life of hours, particularly in comparison to human serum PFOA elimination rate of several years, as first reported by Olsen et al. (2007) made the direct extrapolation of female dose levels in the two-generation study of APFO in rats (Butenhoff et al. 2004b) to humans questionable, as it would be expected that steady state body burden in humans would be

proportionately greater for a given dose. Although this is perhaps the extreme example, all species used in toxicological experiments with PFOS and PFOA had significantly faster elimination rates than humans by at least a factor of 10 (Chang et al. 2012; Hundley et al. 2006; Lau et al. 2007).

Another limitation of the traditional approach was the lack of knowledge regarding the human environmental sources and related intakes from those sources. The amounts of PFOS and PFOA used directly in commercial applications were limited; however, the degradation of what came to be called precursor compounds to form these perfluoroalkyls by either metabolic or environmental processes was not fully understood. Time and the insightful work of numerous investigators increased our understanding of potential sources and associated amounts of exposure, but voluntary manufacturing phase outs and regulatory restrictions as well as *de novo* or increased manufacturing in some areas, e.g., China, have changed patterns of exposure over the last decade. Sometimes a point source of exposure can be identified, as in Hochsauerland in Germany (Kraft et al. 2007) and the mid Ohio River valley (Emmett et al. 2006) between West Virginia and Ohio, but this is generally not the case for most populations. These examples illustrate the limitations of the traditional approach to human health risk assessment for perfluoroalkyls that have poor elimination characteristics in humans once absorbed as compared to the species used in toxicological investigations.

Because the widespread environmental presence of certain perfluoroalkyls, in particular PFOS, PFHxS, and PFOA, was discovered through the development of bioanalytical methodology, particularly blood-based analyses, the early biomonitoring, medical surveillance, and toxicological investigations included measurement of perfluoroalkyls in blood matrices. At the same time, it became increasingly apparent that serum or plasma concentrations of perfluoroalkyls were strongly correlated with administered dose in toxicological studies for PFOS, PFOA, and PFHxS (Andersen et al. 2008). In fact, serum PFOS concentrations were strongly correlated with cumulative administered dose under dosing conditions that did not reach saturation and steady state (Seacat et al. 2002). The rather low serum elimination rates for PFOS, PFHxS, and PFOA in humans (Olsen et al. 2007) suggested that cumulative exposures from all sources would be directly reflected on a proportional basis in their serum concentrations, as a measure related to total body burden. This was supported by the observation that volumes of distribution for those perfluoroalkyls for which pharmacokinetic parameters had been reported were consistent with predominant extracellular distribution (approximately 0.2 L/kg body weight, or 14 L for a 70 kg person). As a result, this property of the perfluoroalkyls circumvented the need for a detailed understanding of exposure sources in order to assess risk for the general population in cases where a known source of exposure was not a factor. The early MOE health risk characterizations for general population exposure to PFOS (3M 2003; Health Canada 2006) and PFOA (Butenhoff et al. 2004a; USEPA 2005) thus relied on population serum/plasma biomonitoring data as an indicator of exposure, comparing the serum perfluoroalkyls concentrations observed in the population to those serum perfluoroalkyl concentrations observed or estimated in toxicological studies at no effect or benchmark doses.

In cases where an external dose has been necessary, such as in setting risk levels for exposure to perfluoroalkyls in drinking water, the doses derived from toxicological studies have been either: (1) used in a traditional approach to set risk levels (UKDWI 2009); (2) have been adjusted with a correction for pharmacokinetic differences between the experimental model and humans (USEPA OW 2009); or (3) have been based on a serum perfluoroalkyl concentration associated with an effect level and adjusted for uncertainty followed by derivation of an external dose using a pharmacokinetic model or relationship (MDH 2009a; NCSAB 2012; Post et al. 2009; Tardiff et al. 2009). Incorporating the concept of internal dose, as represented by serum concentration of the perfluoroalkyls, significantly reduces if not obviates the pharmacokinetic component of uncertainty. However, in less than steady-state conditions, care should be taken to understand the relationship between increasing cumulative dose with repeated exposures and pharmacodynamic response. The majority of risk levels that have been developed using the concept of internal dose have eliminated the approximately half-log (approximately 3) pharmacokinetic component of interspecies uncertainty from the derivation.

The discussion above has concerned perfluoroalkyls for which the elimination rate in humans is significantly lower compared to the species used in toxicological studies. For smaller perfluoroalkyls with fewer perfluorinated carbons for which the human elimination rate approximates that for the species used in toxicological studies, pharmacokinetic adjustments to external dose may not be as necessary. For example, the serum elimination half-lives for PFBA in mice and rats were reported to range from approximately 1–16 h depending on species, dose, and sex versus approximately 40 h for male and female cynomolgus monkeys and approximately 75 h for male and female humans (Chang et al. 2008a). The State of Minnesota Department of Health (MDH) did use pharmacokinetic adjustments in their derivation of a Health Risk Level for PFBA in drinking water (MDH 2011b).

15.3.2 Default Assumptions Regarding Source Contribution for Populations with Known Sources of Exposure

Relative source contribution factors (RSCs), which attribute the proportion of total daily intake of a compound to a specific source, have historically been 10 or 20 % for drinking water by default. Although this default assumption often may be justified in circumstances where exposure sources are not well-characterized, it is notable that Maine has recognized that the availability of robust data representative of environmental background levels of exposure to PFOA in the form of the serum PFOA analyses from the United States Centers for Disease Control (CDC) National Health and Nutrition Examination Survey (NHANES) allowed for a data-driven estimation of RSC in the derivation of Maine's Maximum Exposure Guideline (MEG) for PFOA in drinking water (MEDHHS 2014). After adjusting the external dose POD based on the ratio of human clearance to clearance in species used in toxicological testing in order to derive a lower human equivalent dose (HED) and

then applying an uncertainty factor to the HED to derive a RfD, Maine calculated the estimated steady state serum PFOA for humans associated with the RfD. In so doing, Maine also derived a serum PFOA-based reference concentration (RfC). Subtracting the 95th percentile value from an NHANES data table from this serum PFOA-based RfC and dividing that value by the RfC yielded the proportion of the RfC that would be independent of general population background exposure to PFOA from all sources, which Maine rounded off to 60 %. Maine reasoned that, if a situation did occur where exposure to PFOA was present via a drinking water source in Maine, 60 % of the additional exposure at the RfD would be attributable to the drinking water source.

A similar approach could be taken in situations where biomonitoring data is available for populations with known exposures via drinking water sources as well as for the representative general population. Examples include the mid Ohio River Valley, the east metropolitan area near Saint Paul, Minnesota, and the Hochsauerland in Germany. It could be argued reasonably that the increased serum PFOA in these populations from their specific drinking water source relative to the general population serum PFOA reflects a larger proportion of intake from the drinking water source than suggested by the default assumption of 20 % that usually is applied.

15.3.3 Potential Use of Toxic Equivalency Factors or Hazard Index to Assess Risk from Exposure to Multiple Perfluoroalkyls

It has become evident from biomonitoring studies that humans potentially are exposed to multiple perfluoroalkyls and that these exposures, when represented as measured serum concentrations, are often correlated with each other (Olsen et al. 2003a). Few risk assessments have accounted for these multiple exposures, perhaps, in part, because of limited availability of hazard data for all but a few perfluoroalkyls. Borg et al. (2013) have recently published a risk assessment for the Swedish general population and an occupationally-exposed group of professional ski waxers using a hazard index (HI) approach. Their assessment, which will be described in more detail later, included 17 polyfluoroalkyl compounds by extrapolating hazard data from five of the 17 compounds. Although the hazard index (HI) approach may have value, available data for perfluoroalkyls as well as the broad assumptions used in read-across bring into question the robustness and appropriateness of this methodology.

Scialli et al. (2007) and Peters and Gonzalez (2011) have considered the possibility of combining exposure levels of perfluoroalkyls for risk assessment in a scaling system akin to the Toxic Equivalency Factors (TEFs) which have been developed for polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, and polychorinated dibenzofurans. Scialli et al. (2007) evaluated similar same-species studies performed with different perfluoroalkyls for concordance. They found discordance in endpoints measured for PFOS, PFOA, perfluorobutanesulfonate (PFBS), and perfluorodecanoic acid (PFDA). In addition, pairs of similar rat studies for PFOS,

PFOA, and PFBS, for which dose-response curves could be modeled for the concordant endpoints, did not provide consistent values within an order of magnitude for the same compound. They concluded that available data did not support the combining of perfluoroalkyls exposures in risk assessment.

Peters and Gonzalez (2011) used the analogy of the TEFs to evaluate the suitability of combining exposures of perfluoroalkyls in risk assessment. The TEF system for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and TCDD-like chemicals assigns an order of magnitude estimate for the toxicity of a compound relative to TCDD (Van den Berg et al. 2006). They noted that the conditions required to justify a TEF approach for TCDD and dioxin-like compounds are: (1) demonstration of a toxic response similar to TCDD; (2) a mechanism of toxicity that occurs via interaction with a common receptor (Ah receptor in the case of TCDD); and, (3) substantial experimental evidence showing additive effects of the agents within a factor of about 2. Peters and Gonzalez (2011) noted that data for the perfluoroalkyls likely precludes the use of TEFs, citing the following factors:

> (1) lack of conclusive evidence demonstrating that a single receptor is required to mediate the toxicities of perfluoroalkyl chemicals; (2) the potential influence of species differences in the response to PPARα ligands that would significantly limit this approach; (3) inconsistent toxicities observed with different perfluoroalkyl chemicals; and (4) a limited toxicological database for a number of perfluoroalkyls chemicals (e.g., perfluorinated sulfonamide polymers and perfluorinated sulfonamide-based phosphate fluorosurfactants).

To date, a TEF approach for perfluoroalkyls acids has not been developed and validated for use in risk assessment.

15.4 An Overview of Factors Influencing the Hazard Determination Process as They May Affect Risk Assessment

15.4.1 Toxicological Database for Perfluoroalkyls and Basic Properties Affecting Biological Interactions

The traditional first step in any human health risk assessment is the identification of potential health hazards. Since the confirmation of the widespread presence of perfluoroalkyls in biological samples from non-occupationally exposed human populations in the late 1990s, significant advancements have been made in our understanding of the biological interactions of perfluoroalkyls. Although the majority of investigations have focused on PFOS and PFOA, the range of perfluoroalkyls that have come under study has continued to increase. Moreover, the number and global distribution of investigators has increased correspondingly, resulting in rapid growth of the scientific literature. Toxicological studies with PFOS and PFOA have covered a range of endpoints, including: oncogenesis; hepatotoxicity; metabolic function; immune function; reproduction; development; hormonal changes; neurological effects. A number of mechanistic or mode-of-action studies has been published. It is not the intent of this

chapter to provide a review for all toxicological areas of investigation, which are covered in detail elsewhere in this book and in several reviews (Andersen et al. 2008; DeWitt et al. 2012; Kennedy et al. 2004; Lau et al. 2004, 2007). However, a brief overview in the context of risk assessment can be helpful. In the following discussion, it should be noted that the effects observed are in experimental systems where the doses or concentrations used typically vastly exceed those present in the environment.

By nature, perfluoroalkyls are exceptionally stable and non-reactive under physiological conditions. In fact, this is a property of these compounds that has been exploited in commercial applications (Kissa 2001). Although perfluoroalkyls, in particular, perfluorocarboxylates, resemble free fatty acids, they are not known to be metabolized or enter into the biochemical reactions that use fatty acids (Johnson et al. 1984; Kuslikis et al. 1992; Lau et al. 2007; Ophaug and Singer 1980). However, despite this lack of reactivity, perfluoroalkyls may present themselves as similar to fatty acids in their interactions with ionic binding sites, membranes and membrane transport processes, and interactions with regulators of metabolic processes. With respect to membranes, there may be effects on fluidity (Han et al. 2009; Hu et al. 2003; Starkov and Wallace 2002) and gap junction communication (Hu et al. 2002) at high enough concentrations. Some perfluoroalkyl carboxylates have been demonstrated to utilize membrane transport processes (Nakagawa et al. 2009; Weaver et al. 2010; Yang et al. 2010) and potentially affect the induction and expression of transporters.

Association of these relatively small, rigid, highly electronegative fatty acid-like molecules with ionic binding sites for proteins such as albumin and liver fatty acid binding protein has been demonstrated (Butenhoff et al. 2012d; Han et al. 2003; Jones et al. 2003; Luebker et al. 2002; Ophaug and Singer 1980). Several perfluoroalkyls have also been shown to either directly or indirectly activate nuclear receptors involved in controlling aspects of intermediary metabolism (Bjork et al. 2011; Bjork and Wallace 2009; Elcombe et al. 2010, 2012a; Haughom and Spydevold 1992; Maloney and Waxman 1999; Permadi et al. 1993; Shipley et al. 2004; Sohlenius et al. 1993; Vanden Heuvel et al. 2006; Wolf et al. 2008). Although the lack of metabolism can simplify risk assessment by eliminating the potential for interspecies differences in intermediary metabolism, the potential of perfluoroalkyls to compete for binding and transport with natural substrates and effect the activation of various metabolic processes via nuclear receptors can lead to species differences that affect extrapolation to humans (Andersen et al. 2008).

15.4.2 Species and Sex Differences in Pharmacokinetic Handling and the Role of Organic Anion Transporters

Another important example of the effect of species differences in biological interaction serves as a segue into the topic of pharmacokinetics and can be observed in reviewing the large differences in elimination kinetics that have been observed between species, and by age or sex within species, for PFOA (Hinderliter et al.

2006; Kennedy et al. 2004). While this is discussed elsewhere in this book, species, age, and sex differences in the expression of organic anion transporters most certainly are involved in the different pharmacokinetic profiles that have challenged risk assessors (Andersen et al. 2008; Han et al. 2012). The relatively rapid serum PFOA elimination of the female rat as compared to the male rat has been attributed to a sex-determined expression of renal proximal tubular transporters (Kudo et al. 2002), and data suggest that the male rat, on sexual maturation, has increased expression of a resorption transporter (Hinderliter et al. 2006), thus recapturing PFOA excreted in the urine filtrate (Loccisano et al. 2011, 2012). This resorption process has also been identified for humans as a likely explanation for the relatively long serum elimination half-life of PFOA, but the transporters involved may differ from those in the rat (Han et al. 2012; Yang et al. 2009, 2010). It is also apparent that these processes may be saturable, thus resulting in changes in kinetic parameters at higher levels of exposure (Andersen et al. 2006; Kemper 2003; Kudo et al. 2007).

When considering a series of perfluoroalkyls, for example, perfluoroalkyl sulfonates or perfluoroalkyl carboxylates, large within-species differences in elimination kinetics are observed between the smaller molecules with fewer carbons versus their larger homologs (Lau et al. 2007). Several factors may influence this, including binding affinity to serum carrier proteins and affinity for the key transport processes (Weaver et al. 2010). Branching in the carbon chain for materials manufactured by electrochemical fluorination (ECF) may also affect the elimination kinetics, as has been shown for PFOS in rats (Benskin et al. 2009; De Silva et al. 2009), PFOA in monkeys (3M Company, unpublished data), and PFBA in rats (Ehresman et al. 2007). Most risk assessments have not distinguished between branched and linear forms, and, indeed, much of the available toxicological and pharmacokinetic literature has reported on studies in which mixed linear and branched isomers were present. For PFOS and PFOA made by ECF, the linear content has been approximately 60–70 % of the total for PFOS and approximately 75–80 % for PFOA.

These isomeric differences could also affect target tissue bioavailability and response from differences in transporter affinity, membrane and intercellular binding, and receptor activation characteristics. Because isomeric forms are typically not separately analyzed and reported for pharmacokinetic samples from experimental investigations and in biomonitoring studies used in risk assessment, the impact of this variation in the kinetics of isomeric forms of perfluoroalkyls on risk assessments may not be fully appreciated. In a landmark study, Loveless et al. (2006) compared the effects of dosing mice and rats with linear, linear/branched, and highly branched ammonium PFOA. They were able to conclude from their study that "…the toxicological database developed primarily from testing linear/branched APFO is applicable to linear APFO."

Even though the elimination kinetics vary widely among the perfluoroalkyls that have been studied, although differences exist, there are general similarities in the absorption, distribution, and metabolism. As noted elsewhere, the perfluoroalkyls are resistant to non-metabolic and metabolic degradation pathways relevant to humans. In addition, for those PFAAs studied, the volumes of distribution are in a range that suggests a higher proportion of the body burden being distributed to

extracellular space (Andersen et al. 2008). The association with serum albumin and other carrier proteins in blood appears to constitute a principal distribution sink. As Jones et al. have suggested (Jones et al. 2003), this may have a protective effect at concentrations that do not physiologically impair with the function of natural substrates for these carriers.

Another factor to consider is the bioavailability of perfluoroalkyls to target tissues, such as liver. For example, when comparing the concurrent serum and liver concentrations of PFOS between rats, monkeys, and humans, rats appear to have proportionately higher liver-to-serum PFOS concentration ratios (Chang et al. 2012). Kudo et al. (2007) reported that biliary excretion of PFOA was affected by dose in rats, with lower doses resulting in uptake and distribution to membrane fractions with little biliary excretion, and higher doses resulting in proportionately higher biliary excretion. The concentration of PFBA in liver on dosing of rats with the ammonium salt has been shown to be consistent with predominant distribution in the blood serum contained in the liver (Butenhoff et al. 2012a; Chang et al. 2008a; Das et al. 2008; Foreman et al. 2009). Only two of the PFOS risk assessments that will be discussed attempted a MOE analysis based on liver concentration (3M 2003; Health Canada 2006). While it makes sense to perform MOE based on the target tissue concentration, lack of human-specific data on liver concentration for most perfluoroalkyls precludes meaningful analysis without making assumptions based on experimental studies with laboratory animals.

15.4.3 Activation of Nuclear Receptors

An important example can be taken in the species differences between rodents and humans in the pleiotropic effects resulting from activation of nuclear receptors involved with intermediary metabolism (Bjork et al. 2011; Bjork and Wallace 2009; Corton 2010; Corton et al. 2014; Elcombe et al. 2010, 2012a, 2014; Klaunig et al. 2012; Peters and Gonzalez 2011; Rosen et al. 2009). Several perfluoroalkyls have been shown to be capable of activating both human and rodent peroxisome proliferator activated receptor α (PPARα) (Bjork and Wallace 2009; Vanden Heuvel et al. 2006; Wolf et al. 2008). The activation of liver PPARα in rodent models is typically associated with a hypertrophic and hyperplastic response, while the hyperplastic component of that response appears to absent when the human forms of these receptors are activated (Elcombe et al. 2014). While the lack of the hyperplastic response in human liver has been explained at a molecular level (Gonzalez and Shah 2008), the hypertrophic response, which involves the up regulation of fatty acid metabolism, expansion of the smooth endoplasmic reticulum and, particularly in rodents, the proliferation of peroxisomes, appears to be less pronounced in humans, perhaps due to the known lesser amount of PPARα in human liver. The majority of effects of PFOA in rodents have been attributed to PPARα activation (Rosen et al. 2008b), the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) also appear to be involved (Elcombe et al. 2010; Rosen et al. 2008a).

15.4.4 Enlargement of the Liver as a Critical Effect

Increases in absolute and/or relative liver weight are typical and sensitive responses observed in toxicological investigations of PFAAs in rodents. Because increased absolute or relative liver weight is often the most sensitive finding in toxicological studies with PFOA, many of the examples of risk assessment activity related to PFOA discussed later in this chapter have based the POD on increased liver weight in either rodents or monkeys. The hepatic hypertrophic response to PFAA exposure in rodents is now believed to be due, in large part, to activation of PPARα and CAR/PXR (Bijland et al. 2011; Bjork et al. 2011; Elcombe et al. 2010, 2012a; Foreman et al. 2009; Klaunig et al. 2012; Rosen et al. 2013). PFOA has also been found to increase proliferation of mitochondria in rats and monkeys (Butenhoff et al. 2002; Cai et al. 1996; Walters et al. 2009). Although the use of liver weight increase alone, without indications of overt liver pathology, may be questioned as an appropriate POD for risk assessment (Hall et al. 2012), the relative refractivity of human liver to PPARα activation as compared to the rodent should be considered as a mitigating factor (Corton et al. 2014).

15.4.5 Changes in Serum Lipids as a Critical Effect

Reductions in serum lipids have been noted in toxicological investigations with PFAAs and have been attributed, in large part, to activation of PPARα. The ability of rather specific PPARα agonists, such as the fibrate class of drugs, to reduce low density lipoprotein (LDL) cholesterol (often referred to as bad cholesterol) while maintaining or increasing high-density lipoprotein (HDL) cholesterol (often referred to as good cholesterol), has resulted in the therapeutic use of PPARα agonists. However, some PFAAs have been shown to reduce both LDL and HDL cholesterol. PFOS reduced serum concentrations of both LDL and HDL in experimental studies with monkeys (Seacat et al. 2002) and APOE*3.Leiden.CETP transgenic mice (Bijland et al. 2011), a model developed for atherosclerosis research. This effect has been used by the United States Environmental Protection Agency Office of Water (USEPA OW) and State of Minnesota Department of Health (MDH) as a critical effect in establishing drinking water PFOS concentration values (MDH 2009b; USEPA OW 2009). The hypolipidemic effects of PFOS and PFHxS with respect to LDL and HDL cholesterol can be ascribed to PPARα- and PXR-mediated changes in the formation and clearance of lipoproteins (Bijland et al. 2011). Because serum HDL is involved in reverse cholesterol transport, sustained clinically significant reductions in serum HDL could be considered as potentially adverse. It is interesting to note that, when dosed with ammonium PFOA, no significant change in serum lipids was observed in male monkeys (Butenhoff et al. 2002), decreases in total and HDL cholesterol were noted in mice and rats (Loveless et al. 2006), and decreases in non-HDL cholesterol were noted in human cancer patients involved in a phase I

clinical trial (MacPherson et al. 2011). These observations suggest that care must be taken in evaluating changes in serum lipids as a result of exposure to PFAAs as endpoints for use in risk assessment.

15.4.6 Reproduction and Development as Critical Effects

Effects on reproduction and development have been considered as critical effects in risk assessments. Several perfluoroalkyls have been studied for their potential to disturb reproduction and development (Lau et al. 2004). Overt effects on reproductive function in male and female rats generally have not been observed. Increased early full-liter resorption is one effect noted in female rodents dosed with PFOA during gestation (Lau et al. 2006); however, this may be more the result of an effect on the maintenance of pregnancy in the rodent than an embryotoxic effect (Lau et al. 2005). Because there are significant differences between humans and rodents in the maintenance of pregnancy, it is important to develop a better understanding of this observation.

Since the first observation of perinatal mortality in a multi-generation study of PFOS in rats (Luebker et al. 2005a), there have been numerous developmental studies undertaken to increase understanding of the potential developmental toxicity of perfluoroalkyls (Lau et al. 2007). The prenatal developmental effects of these compounds largely are unremarkable (Case et al. 2001; Lau et al. 2004). Postnatal mortality and developmental delays have been the major focus of research.

A principal role for PPARα in mediating the developmental effects of PFOA in mice has been discovered (Abbott et al. 2007), and the human relevance of these effects in mice requires additional insight and discussion. In the case of PFOS, postnatal developmental effects appear to be either not mediated by PPARα or at least largely independent of PPARα (Abbott et al. 2009), and reduced neonatal survival did not appear to be the result of reductions in lipids, glucose utilization, or thyroid hormones (Luebker et al. 2005b). Potential interference with the functional properties of pulmonary surfactant at birth has been and continues to be a leading hypothesis for the basis of PFOS postnatal mortality (Grasty et al. 2005).

15.4.7 Changes in Serum Concentrations of Thyroid Hormones as a Critical Effect

PFOS-induced hypothyroxinemia in rats appears to be the result of increased displacement from serum carrier proteins and increased uptake and elimination by the liver and kidney as opposed to a direct effect on the hypothalamic-pituitary-thyroid axis (Chang et al. 2007, 2008b; Lau et al. 2003; Yu et al. 2009). Again, differences between rats and humans in the specificity of serum carrier proteins for thyroxine,

with resulting differences in plasma half-life of the hormones, suggest that humans should be less sensitive to this displacement binding effect of PFOS (Capen 1997; Curran and DeGroot 1991; Mendel et al. 1986), a widely-recognized factor in thyroid research that risk assessors should consider.

Changes in serum concentrations of hormones related to thyroid function, namely decreased triiodothyronine (T3) and increased thyrotropin (TSH), observed in a 6-month capsule dosing study in monkeys (Seacat et al. 2002) have been considered as co-critical endpoints by USEPA OW and MDH in establishing risk levels for PFOS in drinking water. The small magnitude of the changes relative to the natural variability of these endpoints and the lack of corresponding histological changes in the thyroid gland led the authors of that study to conclude that any actual change in serum thyroid-related hormones likely was due to non-thyroidal illness syndrome, and, as such, secondary to treatment-related stress.

15.4.8 Genotoxicity and Oncogenicity as Critical Endpoints

With respect to cancer risk, a combination of genotoxicity studies, chronic bioassays, and mechanistic studies is presently available for PFOS and PFOA. Perfluoroalkyls do not possess the chemical/physical properties typically associated with directly genotoxic agents and, in general, have not been found to be genotoxic in the various screening assays used to detect point mutations and chromosomal aberrations (Butenhoff et al. 2014; Lau et al. 2007). At the present time, three Sprague Dawley rat dietary toxicological studies are available that inform us about the oncogenic potential of PFOS and PFOA, two for ammonium PFOA (Biegel et al. 2001; Butenhoff et al. 2012c) and one for PFOS (Butenhoff et al. 2012b). None of these studies has shown a statistically significant increase in any type of malignant tumor. An increase in benign liver tumors was observed in one PFOA study (Biegel et al. 2001) and with PFOS. Based on several mechanistic studies, the origin of liver tumors from exposure of rats to PFOA and PFOS is currently believed to be the result of a combined activation of the xenosensor nuclear receptors, PPARα, CAR, and PXR (Elcombe et al. 2010, 2012a). As discussed above, recent advances in our understanding of differences between rodents and humans with respect to the proliferative response to activation of these receptors allows valuable perspective for human risk assessment in that human PPARα and CAR/PXR support the hepatic hypertrophic response but not the hepatic hyperplastic response, which is necessary for tumor formation (Corton et al. 2014; Elcombe et al. 2014). Pancreatic acinar cell tumors were also increased in one PFOA study (Biegel et al. 2001), and testicular Leydig cell tumors were increased in both PFOA studies. Insights have been gained as to the etiology of these two additional tumor types (Klaunig et al. 2012). It has been reported in secondary sources that female rat mammary tumors were increased by PFOA; however, this was not the conclusion of the study authors, and the lack of an increase in mammary tumors has been confirmed

after a complete audit of the study followed by a pathology working group review (Hardisty et al. 2010). PFOS increased benign thyroid follicular cell tumors (adenomas) only in males for whom dosing was suspended after 1 year and not for males dosed for 2 years. In a follow-up mechanistic studies, PFOS did not increase the S-phase labeling index or decrease apoptotic index of male Sprague Dawley rat thyroid follicular epithelial cells, suggesting that the original observation may have been a chance finding (Elcombe et al. 2012a, b).

To date, risk assessments have treated PFOS and PFOA as non-genotoxic, threshold "carcinogens". Butenhoff et al. (2004a) and Tardiff et al. (2009) used increased incidence of benign testicular Leydig cell adenoma in Sprague Dawley rats as a critical effect in performing MOE analysis and developing RfD values, respectively. No authoritative body has treated PFOS or PFOA as non-threshold carcinogens in risk assessment at this time.

15.4.9 Immunotoxicity

A number of rodent immunotoxicology studies have been published on PFOA and PFOS (DeWitt et al. 2009, 2012). In general, these studies have provided evidence of effects on inflammatory responses, production of proteins involved in immune responses, lymphoid organ weights, and antibody synthesis. Reported findings have been somewhat inconsistent, and have varied with dose, strain, and dosing methodology. Although observed responses have been shown to be driven, in part, by PPARα, the need to study the role of PPARα-independent processes and other factors that may affect the nature of observed responses has become clear (DeWitt et al. 2009).

15.4.10 Neurotoxicity

There have been a number of studies that have incorporated neurotoxicological endpoints (Mariussen 2012). As noted by Mariussen (2012) in a recent review of the neurotoxicological effects of PFAAs:

> Most of the studies that have showed neurobehavioral effects are on prenatally or neonatally animals exposed to doses that have caused other serious effects, such as increased mortality reduced growth and maturation, and birth defects. These effects may lead to the assumption that other toxicological endpoints are of higher importance. The observed neurobehavioral effects also appear subtle and inconclusive.

In general, neurological effects have not been singled out as PODs for risk assessment.

15.4.11 Epidemiological Investigations

The epidemiology of perfluoroalkyls is covered elsewhere in this book. The intention of this section is to comment on the use of epidemiological data in the risk assessment of perfluoroalkyls. To date, risk assessments of perfluoroalkyls have not been based primarily on the results of epidemiological studies.

Identification of organofluorine in human blood, the suggestion of an industrial source, and early results focused on perfluorinated carboxylates as a principal component of blood-borne organofluorine led to early reports on the health of workers at 3M Company engaged in the manufacture of fluorochemicals (Ubel et al. 1980). Until the mid 1990s, 3M Company's medical surveillance of its fluorochemical production workers included periodic measurement of non-specific total organofluorine at three fluorochemical manufacturing plant sites. Beginning in the mid 1990s, medical surveillance at these sites included serum measurements of PFOS and PFOA by high-performance liquid chromatography followed by mass spectrometry (HPLC-MS/MS) methodology. Similarly, such analytical advancements resulted in the speciation of PFOA at the DuPont Company Washington Works plant site in West Virginia, where the ammonium salt of PFOA (C8 or APFO) was used as an emulsifier in the polymerization of tetrafluoroethylene (TFE) to make polytetrafluoroethylene (PTFE) (Woskie et al. 2012). Most of the published articles on occupational fluorochemical biomonitoring (see Chap. 4) and occupational epidemiology (see Chap. 13) have related to the 3M Company and DuPont Company workforces. Although these occupational studies represent the highest known exposures to PFOS and PFOA, the use of these occupational data is rare for the purpose of human health risk assessment for non-occupationally exposed populations. The occupational studies would add valuable context to human health risk assessment.

Similarly, non-occupational epidemiological studies have not been used in health risk assessment of perfluoroalkyls. This is because many of the epidemiological studies of the general population or populations with known local sources of exposure have been cross-sectional in nature, therefore incapable of drawing conclusions with regard to causality. The distribution of perfluoroalkyls to serum carrier proteins and the slow elimination from serum of PFOS and PFOA in humans, likely due to renal proximal tubular resorption, can confound interpretation of cross-sectional epidemiological investigations that make associations between serum/plasma PFOS or PFOA concentrations and various serum clinical measures. Uncontrolled confounding factors that may affect the concentrations of PFOS and PFOA in serum through changes in processes that affect elimination or retention of these compounds, *e.g.*, filtration rate, plasma volume, blood loss, may lead to non-causal associations, especially at low perfluoroalkyls concentrations. An example is the cross-sectional association of serum PFOS and PFOA with subfecundity (as measured by time to pregnancy) in the Danish National Birth Cohort, first reported by Fei et al. (2009). Stratification by parity, taking into account nulliparous births, weakened some of the associations (Fei et al. 2012). Similarly, Whitworth et al.

(2012) found higher odds ratios for subfecundity in the fourth quartile of serum PFOS and PFOA concentrations in parous women of the Norwegian Mother and Child (MoBa) cohort associated with both PFOS (2.1 (95 % CI 1.2–3.8)) and PFOA (2.1 (95 % CI 1.0–4.0)) than among primiparous women (0.7 (95 % CI 0.4–1.3) and 0.5 (95 % CI 0.2–1.2), respectively). Transfer of body burden in prior pregnancies and re-equilibration with the environment in parous women were speculated to contribute to non-causal associations of serum PFOS and PFOA with subfecundity, as no associations were observed in primiparous mothers.

Integration of occupational and non-occupational epidemiological investigations with careful consideration of clinical, mechanistic, and pharmacokinetic factors is necessary. In the authors' opinion, much needs to be done to achieve a framework in which to integrate the toxicological, pharmacological, and epidemiological observations in the context of human health risk assessment for exposure to perfluoroalkyls.

15.4.12 Summary

In summary, there is an expanding understanding of the molecular, biological, metabolic, and physiological bases of responses observed in laboratory toxicological studies. Between and within species differences in the pharmacokinetic and pharmacodynamic properties of perfluoroalkyls have been investigated actively and are important factors to consider and incorporate into the health risk assessment process. Increased liver weight has been used frequently as a critical effect in developing acceptable levels of exposure for risk assessment, yet this is not necessarily reflective of an adverse outcome and may be overly conservative as an endpoint. It is important to incorporate an understanding of epidemiological investigations to gain perspective on the toxicological data; however, due to potential confounding with factors that affect clearance of perfluoroalkyls, care must be taken in interpretation of epidemiological observations, particularly from cross-sectional studies, that associate blood concentrations of perfluoroalkyls with health outcomes. Translating understanding from toxicological systems into a human context will improve our collective ability to understand potential human-health risk from environmental levels of exposures to these agents.

15.5 Perfluoroalkyl Risk Assessment for Non-occupationally Exposed Populations

Although the health status of occupational cohorts exposed to perfluoroalkyls has continued to be followed and updated (Raleigh et al. 2014; Steenland and Woskie 2012), increasing attention has been drawn to potential general population health

risks from background environmental levels of exposure to perfluoroalkyls as well as populations with potential exposure from point sources of exposure from industrial activity. These non-occupational epidemiological investigations are covered in detail in Chap. 13. Following the initial identification of PFOS as the major component of organofluorine in the samples from volunteers non-occupationally exposed to fluorochemicals as reported in 2001 (Hansen et al. 2001), the Organization for Economic Cooperation and Development (OECD) released a hazard profile of PFOS and its salts in which it was recommended that exposure information and risk assessments may be warranted based on widespread occurrence in the environment (OECD 2002).

In the same year, USEPA issued a significant new use rule (SNUR) that restricted the manufacture, use, sale, and importation of PFOS and related precursor materials as well as releasing a revised draft hazard assessment of PFOA and its salts (USEPA 2002). Several actions followed on the OECD hazard profile for PFOS. The UK concluded that PFOS met criteria as persistent, bioaccumulative, and toxic (PBT) (Brooke et al. 2004). The Swedish Chemical Inspectorate recommended a ban under the Stockholm Convention in 2005 (http://www.pops.int/documents/meetings/poprc/meeting_docs/en/POPRC1-INF9-b.pdf). In 2008, Canada banned the manufacture, use, sale, offering for sale or importation of PFOS and its salts as well as compounds containing the perfluorooctanesulfonamide moiety except in certain proscribed exceptions (Canadian Government Department of the Environment 2008). In 2009, PFOS and its precursor compound, perfluorooctanesulfonyl fluoride (POSF) were added to Annex B of the Stockholm Convention (http://chm.pops.int/Implementation/NewPOPs/TheNewPOPs/tabid/672/Default.aspx). This new attention focused on the environmental presence of perfluoroalkyls resulted in a surge of risk assessment activity focused on non-occupationally exposed populations, including populations with potential exposure to perfluoroalkyls via commercial and agricultural sources. This activity is the focus of the remainder of this chapter.

15.5.1 Margin of Exposure Health Risk Characterizations Based on Comparison of Serum or Liver Concentrations Associated with Effect to Those Observed in Biomonitoring Studies

15.5.1.1 Margin of Exposure Health Risk Characterization for PFOS (3M 2003)

In August of 2003, 3M Company submitted an "Environmental and Health Assessment of Perfluorooctane Sulfonic Acid and Its Salts" to the USEPA (3M 2003) that included a MOE characterization of risk. In 2003, the database available for human health risk characterization included a large number of toxicological studies as well as medical surveillance and epidemiological investigations of

exposed workers. The toxicological studies included: subchronic studies in rodents and monkeys; a two-year dietary chronic toxicity and cancer bioassay in rats; an extensive array of genotoxicological tests; reproduction/developmental studies in rats and mice, including a multigeneration reproduction study in rats; fetal developmental studies in rats and rabbits; pharmacokinetic data; and, various investigations into the mode of action of PFOS. In addition, 3M had conducted medical surveillance of fluorochemical production workers for over 25 years. Medical surveillance and epidemiological investigations in workers potentially exposed to PFOS included: medical surveillance of fluorochemical production workers at 3M plants in Decatur, Alabama and Antwerp, Belgium; a mortality study of the Decatur plant workers; a hypothesis-generating study of episodes of medical care based on medical insurance claims from Decatur plant employees. At the time of the MOE analysis, there were no epidemiological studies of the general (non-occupational) population (see Chap. 13). However, based on the biomonitoring data available for the general population, it was reasonable to assume that 3M fluorochemical production workers had the highest level of human exposure to PFOS at the time of the MOE assessment (see Chap. 4).

A unique feature of the MOE analysis was the use of serum/plasma and liver concentrations of PFOS as a measure of internal dose or internal exposure. The use of serum or liver PFOS concentrations as a measure related to integrated exposure to PFOS for risk characterization offered several distinct advantages. Foremost of these was overcoming the uncertainty involved in attempting quantitative estimates of external PFOS exposure from a variety of sources, routes of exposure, and exposure pathways that were not well-characterized at the time. Another important advantage was the ability to compare NOAELs or calculated BMDs, both expressed as the serum or liver PFOS concentration, between studies and species, thus reducing uncertainty in interspecies extrapolation. In the MOE analysis, it was possible to compare human exposure to PFOS as represented by serum or liver PFOS concentration to the serum and liver PFOS concentrations associated with NOAEL or BMD values from toxicological studies. Serum PFOS concentration was used as an integrated measure of exposure over time and related to the probability of toxic response, regardless of source or pathway of exposure. The overall potential variability in using serum PFOS concentrations in risk analysis is likely to be much less than attempting to estimate external exposures to humans from various sources. The MOE analysis was facilitated by the availability of serum/plasma PFOS concentration data in both fluorochemical production workers and the United States general population as well as serum/plasma PFOS measurements made during the course of toxicological investigations. Reported serum PFOS levels in fluorochemical production workers averaged 1,000–2,000 ng/mL, and the highest measured serum PFOS concentration in a worker approached 13,000 ng/mL (Olsen et al. 1999).

3M scientists, in collaboration with others, were able to survey PFOS serum concentrations in the United States general population in four separate studies. (These studies also provided PFOA concentration data used in a similar MOE health risk characterization (Butenhoff et al. 2004a), which is discussed below). Three of

Table 15.1 Geometric mean serum PFOS concentration (ng/mL), range, and upper bound estimate of 95 % tolerance limit in biomonitoring studies of children, adults, and elderly from the general United States population that were used for the 3M (2003) margin-of-exposure health risk characterization

Population (study)	N	Year(s) sampled	Geometric mean (95 % CI)	Range	Upper bound of 95 % tolerance limit estimate
Children (Olsen et al. 2004a)	598	1994–1995	38 (36–39)	7–515	97
Adults (Olsen et al. 2003a)	645	2001	35 (33–37)	<5[a]–1,645	100
Elderly (Olsen et al. 2004b)	238	2001	31 (29–33)	<3[a]–175	104

Tabulated data are adapted from Table 4.4 of 3M (2003)
[a]Analytical method limit of quantitation

these studies provided reasonably good estimates of serum PFOS concentration in the United States across age groups, including: (1) children (N = 598) involved in a Group A Streptococcal clinical trial across 23 states (Olsen et al. 2004a); (2) adult American Red Cross blood donors (N = 645) from six regional collection centers (Olsen et al. 2003a); and, (3) dementia-free elderly (N = 238) from a prospective study of cognitive function (Olsen et al. 2004b). These sample data revealed that approximately 95 % of individual serum PFOS concentrations were less than 100 ng/mL, and the average serum PFOS concentrations in these cohorts ranged between 30 and 40 ng/mL (Table 15.1). The fourth included study serum and/or liver samples from organ donors (N = 31) of which 23 serum and liver samples were paired (Olsen et al. 2003b). The paired organ donor liver and serum PFOS concentration data were valuable in providing insight into the ratio of liver-to-serum PFOS concentration, which allowed for extrapolation to estimate liver concentration in the biomonitoring studies for which only serum PFOS values were available.

Critical effect dose levels were based on either the highest study dose at which the critical effect was not observed (no observed adverse effect level, or NOAEL) or on the modeled benchmark dose (BMD) for the critical effect. The serum and liver PFOS concentrations associated with these dose levels were obtained by direct measurement or through estimation based on pharmacokinetic data and principals. The term benchmark internal concentration (BMIC) was used to represent a serum PFOS concentration corresponding to a BMD value based on administered dose.

The endpoints used for the serum PFOS MOE analysis are presented in Table 15.2 along with the serum PFOS concentration used as the POD for the MOE. For serum comparisons, the lower 95 % CL of the BMIC for a 5 % response ($LBMIC_5$) in reduced post-natal rat pup weight gain during lactation was chosen for the POD. This $LBMIC_5$ value was 31,000 ng/mL. While reduced pup weight gain was the most sensitive endpoint, comparisons were also made for other endpoints. For liver response, the male rat NOAEL for liver effects yielded a serum PFOS-based POD of 44,000 ng/mL PFOS. For liver tumors (benign adenoma) in male and female rats,

Table 15.2 Margins of exposure (MOE) from 3M (2003) health risk characterization based on human serum PFOS concentration in the United States general population

Critical endpoint	POD[a] (ng PFOS/mL serum)	MOE at estimated geometric mean[b] (40 ng PFOS/mL serum)	MOE at estimated upper bound[c] (100 ng PFOS/mL serum)
Pup weight gain	31,000	775	310
Liver effects, rats	44,000	1,100	440
Liver tumors, rats	62,000	1,550	620

Tabulated data are adapted from Table ES-1 of 3M (2003)
[a]Point of departure
[b]Value estimate from Table 15.1
[c]Estimated upper 95 % confidence limit at 95 % tolerance limit from Table 15.1

Table 15.3 Margins of exposure (MOE) from 3M (2003) health risk analysis based on estimated human liver PFOS concentration in the United States general population

Critical endpoint	POD[a] (ng PFOS/g liver)	MOE at estimated geometric mean (68 ng PFOS/g liver)[b]	MOE at estimated upper bound[c] (170 ng PFOS/g liver)[b, c]
Liver effects, monkeys	59,000	868	341

Tabulated data are adapted from Table ES-1 of 3M 2002
[a]Point of departure
[b]Conservative human liver PFOS concentration estimated from serum PFOS concentration in Table 15.1, assuming a liver-to-serum ratio of 1.7:1, which was the upper 95 % CL of liver-to-serum PFOS concentration ratios among 23 paired liver and serum samples from organ donors as reported by Olsen et al. (2003b)
[c]Estimated upper 95 % confidence limit at 95 % tolerance limit from Table 15.1

the $LBMIC_{10}$ (10 % response rate) was associated with a serum PFOS value of 62,000 ng/mL. Thus, the value for pup weight gain in lactation was considered to be protective of liver effects as well.

The serum-based MOE values used estimates of general population serum PFOS at the geometric mean and upper bound 95 % tolerance limit. Based on the data from Table 15.1, 40 and 100 ng PFOS/mL serum were chosen to represent the geometric mean and upper bound 95 % tolerance limit, respectively. MOE values were obtained by dividing the serum PFOS-based POD for an effect by these estimated serum concentrations for the general population (Table 15.2). At the geometric mean for the general population, MOE values ranged from 775 based on reduced rat pup weight gain to 1,550 based on benign liver tumors in rats, and, at the upper bound, MOEs ranged from 310 to 620, respectively.

In estimating the MOE based on liver PFOS concentration (Table 15.3), a POD of 59,000 ng PFOS/g liver was chosen. This liver concentration value corresponded to the study NOAEL for male cynomolgus monkeys in a 6-month oral toxicity study of PFOS (Seacat et al. 2002). Liver concentrations for the general population were conservatively estimated by multiplying the geometric mean and upper bound estimates for serum PFOS by a factor of 1.7, which was the maximum value of the liver-to-serum concentration ratio obtained from the 23 paired organ donor liver and

serum samples (Olsen et al. 2003b). MOE values of 868 and 341 were obtained for the geometric mean and upper bound estimated general population liver PFOS.

15.5.1.2 MOE for United States General Population Exposure to PFOA (Butenhoff et al. 2004a)

Butenhoff et al. (2004a) published a MOE risk characterization for United States general population exposure to PFOA. Measured general population serum PFOA concentrations were obtained from the biomonitoring studies that were used for the PFOS MOE risk characterization discussed previously (3M 2003). Serum concentrations of PFOA averaged approximately 5 ng PFOA/mL with an upper bound of the 95th percentile estimate approximating 11–14 ng PFOA/mL (Table 15.4). The MOE estimates for several endpoints were based on an upper bound 95 % tolerance limit estimate for serum PFOA of 14 ng PFOA/mL serum (Tables 15.4 and 15.5). Dose-response data from toxicological studies were used to estimate serum PFOA concentrations associated with a 10 % benchmark response (BMR) for several key endpoints (Table 15.5). The lower 95 % confidence limits of these benchmark internal concentrations ($LBMIC_{10}$) were then used as a basis for comparison with general population serum PFOA concentrations.

At the time of the assessment, the toxicological database included developmental toxicity, reproductive toxicity, immunotoxicity, genotoxicity, carcinogenicity, pharmacokinetic, and various mode-of-action studies (Kennedy et al. 2004). A review of the toxicological database for PFOA was conducted in order to select studies that covered a variety of endpoints, were sufficiently robust, and provided good dose-response data. The endpoints and associated studies chosen are presented in Table 15.5. Sensitive indicators of response that were chosen for the determination or estimation of $LBMIC_{10}$ were post-natal developmental effects in rats, liver-weight increase in monkeys (not considered by the authors to be an adverse effect in and of itself), body-weight change in monkeys, and increased incidence of benign testicular Leydig cell adenoma in rats. Serum PFOA concentrations for the $LBMIC_{10}$ were based on: (1) measured serum PFOA concentration at presumed steady state; (2) pharmacokinetic estimates of steady-state; or, (3) 24-h mean serum PFOA concentration (24-h area under the curve divided by 24 h). The POD $LBMIC_{10}$ values

Table 15.4 Upper bound estimate of 95 % tolerance limit for serum perfluorooctanoate (PFOA) in biomonitoring studies of children, adults, and elderly from the general United States population that were used for the Butenhoff et al. (2004a) margin-of-exposure health risk characterization

Population (study)	N	Year(s) sampled	Upper bound of 95 % tolerance limit estimate (ng PFOA/mL serum)
Children (Olsen et al. 2004a)	598	1994–1995	11
Adults (Olsen et al. 2003a)	645	2001	14
Elderly (Olsen et al. 2004b)	238	2001	11

Tabulated data are adapted from Table 1 of Butenhoff et al. (2004a)

Table 15.5 Margins of exposure (MOE) from the Butenhoff et al. (2004a) health risk analysis based on estimated upper bound 95 % tolerance limit for human serum perfluorooctanoate (PFOA) concentration (14 ng PFOA/mL serum) in the United States general population

Critical effect	POD (ng PFOA/mL serum)[a]	Margin of exposure[a]
Post-natal effects, rats	29,000[b]	2,100
Liver weight: brain weight ratio[c], monkeys	23,000	1,600
Body-weight change, monkeys	60,000	4,300
Benign leydig cell tumors, rats	125,000	8,900

Tabulated data are adapted from Table 10 of Butenhoff et al. (2004a)

[a]The margin of exposure was calculated by dividing the lower 95 % CL estimate of the benchmark internal concentration (serum concentration associated with the benchmark dose) for a 10 % response ($LBMIC_{10}$ (ng/mL)) by the general population serum PFOA concentration representing the upper 95 % confidence limit of the estimate of the 95th percentile general population serum PFOA (14 ng/mL)

[b]The serum [PFOA] in post-weaning rat pups were estimated conservatively based on adult female rat AUC at the $LBMD_{10}$ value of 22 mg/kg/day for post-natal effects using the relationship of AUC to administered oral dose.

[c]The authors noted that liver-weight increase was not necessary reflective of an adverse effect, as this is a normal adaptive response when other clinical and histological manifestations of liver toxicity are absent. This endpoint was used by the authors as a sensitive indication of biological response in a non-human primate

ranged from 23,000 ng PFOA/mL serum for liver-weight-to-brain-weight ratio increase in monkeys to 125,000 ng PFOA/mL serum for Leydig cell adenoma in rats (Table 15.5).

The MOE values shown in Table 15.5 were estimated by dividing the $LBMIC_{10}$-based POD by the upper bound 95 % tolerance limit estimate of the general population serum PFOA concentration (14 ng/mL). These MOE values ranged from 1,600 for increased liver-weight-to-brain-weight ratio in monkeys to 8,900 for Leydig cell adenoma in rats. The authors noted that MOEs based on the geometric mean serum PFOA (approximately 5 ng PFOA/mL serum) would be approximately three times higher.

15.5.1.3 United States Environmental Protection Agency Preliminary PFOA Health Risk Characterization (2003) and Draft PFOA Risk Assessment (2005)

In 2003, a preliminary MOE risk assessment was released by USEPA (2003), which presented a range of MOE values for developmental toxicity based on comparisons of human serum concentrations of PFOA and the serum concentrations in samples taken from rats involved in a two-generation reproduction and development study that was submitted to USEPA and later published by Butenhoff et al. (2004b). For

this preliminary assessment, USEPA used the adult American Red Cross blood donor and children studies published by Olsen et al. (2003a, 2004a) that were also used in the previously discussed MOE assessments for PFOS (3M 2003) and PFOA (Butenhoff et al. 2004a). USEPA used the mean and geometric mean serum PFOA concentrations for both sexes combined from the American Red Cross blood donor and children biomonitoring studies for MOEs calculated for women of childbearing age and children, respectively. Because there were no effects on reproductive parameters in the parental (F0) and F1 generation in rats, endpoints related to development were considered relevant for the preliminary risk assessment. These included significant mean body weight reductions with respect to controls during the lactation period, with the additional and likely related observation of post-weaning mortality and delayed sexual maturation. The study LOAEL for developmental effects was given as 10 mg/kg/day with a study NOAEL of 3 mg/kg/day.

Mean serum PFOA concentrations of the parental male and female rats from the two-generation reproduction and development study at 10 mg/kg/day dose group (51,100 ng/mL and 370 ng/mL, respectively) were used as POD values for calculating the MOEs. For women of childbearing age, the ranges of MOEs based on arithmetic mean and geometric mean serum PFOA were 66–9,125 and 80–11,109, respectively. For children, the ranges of MOEs based on the arithmetic and geometric mean serum PFOA were 66–9,125 and 75–10,429, respectively. However, the USEPA cautioned that the MOE values in their preliminary assessment should not be considered to represent the range of possible MOE values for general populations because of uncertainties resulting from the lack of appropriate pharmacokinetic data in weanling rats and their relationship to human serum levels of PFOA:

> It is important to note that MOEs that were calculated from the serum levels in the F0 female and male rats provide a means to bracket the low and high ends of experimental animal exposures. This is an unusual situation in that MOE estimates, which typically represent point estimates, are described here as a range of potential values due to uncertainties in the rat serum data. This situation arises from the fact that the available data do not allow selection of a particular departure point for the MOE calculations. It is likely that MOEs calculated using the F0 female rat serum level are lower than what would be anticipated in the human population, and it is likely that MOEs calculated using the F0 male rat serum level are higher than what would be anticipated in the human population. As uncertainty around the rat serum values decreases the end brackets are likely to shift towards the middle of the current range. Therefore, MOE values presented in this document should not be interpreted as representing the range of possible MOEs in the US population. It is likely that when more extensive rat kinetic data are available, the resultant, refined estimated range of MOEs will constitute a narrower subset of the range presented here. Interpretation of the significance of the MOEs for ascertaining potential levels of concern will necessitate a better understanding of the appropriate dose metric in rats, and the relationship of the dose metric to the human serum levels.

The USEPA followed with a draft risk assessment in 2005 (USEPA 2005). The 2005 draft risk assessment also derived MOE values but has not been finalized to date.

Table 15.6 Margins of exposure based on serum PFOS concentrations at the mean and 95th percentile for Canadian adults and United States children from the Health Canada (2006) screening health risk characterization

Critical effect(s), species	Point of departure (ng/mL serum)	Margin of exposure			
		Adults		Children	
		Mean (28 ng/mL)	95th percentile (63.1 ng/mL)	Mean (37.5 ng/mL)	95th percentile (97 ng/mL)
Histological changes in liver, ♂ and ♀ rats[a]	13,900	496	220	371	143
Multiple effects, monkeys[b]	14,500	578	230	387	149

Tabulated data are adapted from Health Canada (2006)
[a]Based on data from a 104-week dietary study of potassium PFOS in Sprague Dawley rats (Butenhoff et al. 2012b; Seacat et al. 2002)
[b]Thymic atrophy (♀), reduced serum high density lipoprotein (♂), cholesterol (♂), triiodothyronine (♂) and total bilirubin (♂). Based on a 6-month capsule dosing study of potassium PFOS in cynomolgus monkeys (Seacat et al. 2002)

15.5.1.4 Health Canada Screening Level Health Assessments for PFOS (Health Canada 2006)

In January, 2006, Health Canada released a "State of the Science Report for a Screening Health Assessment" of PFOS and its salts and precursors containing the perfluorooctanesulfonyl or perfluorooctanoate moiety (Health Canada 2006). In this screening MOE assessment, mean and 95th percentile serum PFOS values from a pilot biomonitoring study of Canadian adults (Kubwabo et al. 2004) and United States children (Olsen et al. 2004a) were used to represent the Canadian population (Table 15.6). Mean and 95th percentile serum PFOS values in ng/mL were 28 and 63.1 for adults, respectively, and 37.5 and 97 for children, respectively. In addition, the mean liver PFOS concentrations in a group of 30 organ donors from the United States (Olsen et al. 2003b) (18.8 ng PFOS/g liver) was used to estimate MOEs based on liver concentration data associated with the critical effects.

Two toxicological studies were chosen to assign critical endpoints and obtain associated serum and liver PFOS concentration data for use as PODs. These were a 104-week dietary study of potassium PFOS in Sprague Dawley rats (Butenhoff et al. 2012b; Seacat et al. 2003) and a 6-month capsule dosing study of potassium PFOS in cynomolgus monkeys (Seacat et al. 2002). Microscopic changes in the livers of male and female rats were chosen as the critical endpoint from the chronic dietary study, and combined average male and female serum and liver PFOS concentrations associated with this effect were used as the POD values for serum (13,900 ng PFOS/mL serum) and liver (40,800 ng PFOS/g liver). From the monkey study, thymic atrophy in females, and, in males, reductions in serum high density lipoprotein cholesterol, total cholesterol, triiodothyronine, and total bilirubin were considered as critical effects. Serum and liver concentrations associated with these effects were 14,500 ng/mL and 19,800 ng/g, respectively. Margins of exposure

based on serum PFOS are presented in Table 15.6 and varied from 371 to 578 at the mean values for serum PFOS for Canadian adults and United States children and from 143 to 230 at the 95th percentile serum PFOS values for these two groups.

The MOE values obtained based on liver PFOS concentration were 2,170 and 1,053 when comparing mean liver PFOS from the 30 organ donor samples to the liver PFOS concentration associated with the critical effects in the rat chronic dietary study and the monkey six-month capsule dosing study, respectively. In a footnote to the table displaying the MOE values, Health Canada noted that the MOEs based on the highest liver PFOS among the 30 donor samples (57 ng/g) would be 716 and 347 based on POD values from the rat and monkey studies, respectively.

In considering potential risk based on the MOE values obtained, Health Canada concluded:

> These margins are considered adequate to address elements of uncertainty, including intraspecies variation, interspecies variation and biological adversity or severity of the effects considered critical here. These margins will also be protective for the increased incidence of tumours observed in the chronic study of PFOS in rats, since the tumours were observed only at doses of PFOS that were higher than those that induced non-neoplastic effects and since the weight of evidence indicates that PFOS (and its precursors) are not genotoxic. While the margins for blood levels in children are somewhat less (approximately 145 for the 95th-percentile values), more appropriate margins for comparison with the effect level from long-term studies are those for adults (approximately 225 for the 95th-percentile values), since they are exposed for a greater portion of their life span. In addition, the critical lowest-observed-effect levels selected for development of these margins of exposure are very conservative, being about an order of magnitude less than values in other studies (i.e., for effects observed in reproductive studies with rats).

15.5.1.5 Health Canada Screening Level Health Assessment for PFOA (Health Canada 2012)

The screening assessment for PFOS was followed in 2012 by a screening level MOE assessment for exposure of Canadians to PFOA (Table 15.7). This assessment was also based on a MOE comparison of serum PFOA concentrations associated with toxicological studies with serum or plasma PFOA concentrations observed in adults, infants, and children. Adult geometric mean and 95th percentile plasma PFOA was based on results of the Canadian Health Measures Survey Cycle 1 (2007–2009) (Health Canada 2010). Geometric mean and maximum PFOA values for Inuit children in Canada aged 12–54 months were from a contaminant nutrient interaction study (Turgeon-O'Brien et al. 2010). Median and 95th percentile serum PFOA values for 6-month-old infants in Munich, Germany from samples collected between the years 2007–2009 were also used.

Serum PFOA concentrations associated with lowest observed effect levels for several critical endpoints were used as the reference POD for the MOE analyses. These are summarized in Table 15.7, and include increased liver weight in mice, changes in serum lipids in rats, increased liver weight in pregnant mice as well as developmental effects in their offspring, and increased liver weight in monkeys. These serum PFOA-based PODs varied from 13,000 to 77,000 ng/mL. Resulting

Table 15.7 Margins of exposure based on either geometric mean (GM), 95th percentile (95th %tile), maximum (MAX), or median (MED) serum PFOA concentrations for Canadian adults, Inuit children (12-months old), and German infants (6 months old) from the Health Canada (2012) screening health risk characterization

		Margin of exposure					
		Canadian adults (20–79 years old)		Inuit children (12–54 months old)		German infants (6-months old)	
Critical effect(s) sex and species	Point of departure (ng/mL)	GM 2.52	95th %-tile 5.50	GM 1.62	MAX 11	MED 6.9	95th %-tile 19.5
↑ Liver weight, ♂ mice[a]	13,000	5,159	2,364	8,024	1,182	1,884	667
Serum lipids, ♂ rats[a]	20,000	7,937	3,636	12,346	1,818	2,899	1,026
↑ Liver weight, ♀ mice, and development, ♂ mice[b]	21,900	8,690	3,982	13,519	1,991	3,174	1,123
↑ Liver weight, ♂ monkeys[c]	77,000	30,556	14,000	47,531	7,000	11,159	3,949

Tabulated data are adapted from Table 8 Health Canada (2012)
All PFOA concentrations are in ng/mL
[a]Based on the 14-day gavage study of Loveless et al. (2006)
[b]Increased liver weight (♀ mouse dams) and delayed fetal ossification and early puberty in male mouse pups from Lau et al. (2006)
[c]Based on the 6-month capsule dosing study of Butenhoff et al. (2002)

MOE values ranges from 1,884 to 30,556 at the central estimates and from 667 to 14,000 at the upper bound (Table 15.7). Health Canada concluded from these MOE data that:

> Based on the available information on the potential to cause harm to human health and the resulting margins of exposure, it is concluded that PFOA and its salts are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

15.5.2 Health Risk Characterizations Based on Dietary Intake of Perfluoroalkyls

Dietary sources represent an exposure pathway for PFOS and PFOA as reviewed by Domingo (2012); however, the proportion of total exposure to PFOS and PFOA that is contributed by diet may vary. Some authors have suggested diet as a principal source of exposure for the general population unaffected by point sources of exposure from industrial or agricultural activity. It is likely that there are regional

differences. Three examples of risk assessments based on estimated dietary intake follow.

15.5.2.1 United Kingdom Committee on Toxicology (COT) Statement on the Tolerable Daily Limit (TDI) for Intakes of PFOS and PFOA

The UK Committee on Toxicity of Chemicals in Food, Consumer Products and The Environment (COT) released their "COT Statement on the Tolerable Daily Intake for Perfluorooctane Sulfonate" and their "COT Statement on the Tolerable Daily Intake for Perfluorooctanoic Acid" in October, 2006 (COT 2006a, b). In considering PFOS, the NOAEL for reduced serum triiodothyronine (0.03 mg/kg) from the six-month capsule dosing study in cynomolgus monkeys of Seacat et al. (2002) was used as the critical effect. COT considered the pharmacokinetic data available for PFOS in the cynomolgus monkey model, noting that, at study term, monkeys in the 0.03 mg/kg dose group would have been at approximately one-half steady state. Because the critical effect chosen was considered mild, an uncertainty factor for incomplete attainment of steady state was not applied, and a total uncertainty of 100 was used to derive a provisional TDI of 0.3 μg/kg (0.0003 mg/kg). Because of the accumulative properties of PFOS, COT recognized that exposures should be averaged over prolonged times for comparison with the provisional TDI. In composite samples of food from a UK 2004 Total Diet Survey, PFOS was detected above the method detection limit only in potatoes, canned vegetables, eggs, and sugars and preserves (FSA 2006).

Using the latter data, the UK Food Standards Agency (FSA) estimated the ranges of average and high adult PFOA intake from whole diet to be 0.01–0.1 μg/kg body weight and 0.03–0.2 μg/kg body weight daily, respectively, where the ranges represent lower and upper bound values. For toddlers (1.5–2.5 years old), the estimated high dietary intake was 0.1–0.5 μg/kg body weight daily. Only 10–20 % of the estimated intake was assumed to be from the four food groups. Based on estimated food intake of PFOS and the provisional TDI, the COT concluded that, "on the basis available information this provisional TDI is adequate to protect against the range of identified effects." However, COT went on to note that some groups of consumers may exceed the recommended TDI. COT further noted that there were "considerable uncertainties in the dietary intake estimates, and therefore these potential exceedances do not indicate immediate toxicological concern."

In considering PFOA, a point of departure of 0.3 mg/kg body weight daily was established based on several endpoints (hepatic, renal, hematological, and immunological). To this, a total uncertainty factor of 100 was applied to derive a TDI of 3 μg/kg body weight daily (0.003 mg/kg). The COT noted that PFOA was only detected in potatoes above the method limit of detection in the analysis of composite food group samples from the UK 2004 Total Diet Study. The FSA estimated the

ranges of average and high PFOA adult intake from whole diet to be 0.001–0.07 μg/kg body weight daily and 0.003–0.1 μg/kg body weight daily, respectfully, where the ranges represent lower and upper bound values. For toddlers, the estimated high dietary intake was 0.01–0.3 μg/kg body weight daily. An analysis based on estimated intake of PFOA from food as compared to the TDI led the COT to conclude that "the estimated intakes are not of concern regarding human health."

In 2009, at the request of the UK Drinking Water Inspectorate (DWI), COT reconsidered their TDI for PFOA and lowered this to 1.5 μg/kg body weight daily after adding an additional pharmacokinetic uncertainty factor of 2 (COT 2009). COT also reevaluated the provisional TDI for PFOS at that time, and COT came to the conclusion that a change in the PFOS TDI was not warranted.

15.5.2.2 Health Canada Health Risk Characterization for Exposure of Canadians to PFOS and PFOA from Consumption of Various Food Items (Tittlemier et al. 2007)

Tittlemier et al. (2007) reported on the analysis of 54 solid food composite samples from the Canadian Total Diet Study collected between 1992 and 2004 for PFOS and perfluorocarboxylates (PFCAs), including PFOA. Nine of the composite samples contained quantifiable amounts of perfluoroalkyl compounds, with PFOS and PFOA being detected with greatest frequency. From the 25 composite samples in 2004, only six of which had detectable perfluoroalkyls, they estimated that the dietary intake of PFCAs and PFOS to be 250 ng/day and concluded that diet was an important source, with PFOS contributing 110 ng/day and PFOA and perfluorononoate (PFNA) each contributing 70 ng/day. This was in consideration of data for estimated daily exposure via water, dust, solution-treated carpeting, treated apparel, and air, which totalled 160.3 ng/day in Table 6 of their published article.

Tittlemier et al. used their analytical data and estimates of dietary exposure to perform MOE risk characterizations for Canadians ≥12 years old for dietary exposure to PFOS and perfluorocarboxylates. For the latter, it was assumed that all perfluorocarboxylates had the same biological activity as PFOA. For the PFOS POD, the 0.03 mg/kg/day dose from the six-month capsule dosing study in monkeys (Seacat et al. 2002) was taken as the LOEL, consistent with the Health Canada screening assessment discussed previously (Health Canada 2006). The POD for the perfluorocarboxylates was based on the $BMDL_{10}$ for PFOA of 0.6 mg/kg/day for increased liver weight in rat parental and F1 offspring from the two generation study of Butenhoff et al. (2004b) as calculated by Butenhoff et al. (2004a). The calculated MOE values were greater than 1.6×10^4 and 2.7×10^5 for PFOS and PFOA, respectively. The authors noted that MOE values $\geq 1.0 \times 10^4$ were considered by the EFSA to be of low concern (EFSA 2005). The authors also noted that, for infants and children, a separate exposure evaluation involving a broader array of composites would be warranted.

15.5.2.3 European Food Safety Agency (EFSA) Scientific Panel on Contaminants in the Food Chain (CONTAM) Health Risk Characterization for PFOS and PFOA (Alexander et al. 2008; EFSA 2012)

An initial risk assessment for the exposure of the European population to PFOS and PFOA via food was published in 2008 by the European Food Safety Agency (EFSA) Scientific Panel on Contaminants in the Food Chain (CONTAM) (Alexander et al. 2008). At the time of their assessment, CONTAM had established TDI values for PFOS and PFOA of 0.15 μg/kg body weight per day and 1.5 μg/kg body weight per day, respectively.

The TDI for PFOS was based on the 6-month capsule dosing study in cynomolgus monkeys (Seacat et al. 2002), from which CONTAM identified a NOAEL of 0.03 μg/kg body weight per day as suitable for deriving the TDI. CONTAM used an overall uncertainty factor (UF) of 200, 100 for inter and intra-species differences plus an additional factor of two to compensate for uncertainties in connection to the relatively short duration of the study with respect to the internal dose kinetics, yielding a TDI of 0.15 μg/kg body weight per day for PFOS. CONTAM noted difficulties in obtaining robust estimates of daily PFOS intake and reported indicative dietary exposures of 0.060 μg/kg body weight per day based on average food consumption to 0.200 μg/kg body weight per day for higher consumption of fish. CONTAM noted that the average consumption values were below the TDI of 0.150 μg/kg body weight, but that the highest exposed people within the general population might slightly exceed this TDI.

CONTAM recognized that limited quantitative data were available for the occurrence of PFOA in food. Because serum PFOA concentrations measured in non-occupational populations in Europe were approximately three orders of magnitude lower than those associated with thresholds for effects in rats, CONTAM considered it unlikely that adverse effects of PFOA were occurring in the general population.

Importantly, CONTAM pointed out the limited availability of data on exposure via food and suggested that more data on the occurrence of polyfluoroalkylated substances (PFASs) be collected to facilitate a more accurate assessment of risk. This resulted in the European Commission's issuance of Commission Recommendation 2010/161/EU which called for member states to collect data on the occurrence and concentration of PFASs in a broad range of foods. These monitoring data were collected and assessed by EFSA, resulting in EFSA's updated risk assessment, which was published in 2012 (EFSA 2012).

For their 2012 risk assessment, EFSA had available 54,195 analytical determinations covering 27 PFASs made across 7,560 samples of food that were submitted by 13 European member states. Not all analytes were monitored in all samples. Of the 27 PFAS analytes, only 16 were present at concentrations that allowed quantitation. All analyses for the other 11 PFASs were either below the limit of quantitation or the limit of detection. Analytical determinations for PFOS (N=7,523) and PFOA (N=7,536) were most frequent. PFOS was by far the most frequently quantified

PFAS in food (29 %). Foodstuffs with the highest frequency of reported PFAS analytes were fish and other seafood, meat and meat products, with lesser frequencies in other food groups. Highest concentrations were in edible offal, especially liver. EFSA reported the range of PFOS concentrations in samples varied from a low of 0.00034 μg/L in a drinking water sample to a high of 3,480 μg/kg for wild boar liver.

In their assessment of risk from exposure via food, EFSA used lower and upper bound mean concentrations of PFASs. Because of a "very low proportion of quantified results", EFSA concluded that, with the exception of PFOS and PFOA, chronic dietary exposure to the additional 25 PFASs would be on the order of low ng/kg body weight per day or lower, and that the lack of TDI values for these 25 PFASs disallowed evaluation of the relevance of their contribution to dietary exposure to human health. The results of the EFSA risk analyses for PFOS and PFOA with respect to adults and children are shown in Table 15.8. With respect to dietary exposure to PFOS and PFOA, EFSA concluded that:

> The low proportion of quantified results prevented calculation of a more realistic dietary exposure. The upper bound result are highly overestimated, but still the exposure estimates in all age classes and for both mean and 95th percentile consumers were well below the TDIs for PFOS (150 ng/kg b.w. per day) and PFOA (1500 ng/kg b.w. per day) set by the EFSA Scientific Panel on Contaminants in the Food Chain.

15.5.3 Risk Assessment for Members of the Swedish Population Using a Hazard Index Approach

Using a hazard index (HI) approach, Borg et al. (2013) have recently reported on a risk assessment for 17 polyfluoroalkylated chemicals identified in samples from the Swedish general population as well as a group of occupationally-exposed ski waxers. RfD values for liver and reproductive (developmental) effects were established for compounds for which appropriate toxicological data existed (5 of the 17) via selection of POD values based on NOAELs where feasible and through application of assessment factors (AF = uncertainty factors) based on REACH guidelines. Read-across methodology was used to estimate the RfD values for the 12 compounds which lacked appropriate data. This involved use of an AF of three to go from shorter-chain congener to longer-chain, while no AF was used when extrapolating from longer-chain to shorter-chain congeners. Because RfD values based on serum concentration of the chemicals were derived, an AF for pharmacokinetic differences between species was not used. Hazard quotients (HQs) for "hepatotoxicity" and "reproductive toxicity" for the general population and an occupationally-exposed group were calculated for 15 of the 17 compounds by taking the ratio of the highest population serum level from 5 general population biomonitoring studies and one occupational study of ski waxers to the serum concentration-based RfD. Individual perfluoroalkyl HQ values >1.0 were considered cause for concern by the authors. These HQ values were summed to yield the HI, such that an HI value

Table 15.8 Estimated highest upper bound mean and highest 95th percentile daily dietary exposures of adults and toddlers to PFOS and PFOA in ng/kg body weight at and the corresponding percent of the TDI represented by these exposures from the EFSA (2012) risk characterization

	TDI	Highest upper bound mean daily dietary intake						Highest 95th percentile daily dietary intake					
		Adult			Toddler			Adult			Toddler		
	(ng/kg)	ng/kg	%TDI	MOE[a]	ng/kg	%TDI	MOE	ng/kg	%TDI	MOE	ng/kg	%TDI	MOE
PFOS	150	5.2	3.5	29	14.0	9.3	10.7	10.0	6.7	15.0	28.5	19.0	5
PFOA	1,500	4.3	0.3	349	16.5	1.1	90.9	7.7	0.5	194.8	31.5	2.1	48

[a]Margins of exposure based on the TDI have been calculated by the authors from the data

of >1.0 would be considered by the authors as a cause for concern for the combined exposures. PFOS was the largest individual contributor to the HI. The HIs for "hepatotoxicity" and "reproductive toxicity" for the general population did not show cause for concern, except for a small sub-population of high fish consumers. HIs for ski waxers were above 1 for "hepatotoxicity" (1.3–1.4) and "reproductive toxicity" (1.1).

15.5.4 Conclusions Regarding General-Population Risk Assessments

The general populations risk assessments that have been presented in this section support a conclusion that untoward health risk is unlikely at the levels of exposure found from serum or liver biomonitoring or in diet. The MOE analyses based on serum PFOS and PFOA levels found in the general population provide a direct indication of the extent of exposure of these populations from all sources in the environment. The strength of these assessments is dependent on the degree to which the serum biomonitoring data represent the population. In the United States, the serum PFOS and PFOA biomonitoring conducted with sub-samples from NHANES is considered to be representative. Early MOE risk assessment for PFOS (3M 2003) and PFOA (Butenhoff et al. (2004a) and USEPA (2003)) used data from the biomonitoring studies of Olsen et al. (2003a, 2004a), in particular approximately 600 American Red Cross adult blood donors, approximately 100 from each of 6 regional collection centers, to estimate serum concentrations of PFOS and PFOA. Serum PFOS and PFOA distributions in the American Red Cross adult blood donor samples have tracked well with the NHANES data for concurrent time periods, and, although they are not fully representative, they are a good approximation for adults. Similarly, the Health Canada MOE risk assessment of 2012 used representative data for Canadian adults (Health Canada 2012). In all of these MOE assessments, less representative serum PFOS and PFOA data have been available for infants and children; however, the data that were available suggested that the serum concentrations in children were not greatly different than those in adults.

For the risk assessments based on dietary exposure, strength of the assessment is dependent on the representativeness of the estimates for dietary exposure. The 2012 risk assessment from EFSA provided a much broader view of the magnitude of exposure via diet for PFOS and PFOA within Europe. The composite food samples used in the UK and Canadian risk assessments were designed to be reasonably representative of diets in those regions. However, it is likely that there are regional differences that may affect exposure patterns, e.g., higher consumption of fish.

It should also be noted that the risk assessments based on dietary exposure are dependent on the methods used to establish the TDI values. The choice of critical endpoint and the POD based on the critical endpoint as well as uncertainty factors chosen can vary considerably.

15.6 Establishment of Regulatory Risk Levels for Exposure to Perfluoroalkyls via Drinking Water

Consumption of water containing PFOA, and, to a lesser extent, PFOS, has been a documented source of exposure for several populations. Most notable among these populations were communities in the mid Ohio River Valley that were exposed to PFOA in their water supplies as a result of discharges from an industrial facility that used ammonium PFOA as a processing aid in the production of polytetrafluoroethylene (PTFE). Exposure via drinking water and the resulting serum PFOA concentrations in these mid Ohio River Valley communities have been well described (Emmett et al. 2006; Frisbee et al. 2009; Steenland et al. 2009). In another circumstance, a community in the vicinity of Arnsberg in the state of North Rhine-Westphalia in Germany was also exposed to PFOA, with lesser concentrations of PFOS, in drinking water after a soil enhancer that had been mixed with industrial waste was applied to agricultural land on the upper Moehne River (Brede et al. 2010; Hölzer et al. 2008, 2009; Skutlarek et al. 2006). In 2009, the Minnesota Department of Health released the results of a pilot biomonitoring study that covered residents in an area with potential exposure via consumption of ground water containing PFOA, and lesser concentrations of PFOS, as a result of leaching from landfill waste sites (MDH 2009c). Concern for exposure to perfluoroalkyls through drinking water has prompted several jurisdictions to develop guidelines or advisories for the presence of certain perfluoroalkyls in drinking water, predominantly PFOA, but also for PFOS, and sometimes with consideration of other perfluoroalkyls. These guidelines are based on daily intake of the perfluoroalkyl(s) via consumption of water. Several approaches to the development of drinking water guidelines and advisories have been taken, with most incorporating a means of adjusting for pharmacokinetic differences between humans and the species used in the study from which the critical effect(s) and POD has been chosen. Notable examples of drinking water guidelines/advisories developed for PFOS and PFOA are presented below, and several of these are summarized in Tables 15.9 and 15.10.

15.6.1 United States Environmental Protection Agency Office of Water Provisional Health Advisories for PFOS and PFOA

In 2009, United States Environmental Protection Agency (USEPA) Office of Water (OW) derived Provisional Health Advisory (PHA) values for PFOS and for PFOA in drinking water (USEPA OW 2009), which are summarized in Tables 15.9 and 15.10, respectively. In deriving the PHA for PFOS, USEPA OW considered as critical three clinical chemistry endpoints observed in females from a 6-month capsule dosing study of potassium PFOS in male and female cynomolgus monkeys (*Macaca fascicularis*) (Seacat et al. 2002): decreased serum concentration of high-density

Table 15.9 Factors used by the United States, the State of Minnesota, and the United Kingdom in deriving guidance values for exposure to PFOS via drinking water

	United States	Minnesota	United Kingdom
	EPA OW[a]	MDH[b]	DWI[c]
Study	Seacat et al. (2002)	Seacat et al. (2002)	Seacat et al. (2002)
Species	Monkey	Monkey	Monkey
Sex	Female	Female	Female
Endpoint(s)	↓HDL, ↑TSH, ↓TT3	↓HDL, ↑TSH, ↓TT3	Multiple
Basis of POD	NOAEL (oral gavage)	BMCL10 (serum [PFOS])	NOAEL
POD	0.03 mg/kg/day	35 μg/mL	0.03 mg/kg/day
PK adjustment			
Clearance ratio	13		
HED	0.0023 mg/kg/day	Direct calculation[d]	Direct calculation
Uncertainty			
Inter-individual	10	10	10
Interspecies	3	3	10
Total uncertainty	30	30	100
RfD or TDI	0.000077 mg/kg/day	0.00008 mg/kg/day	0.0003 mg/kg/day[e]
Guideline factors			
Daily water	0.1 L/kg/day (10 kg child)	0.049 L/kg/day[f]	0.1 L/kg/day (10 kg child)
RSC	0.2	0.2	0.1
Guidance level	0.2 μg/L	0.3 μg/L	>0.3 μg/L (Tier 2)[g]

[a]Environmental Protection Agency Office of Water
[b]Minnesota Department of Health
[c]Drinking water inspectorate
[d]Based on one-compartment, first order kinetics using a human serum PFOS elimination half-life of 1,971 days (5.4 years) and a volume of distribution of 0.2 L/kg
[e]From UK FSA COT (2009)
[f]Estimated TWA 95th percentile intake rate for the first 27 years of life. For a 70 kg adult, this would be 3.4 L/day
[g]Tier 2 (DWI notes that drinking water suppliers should include PFOS in their Regulation 27 risk assessments as a minimum (Tier 1). If the value for Tier 2 is exceeded, monitoring and consultation with health authorities is required as a minimum. Tier 3 and Tier 4 values and their required actions are discussed in the text)

Table 15.10 Factors used by the United States and the States of Maine, Minnesota, New Jersey, and North in deriving guidance values for exposure to PFOA via drinking water

	United States	Maine	Minnesota	New Jersey	North Carolina
	EPA OW	DHHS	MDH	DEP	NCDENR
Study	Lau et al. (2006)	Multiple studies	Butenhoff et al. (2002)	Butenhoff et al. (2012c)	Butenhoff et al. (2002)
Species	Mouse	Mouse and rat	Monkey	Rat	Monkey
Sex	Female (maternal)	Male and female	Male	Female	Male
Endpoint(s)	↑ Liver weight	↑ Liver weight/hepatocellular hypertrophy	↑ Relative liver weight (liver: brain weights)	↓ Body weight	↑ Liver weight
Basis of POD	$BMDL_{10}$ (gavage dose)	$BMDL_{10}$[a] (gavage and dietary dose)	$BMDL_{10}$ (serum [PFOA])	NOAEL (dietary dose)	BMC_{10}[b] (serum [PFOA])
POD	0.46 mg/kg/day	0.42 mg/kg/day[c]	23 μg/mL	1.8 μg/mL[d]	40 μg/mL
PK adjustment					
PBPK					0.12 μg/kg body weight per μg/mL[e]
HEss dose[f]			Direct calculation[g]		
Clearance ratio	81	226[h]			
HED	0.0057 mg/kg/day	0.0018 mg/kg/day[a]	0.0023 mg/kg/day		0.0048 mg/kg/day
Uncertainty					
Inter-individual	10	10	10	10	10
Interspecies	3	3	3	10	3
Total uncertainty	30	300 (includes a factor of 10 for database uncertainty)	30	100	30
RfD or TDI	0.00019 mg/kg/day	0.000006 mg/kg/day	0.000077 mg/kg/day	18 ng/mL[i]	0.00016 mg/kg/day

(continued)

Table 15.10 (continued)

	United States	Maine	Minnesota	New Jersey	North Carolina
	EPA OW	DHHS	MDH	DEP	NCDENR
Guideline factors					
Daily water	0.1 L/kg/day[j]	0.029 L/kg/day[k]	0.053 L/kg/day[l]	Not used	0.029 L/kg/day[k]
RSC	0.2	0.6	0.2	0.2	0.2
Guidance level	0.4 μg/L	0.1 μg/L	0.3 μg/L	0.04 μg/L[m]	1 μg/L

[a]Geometric mean among six $BMDL_{10}$ values obtained from various gavage and dietary studies
[b]Central estimate of BMC_{10} based on serum PFOA concentration
[c]Geometric mean among six $BMDL_{10}$ values obtained from various gavage and dietary studies (individual $BMDL_{10}$ values are: 0.46, 0.4, 0.29, 0.44, 0.74, and 0.31)
[d]Based on estimate of serum PFOA concentration at NOAEL of 1.6 mg/kg/day using modelled AUC from USEPA (2005) (1.6 mg/kg/day gives AUC of 44 μg*h/mL)
[e]From Clewell et al. (2006)
[f]Human equivalent steady state dose
[g]Based on one-compartment, first order kinetics using a human serum PFOA elimination half-life of 1,387 days (3.8 years) and a volume of distribution of 0.2 L/kg
[h]Geometric mean of gavage and dietary study clearance ratios (individual values were 82, 277, 277, 277, 277, and 277)
[i]Target human blood concentration (assume serum concentration)
[j]10 kg child
[k]2 L/day, 70 kg adult
[l]Estimated TWA 95th percentile intake rate for the first 19 years of life
[m]0.18 μg/L based on observation by Emmett et al. (2006) of the relationship in Mid-Ohio Valley community samples of 1 μg/L contaminated water results in approximately 100 μg/L serum PFOA if water accounts for 100 % exposure. Applying a RSC of 20 % lowers to 0.04 μg/L.

lipoprotein (HDL) cholesterol; decreased total triiodothyronine (TT3); increased thyrotropin (thyroid stimulating hormone, or TSH). A NOAEL of 0.03 mg/kg/day was used as the POD, from which a human equivalent dose (HED) of 0.0023 mg/kg/day was obtained after a pharmacokinetic adjustment of 13 based on the monkey: human clearance ratio. To this HED, a total uncertainty factor of 30 was applied to yield a RfD of 0.000077 mg/kg/day. A water consumption rate of 1 L/day based on a 10 kg child was used along with a default relative source contribution (RSC) factor of 20 %. The RSC attributed the proportion of the daily intake from all sources that is contributed, in this case, by consumption of water. Therefore, in using the default of 20 %, it is assumed that one-fifth of the total daily intake from all sources is from drinking water. The Provisional Health Advisory (PHA) guidance level therefore was set at 0.2 μg/L.

In deriving a PHA for exposure to PFOA from drinking water, USEPA Office of Water used increased maternal liver weight as the critical endpoint from a mouse developmental study (Lau et al. 2006). The derivation of this PHA is summarized in Table 15.10. A $BMDL_{10}$ value of 0.46 mg/kg/day was used as the POD, from which a HED of 0.0057 mg/kg/day was obtained after a pharmacokinetic adjustment of 81 based on the mouse: human clearance ratio. To this HED, a total uncertainty factor of 30 was applied to yield a RfD of 0.00019 mg/kg/day. Using a water consumption rate of 1 L/day based on a 10 kg child and a RSC of 20 %, the Provisional Health Advisory guidance level was set at 0.4 μg/L.

15.6.2 Drinking Water Guidance Values from States Within the United States

15.6.2.1 Maine

The Maine Department of Health and Human Services developed a health-based Maximum Exposure Guideline (MEG) for PFOA in drinking water (MEDHHS 2014). The MEG for PFOA was based on liver effects in six toxicological studies with mice and rats (Table 15.10). $BMDL_{10}$ values for various liver effects obtained from multiple gavage and dietary studies in rodents and reported by EFSA were used in the derivation. For each study, the $BMDL_{10}$ divided by a pharmacokinetic (PK) adjustment factor based on the estimated rodent (mouse or rat): human clearance ratio was used as the HED. These HED values ranged from 0.0010 to 0.0056 mg/kg/day with a geometric mean of 0.0018 mg/kg/day. This geometric mean value was used to derive the RfD of 0.000006 mg/kg/day after applying a total uncertainty factor of 300. The MEG of 0.1 μg/L was calculated from the RfD through application of a standard 70 kg adult body weight and 2 L/day water intake rate with a relative source contribution (RSC) factor of 60 % of exposure via drinking water.

It is noteworthy that the deviation from the usual default RSC of 20 % was data-driven. Maine reasoned that there were adequate background exposure data to

derive a PFOA-specific RSC value. Pursuant to this, Maine took the upper 95th percentile serum PFOA concentration level from the updated tables issued in September 2013 for most recent United States Center for Disease Control's National Health and Nutrition Examination Survey (CDC_NHANES 2009). (These updated tables were withdrawn by CDC NHANES and replaced with new updated tables in August, 2014.) This serum PFOA concentration (7.5 ng PFOA/mL serum), was considered as the upper bound serum PFOA concentration associated with background PFOA exposure of the United States general population from all sources. Maine first converted the RfD (0.006 μg/kg/day) to a "drinking water equivalent level" (DWEL), assuming that 100 % of the RfD is contributed by water. A DWEL value of 0.21 μg/L was derived based on consumption of 2 L of water containing an amount of PFOA representing the RfD for a 70 kg person. The DWEL was then converted to a corresponding serum PFOA concentration using the 100:1 serum PFOA: drinking water PFOA concentration level relationship described by Emmett et al. (2006) for a population in the mid Ohio Valley with exposure to PFOA through drinking water as a principal source. Applying this 100:1 relationship resulted in a corresponding serum PFOA concentration of 21 μg/L. The PFOA-specific RSC was then obtained by dividing the serum concentration associated with drinking water PFOA concentrations at the RfD (21 μg/L or 21 ng/mL) minus the NHANES upper 95th percentile serum PFOA from background exposure (21 ng/mL−7.5 ng/mL = 13.5 ng/mL) by the serum concentration associated with drinking water PFOA concentrations at the RfD times 100 (13.5 ng/mL/21 ng/mL 100=64.3 %). The resulting value of 64.3 % was rounded to 60 %. Maine is the only government authority to date that has taken such a data-driven approach to developing a RSC.

15.6.2.2 Minnesota

Perfluoroalkyls, notably PFOS, PFOA, and perfluorobutyrate (PFBA), have been found to impact the groundwater used as a supply for drinking water in several Minnesota communities (MDH 2008). Landfill leachate was thought to contribute to the PFOS and PFOA exposure via this groundwater. The Minnesota Department of Health (MDH) has derived Health Risk Limits (HRLs) for four perfluoroalkyls: PFOS (MDH 2009b); PFOA (MDH 2009a); perfluorobutanesulfonate (MDH 2011a); and, perfluorobutyrate (MDH 2011b). Derivation of HRLs for PFOS and PFOA are summarized in Tables 15.9 and 15.10, respectively, and below.

In developing an HRL for PFOS, the MDH chose critical effects of decreased serum HDL cholesterol, decreased serum TT3, and increased serum TSH from the 6-month oral capsule dosing study of potassium PFOS in cynomolgus monkeys (*Macaca fascicularis*) (Seacat et al. 2002) (Table 15.9). MDH considered a number of co-critical effects, additivity endpoints, and secondary effects in their derivation. A serum PFOS concentration-based $BMDL_{10}$ of 35 mg/L (equivalent to 35 μg/mL) was used by MDH as the POD. This serum PFOS concentration was used to derive the estimated HED by assuming that the 35 μg/mL represented steady state and

calculating the estimated daily dose in humans that would be associated with that steady-state concentration. For this calculation, MDH assumed first order elimination kinetics, a non-compartmental model, an arithmetic mean human serum PFOS elimination half-life of 1,971 days, based on Olsen et al. (2007), and a human volume of distribution of 0.2 L/kg. The resulting HED was 0.0025 mg/kg/day, to which a total uncertainty factor of 30 was applied to derive an RfD of 0.00008 mg/kg/day. To obtain the HRL, MDH used a time-weighted average water consumption calculated at the 95th percentile water consumption rate over the first 27 years of life (0.049 L/kg/day). The latter time period was considered to be that representing attainment of steady state. For a 70 kg person, this represents a consumption rate of 3.4 L/day. MDH applied a RSC of 20 % to yield the HRL of 0.3 μg/L.

For drinking water exposure to PFOA, The Minnesota Department of Health (MDH) chose a critical effect of increased relative liver weight from the 6-month oral capsule dosing study of ammonium PFOA in cynomolgus monkeys (*Macaca fascicularis*) (Butenhoff et al. 2002), chosen as the critical study (Table 15.10). MDH considered a number of co-critical effects, additive endpoints, and secondary effects in their derivation. A serum PFOA concentration-based $BMDL_{10}$ of 23 mg/L (equivalent to 23 μg/mL) as derived by Butenhoff et al. (2004a) was used by MDH. This serum PFOA concentration was used to derive the estimated HED by assuming that the 23 mg/L represented steady state and calculating the estimated daily dose in humans that would be associated with that steady-state concentration. For this calculation, MDH assumed first order elimination kinetics, a non-compartmental model, an arithmetic mean human serum PFOA elimination half-life of 1,387 days, based on Olsen et al. (2007), and a human volume of distribution of 0.2 L/kg. The resulting HED was 0.0023 mg/kg/day, to which a total uncertainty factor of 30 was applied to derive an RfD of 0.000077 mg/kg/day. To obtain the HRL, MDH used a time-weighted average water consumption calculated at the 95th percentile water consumption rate over the first 19 years of life (0.053 L/kg/day), a time period in which MDH reasoned that steady state serum PFOA would be reached. For a 70 kg person, the corresponding consumption rate is 3.7 L/day. MDH then applied a RSC of 20 % to yield the HRL of 0.3 μg/L.

15.6.2.3 New Jersey

The New Jersey Department of Environmental Protection developed a health-based drinking water concentration for PFOA, which was published in 2009 (Post et al. 2009), as part of an overall evaluation of the occurrence of PFOA in New Jersey public water systems (Table 15.10). The exposure assessment and health-based water PFOA concentration were based on an observed relationship between concentrations of PFOA in drinking water and PFOA concentrations in humans exposed to drinking water containing PFOA (Emmett et al. 2006). In determining the POD for derivation of the health-based value, the 2-year dietary study of ammonium perfluorooctanoate in male and female rats (Butenhoff et al. 2012c) was chosen as the critical study. From this study, a NOAEL of 1.6 mg/kg/day for decreased body weight

in female rats was chosen as the critical effect. The estimated serum PFOA at this NOAEL was calculated from a modeled AUC taken from the USEPA draft risk assessment for PFOA (USEPA 2005), which was 44 μg·h/mL at the 1.6 mg/kg/day dose, yielding 1.8 μg/mL as an average serum concentration over a 24-h period. This serum PFOA concentration was used as the POD. Uncertainty factors of 10 for inter-individual variation and 10 for interspecies variation were applied to yield a total uncertainty of 100 and a RfD of 18 ng PFOA/mL serum. A RSC of 20 % was assumed for exposure from drinking water. Based on the observation of Emmett et al. (2006), a population exposed to PFOA in drinking water at approximately 1 μg/L (equal to 1 ng/mL) had serum PFOA concentrations of approximately 100 ng/mL. Assuming that the later observation applied to 100 % of exposure via drinking water and using this relationship, the POD of 18 ng/mL (0.018 μg/mL) would correspond to a drinking water concentration of approximately 0.18 μg/L. Applying a RSC of 20 % attributable to exposure via water consumption, New Jersey DEP derived a health-based concentration of 0.04 μg PFOA/L water.

15.6.2.4 North Carolina

In their original 2006 derivation of an Interim Maximum Allowable Concentration (IMAC) for PFOA in ground water, the North Carolina Science Advisory Board (NCSAB) derived a RfD for PFOA of 0.0003 mg/kg/day (Williams 2006), which was based on increased liver weight observed in rats from an oral (gavage) two-generation reproduction and development study that used ammonium perfluorooctanoate (APFO) as the test agent (Butenhoff et al. 2004b) (Table 15.10). The State of North Carolina found that the lowest observed adverse effect level (LOAEL) for increased liver weight was 1 mg/kg/day. Using this dose as the POD, four uncertainty factors were applied: (1) Ten for inter-individual variation; ten for interspecies variation; ten to account for the lack of a no observed adverse effect level (NOAEL) for liver weight increase; three to account for perceived deficiencies in the database. The resulting total uncertainty factor after multiplying the individual factors together was 3,000. Dividing the 1 mg/kg/day POD by the total uncertainty of 3,000 yielded a RfD of 0.0003 mg/kg/day for PFOA. On August 10, 2012, the NCSAB issued a revised IMAC for PFOA in ground water which is currently pending approval (NCSAB 2012). The derivation of this revised IMAC is summarized in Table 15.10. Using increased liver weight (with increased liver to brain weight ratio) observed in male monkeys as the critical effect (Butenhoff et al. 2002), a central estimate of BMC_{10} on serum PFOA concentration at 40 μg/mL was determined to be the basis of POD for the IMAC derivation. With PBPK (0.12 μg PFOA/kg body weight per μg PFOA/mL serum) and uncertainty factor (30) adjustments, the IMAC of 1 μg/L was proposed in North Carolina assuming a 70-kg adult with 2 L daily water consumption and a 20 % relative source contribution for exposure from drinking water.

15.6.2.5 West Virginia

The State of West Virginia was among the first jurisdictions to develop health-based guidelines for PFOA concentration in drinking water. The use of ammonium PFOA as a processing aid in the production of tetrafluoroethylene at an industrial facility in Parkerburg, West Virginia resulted in the presence of PFOA in drinking water sources in several mid Ohio River Valley communities (Emmett et al. 2006; Shin et al. 2011). The West Virginia Department of Environmental Protection released a report on the establishment of preliminary risk screening levels for PFOA in drinking water in the mid Ohio River Valley communities near a the PTFE production facility that used ammonium PFOA as a processing aid (WVDEP 2002). This report documented the results of an expert workshop of the Ammonium Perfluorooctanoate (C8) Assessment of Toxicity Team (or, CATT). The CATT was established by a consent order between E. I. DuPont de Nemours, Inc. and two West Virginia departments, the Department of Environmental Protection and the Department of Health and Human Resources. Three objectives were established for the CATT, as stated in the Executive Summary of the CATT report: "(1) determine risk-based human health protective screening levels (SLs) for this unregulated chemical in air, water, and soil; (2) provide health risk information to the public; and (3) determine an ecological health protective SL for C8 in surface water." Human health provisional risk factors for oral (RfD) and inhalation (RfC) exposures were derived by the CATT. From these RfD risk factors, health protective screening levels (SLs) were developed based on then current USEPA Region 9 standard methodology. For the oral route of exposure, an RfD of 0.004 mg/kg of body weight daily was determined, and a provisional RfC of 1 μg/m^3 of air was established. The RfD was used to derive SLs of 150 μg/L (parts per billion, or ppb) for drinking water. All water samples collected in the vicinity of this facility were below the risk screening level of 150 μg/L derived for drinking water in this process. Water samples from the 50 private wells and cisterns used for drinking water and the nine public water supplies were below 3 μg/L.

15.6.3 United Kingdom Drinking Water Inspectorate Guidance on Water Supply Regulations for PFOA and PFOS

The UK Drinking Water Inspectorate (DWI) under the Department of Environment, Food, and Rural Affairs (DEFRA) originally issued guidance for concentrations of PFOA and PFOS in drinking water in 2007 which was then revised in 2009 to be consistent with the revised UK COT TDI for PFOA, which was lowered from 3.0 to 1.5 μg/kg/day after consideration of the EFSA TDI for PFOA, which included an additional pharmacokinetic adjustment factor (UKDWI 2009). The DWI guidance includes a multi-tiered approach consisting of four tiers with related minimum

actions to be taken. For PFOA and PFOS, Tier 1 is not associated with a water concentration; however, this tier calls for consideration of PFOA and PFOS as part of a statutory risk assessment for water companies as well as the consideration of monitoring water for PFOA and PFOS where appropriate. Tier 2 establishes a concentration of PFOA and PFOS in water, 0.3 μg/L, above which further sampling, investigation, and consultation with local health authorities is appropriate. This Tier 2 concentration for both PFOA and PFOS is based on the derived Tier 2 concentration for PFOS (see below and Table 15.9), which, in turn, is based on a RSC of 10 % of the UK COT TDI for PFOS (0.03 μg/kg) allocated to 1.0 L of drinking water consumed daily by a 10 kg child.

Tier 3 considers the wholesomeness of water and establishes a concentrations of PFOA and PFOS in water below which it is assumed that a "potential danger to human health" does not exist. For PFOA, DWI established a wholesomeness level of 5.0 μg/L or less as protective of "the whole range of consumers". This level was based on a RSC of 50 % of the TDI allocated to 0.75 L/day of water consumed by a 5 kg bottle-fed infant. In the case that PFOA concentrations in water are above 5.0 μg/L, the guidance instructs that water companies should discuss appropriate actions with local health authorities aimed at reducing exposure to PFOA via drinking water, put these exposure-reduction strategies in place as soon as practicable, an monitor PFOA in drinking water. In considering a Tier 3 level for PFOS, DWI considered how best to ascribe the source contribution for water for young children. In so doing, DWI noted that, taking worst case estimates of dietary exposure to PFOS for small adults, a Tier 3 level of 3.0 μg/L would still be protective. However, DWI noted "considerable" uncertainty in estimates of dietary intake of PFOS for small children, and that drinking water exposure to PFOS would "be appropriately restricted by establishing a value in the range zero and 2.5 μg/L". DWI further noted that, based on current toxicological expert advice, a Tier 3 water PFOS concentration of 1.0 μg/L would meet the wholesomeness requirement. The same actions would be required as for PFOA if drinking water were to exceed the 1.0 μg/L PFOS Tier 3 level.

Tier 4 requires notification by water companies of any event which has or may adversely affect the quality of water. Tier 4 also establishes a level of exceedance that would require more immediate action and notification of relevant stakeholders. For PFOA, this notification level was set to reflect allocation of the whole TDI for PFOA (0.15 μg/kg/day) to 2 L/day of drinking water consumed by a 60 kg adult (>45 μg/L). The DWI also provided water concentrations of PFOA considered to be unfit for human consumption and subject to potential prosecution. These PFOA water concentrations were noted as: 2,000 μg/L for bottle-fed babies; 3,000 μg/L for 1-year old children; 9,000 μg/L for adults. Similarly, for PFOS, in allocating all of the TDI to 2 L of drinking water per day for a 60 kg adult, the notification level is >9.0 μg/L. Concentrations of PFOS in drinking water considered unfit for human consumption and potentially subject to prosecution were noted as: 67 μg/L for bottle-fed babies; 100 μg/L for 1-year old children; 300 μg/L for adults.

15.6.4 German Drinking Water Commission

After the discovery of PFOA in drinking water at concentrations up to 0.56 μg/L in the Hochsauerland district in Germany (Skutlarek et al. 2006), the Public Health Department of Hochsauerland (Gesundheitsamt des Hochsauerlandkreises) asked the Drinking Water Commission (Trinkwasswerkommission, or TWK) of the Federal Environment Agency (Umweltbundesamt, or UBA) to determine maximum tolerable concentrations of PFOA in drinking water. This resulted in a July 13, 2006 provisional guideline issued by TWK (2006). Because PFOS was also detected in water at lower concentrations than those found for PFOA, the TWK guidance reflected the composite concentrations of both PFOA and PFOS. Four guidance values were presented. One of these was based on the 2003 UBA admissible health guidance value (Gesundheitlicher Orientierungswert, or GOW) of 0.1 μg/L for non- or low-potency genotoxic substances, which was considered applicable to lifetime exposure to combined total concentrations of PFOA and PFOS via drinking water. In addition, for less than lifetime exposure, two precautionary action values (Vorsorgemaβnahmewert, or VMW) were recommended based on UBA's action value guidance (Maβnahmewert-Empfehlung). A VMW_0 (Vorsorge-Maβnamewert für Erwachsene) of 5 μg/L is used to indicate when immediate action is required to reduce exposure to PFOA and PFOS via drinking water. For infants and pregnant women, the VMW_0 of 5 μg/L is reduced by a factor of 10 to yield an infant and pregnancy VMW_s (Vorsorge-Maβnamewert für Säuglinge) of 0.5 μg/L. In addition to these GOW and VMW values, a specific health-based value (Lietwert, or LW) for PFOA and PFOS of 0.3 μg/L was derived based on toxicological data. TDI values of 0.1 μg/kg/day for PFOA and PFOS were developed based on consideration of the NOAELs from the 2-year dietary study (Butenhoff et al. 2012c) and two-generation reproduction and development study of ammonium PFOA (Butenhoff et al. 2004b), both in rats, and the NOAEL from the 2-year dietary study in rats of potassium PFOS (Butenhoff et al. 2012b). NOAELs of 0.1 mg/kg/day and 0.025 mg/kg/day were selected for PFOA and PFOS, respectively. Total uncertainty factors of 1,000 for PFOA (10 for inter-individual, 10 for interspecies, and 10 for additional pharmacokinetic uncertainty) and 300 for PFOS (10 for inter-individual, 10 for interspecies, and 3 for additional pharmacokinetic uncertainty) were used. An RSC of 10 % was used, allocated to 2 L/day of water consumed by a 70 kg adult.

15.6.5 Conclusions Regarding Establishment of Regulatory Risk Levels for Exposure via Drinking Water

As can be seen from an inspection of Tables 15.8 and 15.9, the RfD or TDI values derived for PFOS and PFOA vary by a factor of 3–4. For PFOS (Table 15.9) the same study has been used as the critical study (Seacat et al. 2002), and the same endpoints have been considered as critical (reduced serum HDL, reduced serum Total T3, and increased serum TSH). For PFOA, increased liver weight was the

critical endpoint used in the five examples given in Table 15.10. The studies differed, with USEPA OW using the Lau et al. (2006) mouse developmental study, MDH and NCDENR using the Butenhoff et al. (2002) monkey study, Maine using multiple studies, and New Jersey DEP, which did not develop a RfD, using female data from the 2-year Sprague Dawley rat dietary study (Butenhoff et al. 2012c). When these values are used to develop safe drinking water levels, for PFOA, the μg/L values vary by a factor of 25. Again, considering that these values for PFOA are based on liver weight increase, which is not necessarily an adverse outcome, this degree of variability in the resulting safe drinking water levels raises questions about the appropriateness of the process and its potential impacts in terms of risk management.

15.7 Conclusion

In this chapter, the major human health risk assessment activities that have been undertaken for human exposure to perfluoroalkyls have been summarized. Comments have been made on several factors influencing risk assessment. It becomes apparent that the methods used to assess human health risk from exposure to perfluoroalkyls have been evolving and will likely continue to develop as new information and approaches are introduced. Perhaps the most important direction that risk assessment for perfluoroalkyls has taken has been in the use of internal dose metrics to bridge differences in pharmacokinetic elimination kinetics between species. This practice also has the benefit of integrating contributions to exposure from all sources. Although a large and robust database exists for PFOA and PFOS that covers multiple health endpoints, data are more limited for other perfluoroalkyls. Increased liver weight is a frequent and sensitive effect observed in toxicological studies with perfluoroalkyls, particularly in rodents, and data have been developed to attribute this to increased activation of the nuclear receptors PPARα and CAR/PXR. A number of the risk assessment activities discussed in this chapter considered increased liver weight as an effect appropriate for establishing a POD; however, the use of increased liver weight to represent an adverse effect in the absence of other indications of liver toxicity is not consistent with past or current guidance for the evaluation of liver weight increase as adaptive versus adverse (Hall et al. 2012). Moreover, the notable differences between the human and rodent liver response to increased activation of PPARα and CAR/PXR argue for mitigation of concern in translating liver weight increases for rodent exposure to perfluoroalkyls to humans (Corton et al. 2014; Elcombe et al. 2014; Klaunig et al. 2012). There is a need to better inform epidemiological investigations with the understanding obtained from toxicological and pharmacokinetic investigations and principals. Translating our understanding from toxicological systems into a human context will improve our collective ability to understand potential human health risk from environmental levels of exposure to perfluoroalkyls.

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Chapter 16
Systematic Review, An Illustration of Increased Transparency in a Framework for Evaluating Immunotoxicity Associated with PFOA and PFOS Exposure

Andrew A. Rooney, Abee L. Boyles, and Vickie R. Walker

Abstract Background: Systematic review methodologies were first developed to assess the efficacy of health care interventions, but these approaches can be adapted to evaluations of environmental health questions such as immunotoxicity associated with PFOA and PFOS exposure. This structured approach provides objectivity and transparency to the process of collecting, synthesizing, and reaching conclusions based on the scientific evidence available.

Objectives: To outline the process of systematic review and evidence integration and demonstrate each step by following a single research question from start to finish. The example systematic review will evaluate the evidence that PFOA and PFOS exposure are associated with immunotoxicity – using a subset of the available evidence to illustrate concepts, not to develop hazard identification conclusions.

Methods: The Office of Health Assessment and Translation (OHAT) Approach to evaluating the scientific evidence for immunotoxicity of PFOA and PFOS is detailed in a protocol that is laid out in seven steps: scoping and problem formulation, search for and select studies for inclusion, extract data from studies, assess quality of individual studies, rate confidence in the body of evidence, translate confidence ratings into level of evidence, and integrate evidence to develop hazard identification conclusions incorporating human, animal, and mechanistic evidence.

A.A. Rooney (✉)
Office of Health Assessment and Translation, Division of the National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), Research Triangle Park, NC, USA

NIEHS, P.O. Box 12233, Mail Drop K2-04, Research Triangle Park, NC 27709, USA
e-mail: Andrew.Rooney@nih.gov

A.L. Boyles • V.R. Walker
Office of Health Assessment and Translation, Division of the National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), Research Triangle Park, NC, USA

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_16

ED_002330_00119699-00426

Results and Discussion: Eligibility criteria for identifying important exposures and outcomes were presented as the basis for assembling the relevant studies for evaluating whether or not PFOA or PFOS exposure is associated with immunotoxicity (human, n = 18; animal, n = 80; and mechanistic/in vitro assays, n = 19). A tool for assessing study quality in terms of risk of bias or internal validity was tailored to the research question – particularly for evaluating PFC exposure and assessing immunological outcomes. An example of an evidence profile is provided to illustrate the basis for confidence ratings using a hypothetical set of studies of PFOS and functional antibody response. Finally, a discussion is presented on how the hazard identification conclusions would be reached and interpreted by integrating the human, animal, and mechanistic evidence.

Conclusion: The OHAT Approach to hazard identification of health effects of PFCs is illustrated with a case study on PFOA/PFOS and immunotoxicity. Communication of the evaluation process is enhanced by using objective, reproducible methods that transparently document scientific judgments and the scientific basis for hazard identification conclusions.

Keywords Systematic review • Perfluorinated chemicals • Immunotoxicity • Risk of bias • Hazard identification • PFOA • PFOS

16.1 Introduction

The strength and reliability of hazard conclusions on the potential human health effects from environmental exposures can be hindered by inconsistent or unclear methods of how the evaluation was performed. Systematic-review methodologies provide a structure that increases transparency and objectivity in the process of collecting and synthesizing scientific evidence for literature-based evaluations. Multiple organizations have adopted (Birnbaum et al. 2013; Woodruff and Sutton 2014) or recommended (EFSA 2010; NRC 2013a, b; Rhomberg et al. 2013; US EPA 2013a) the use of systematic review methods for evaluating the association between health effects and environmental exposures. First developed and established in clinical medicine to assess data for reaching health care recommendations (AHRQ 2013; Guyatt et al. 2011; Higgins and Green 2011), systematic-review methodologies typically addressed data from clinical trials and focus on human data alone.

The data available to evaluate potential health effects from exposure to environmental chemicals comes from diverse sources and rarely include experimental trials in humans. Human data are typically from observational studies that include cohort, cross sectional, case control, and even case report study designs. Animal data, primarily from *in vivo* laboratory studies in rodents, provide a large percentage of the toxicology data used for hazard identification and risk assessment. Mechanistic or other relevant data from *in vitro* and *in vivo* studies on molecular and cellular

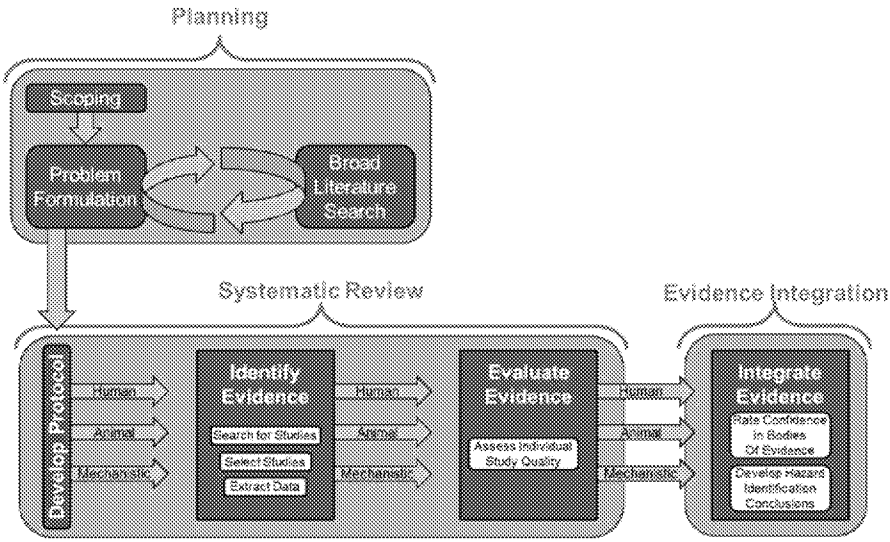

Fig. 16.1 OHAT framework for systematic review and evidence integration
An evaluation begins with the planning process and development of a detailed protocol to guide each step in the assessment. The systematic review identifies, collects and evaluates the evidence from individual studies. Evidence integration is the process where bodies of evidence and multiple lines of evidence gathered in the systematic review (human, animal, and mechanistic studies) are integrated to develop conclusions

events also inform the hazard conclusions as part of the overall database (NRC 2013b). The National Toxicology Program's (NTP) Office of Health Assessment and Translation (OHAT) developed a framework for examining environmental health questions (Fig. 16.1) using systematic review procedures that address the breadth of relevant data (e.g., human, animal, and mechanistic studies), and evidence integration procedures to consider the collective evidence in developing conclusions (Rooney et al. 2014).

16.2 Systematic Review

Use of systematic review methodology adds a level of objectivity and transparency to established principles of hazard assessment (WHO 1999) and continual improvement in communicating the basis for scientific judgments within an assessment of potential health effects of environmental chemicals (NRC 2009, 2013b). A planning process of scoping and problem formulation lays the groundwork for the systematic review by framing the specific research question to be addressed and the analytical approach: hazard assessment, risk assessment, or to identify data gaps where the scientific evidence base is small or narrow (Fig. 16.1). Then, systematic review procedures use transparent, rigorous, objective, and reproducible methodology to

identify, select, assess, and analyze results of relevant studies to complete the literature-based evaluation. These methods do not eliminate the need for scientific judgment; rather, they offer an increased level of transparency for understanding the basis of decisions and the overall confidence in the conclusions.

16.3 Evidence Integration

Evidence integration begins with identifying groups of studies with data on an outcome (or biologically related outcomes), or "bodies of evidence." Confidence ratings are then developed for the human and animal bodies of evidence separately by considering the strengths and weaknesses of the relevant studies. Ratings reflect confidence that the study findings accurately represent the true association between exposure to a substance and an effect. If the evidence base is insufficient, the evaluation can result in a summary of the data gaps. If the evidence base is sufficient, it can proceed to the last step in evidence integration – to develop hazard conclusions from the confidence ratings by integrating the animal and human evidence with consideration of the impact of mechanistic data.

This document will use an evaluation of the evidence that PFOA and PFOS exposure are associated with immunotoxicity to illustrate the systematic review and evidence integration process. While this is not intended to be a complete evaluation, the concepts and procedure presented give substance to the OHAT framework. No hazard conclusions are developed in this example because only subsets of the evidence are used to illustrate these concepts of this systematic review approach.

16.4 Systematic Review of PFOA and PFOS Immunotoxicology

16.4.1 Scoping and Problem Formulation

The foundation of an evaluation relies on focused questions that have been developed and refined through a process of scoping and problem formulation. Scoping procedures define the needs and goals of the evaluation, such as whether it will address occupational exposure or the general public, and whether the goal is to support hazard identification conclusions, a complete risk assessment, or government regulations. Outreach and consultation with subject-matter experts and interested parties, (which may include the public and stakeholders depending on the policies of the review organization), help assure that the product meets the needs of the risk manager (US EPA 1998b) and all available information is considered (especially existing analyses or reviews). Problem formulation is the process of refining the objectives of the evaluation, clearly stating the key questions to be answered, and

outlining how they will be addressed (NRC 2009). The questions define eligibility criteria for the populations, exposures, comparators, outcomes, timings, and settings of interest (PECOTS) for the evaluation (Matcher 2012; Samson and Schoeles 2012).

16.4.1.1 Scoping and Problem Formulation for PFOA/PFOS Immunotoxicity

The planning process for an evaluation of immunotoxicity associated with PFOA and PFOS would address the basic requirements described above including outreach to obtain input on the need for an assessment and availability of data. As part of exposure considerations, the persistence and wide environmental distribution of PFOA and PFOS would be key factors (see Chaps. 2, 3, 4, and 5 of this Book). Given the voluntary agreements by the primary manufacturers to phase out production of PFOA and PFOS is the United States by 2015 (ATSDR 2009; US EPA 2006, 2009, 2013b, 2014), the potential for future exposure would also be considered. Although emissions have been dramatically reduced, the persistence and bioaccumulation of both PFOA and PFOS still result in detectable levels in the U.S. population and therefore are of potential human health relevance (US EPA 2014).

Overview of Scientific Information on PFOA/PFOS Immunotoxicity

During problem formulation the extent of available health effect data would be outlined including whether or not the database is likely to be sufficient to develop conclusions. Several publications from 2012 to 2014 link PFOA and PFOS exposure to functional immune changes in humans that are consistent with evidence of immunotoxicity from animal studies. Immune-related health effects including suppression of the antibody response to vaccines and increased incidence of autoimmune ulcerative colitis have been reported in adults living in an area of Ohio and West Virginia where public drinking water had been contaminated with PFOA (Looker et al. 2014; Steenland et al. 2013). PFOA- and PFOS-associated antibody suppression were also described in prospective cohort studies of children in Norway (Granum et al. 2013) and the Faroe Islands (Grandjean et al. 2012).

Suppression of the antibody response in mice has been reported at blood concentrations of PFOS occurring in the general U.S. population (e.g., CDC 2009, 2014; DeWitt et al. 2012; Fair et al. 2011; Peden-Adams et al. 2008). Experimental studies of PFOA and PFOS in laboratory animals have also demonstrated exposure-related suppression of the antibody response among other immune changes including altered inflammatory response, cytokine signaling, and measures of both innate and adaptive immunity (reviewed in DeWitt et al. 2012). Wildlife studies in species ranging from loggerhead sea turtles to sea otters have also reported widespread exposure and altered immune measures associated with PFOA and PFOS (e.g., Hart et al. 2009; Kannan et al. 2006; Keller et al. 2005). Mechanistic and in vitro exposure

studies of PFOA and PFOS are primarily focused on cytokine secretion (Ahuja et al. 2009; Corsini et al. 2011, 2012; Han et al. 2012); although more predictive measures of immunotoxicity (e.g., immune function), such as natural killer cell activity have also been studied after *in vitro* exposure (Wirth et al. 2014).

Objectives and Key Questions for Evaluating PFOA/PFOS Immunotoxicity

The objective of this illustration is to develop hazard identification conclusions regarding exposure to PFOA or PFOS and potential associations with immunotoxicity or immune-related health effects. Although PFOA and PFOS are both considered in this example, conclusions would be developed separately for each chemical. The objectives would be addressed by answering key questions listed below.

- What is our confidence in the body of evidence from human studies for the association between exposure to PFOA or PFOS and immunotoxicity or immune-related health effects?
- What is our confidence in the body of evidence from animal studies for the association between exposure to PFOA or PFOS and immunotoxicity or immune-related health effects?
- How does the evidence from other relevant studies (e.g., mechanistic or in vitro studies) support or refute the biological plausibility of the association between exposure to PFOA or PFOS and immunotoxicity or immune-related health effects?

The available studies for each of the three evidence streams (human, animal, and mechanistic or other relevant studies) would be evaluated separately. Then, hazard identification conclusions for PFOA-associated immunotoxicity and PFOS-associated immunotoxicity would be developed by integrating the human and animal evidence with consideration of the impact of mechanistic or other relevant data.

16.4.2 Protocol

The evaluation is structured to answer the key questions and a detailed protocol is developed to guide the evaluation process from the literature search, through analysis, and finally the process of integrating the evidence to develop conclusions. Subject-matter experts, particularly scientists with backgrounds in exposure and relevant health effects for the chemical under review, should be consulted in establishing the protocol before proceeding with the evaluation. The protocol's "*a priori*" guidance reflects the scientific knowledge in the field and forms the basis for scientific judgments throughout the evaluation; however, if unanticipated issues arise during the evaluation the protocol can be modified. Pilot-testing and refining the procedures outlined in the protocol on a small subset of studies is recommended at multiple steps, particularly: applying inclusions/exclusion criteria, data

extraction, and risk of bias assessment of individual studies. The transparency principals of systematic review dictate that any revisions are documented and justified including when in the evaluation process the decision was made, not that initial decisions are locked.

16.4.3 Search for and Select Studies for Inclusion

Systematic review requires a comprehensive and transparent literature search strategy and a clear statement of the inclusion and exclusion criteria used to determine if a study is relevant for the evaluation. The protocol outlines the search and selection procedures in sufficient detail such that the literature retrieval could be clearly understood and reconstructed by a third party, including the basis of scientific judgments. The search strategy details the exact search terms used as well as the specifics of the literature search process including which databases will be searched, limits in language or dates of publication, and how unpublished studies will be treated. The eligibility criteria reflect the scoping and problem formulation decisions and specifies the types of human and animal studies (e.g., experimental only or also including wildlife studies), exposure metrics (e.g., potentially excluding occupational exposures or ecological studies without individual exposure measurements), and outcomes that will be used to address the key questions. The protocol also states the procedures for screening references for inclusion, resolving conflicts between reviewers, and documenting the reasons references were excluded. Screening is typically a two-step process starting at the title and abstract level to exclude references that are clearly not relevant, and then proceeding to more detailed review of the full text of studies that passed the first screen. The title and abstract of each reference are reviewed for relevance and eligibility by two screeners independently, with conflicts resolved through discussion or consultation with a third reviewer. Exclusion decisions during full text review should be documented in the form of a flow diagram (Fig. 16.2) tracking the number of references retrieved and exclusion during the screening process up to the point references are selected for data extraction (Liberati et al. 2009; Moher et al. 2009).

16.4.3.1 Searching for and Selecting PFOA/PFOS Immunotoxicity Studies

The search terms for both PFOA and PFOS exposure and immune effects for this example were identified by (1) reviewing Medical Subject Headings (MeSH) for relevant terms, (2) extracting key terminology from reviews and a sample of relevant primary data studies, and (3) consulting a review of PFOA search terms from early drafts of a systematic review of developmental PFOA exposure and fetal growth (Johnson et al. 2013, 2014; Koustas et al. 2014). Although a published search strategy of PFOS was not located, the PFOA strategy was used by analogy as the

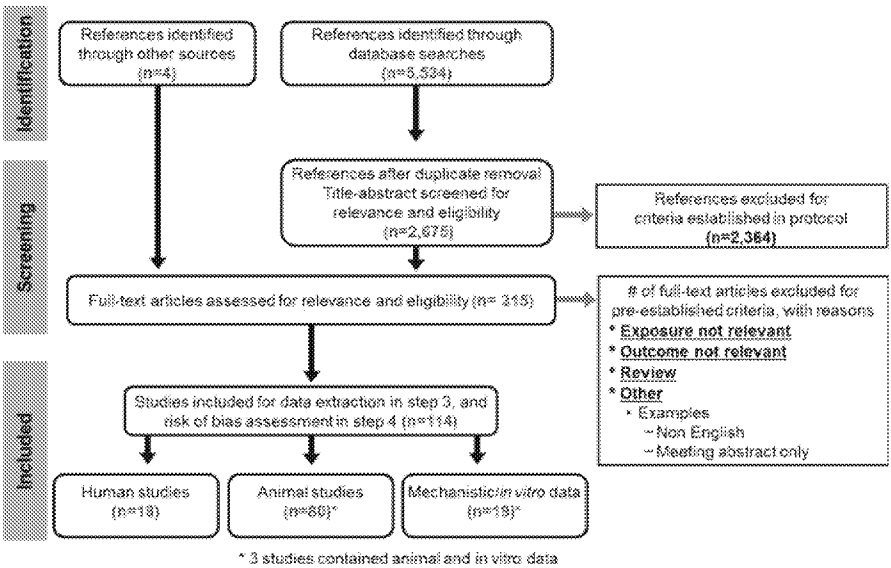

Fig. 16.2 Study tracking based on the PRISMA reporting standard
Clear documentation of the literature selection process can be accomplished with a simple flow chart following the PRISMA reporting standard for tracking references obtained from the literature search, through inclusion or exclusion for relevance or eligibility

basis for developing terms for PFOS exposure. A list of relevant subject headings and keywords were identified that combined exposure and immune or immunotoxicology terms (Table 16.1). A small set of relevant studies was used to test the search terms to ensure the strategy retrieved 100 % of previously identified "relevant" references. The search strategy presented in Table 16.1 would have to be tailored for each database.

Evaluations identify eligible studies from the PECOTS statement developed in problem formulation by clearly stating the populations, exposures, comparators, outcomes, timings, settings and important considerations such as appropriate study designs considered relevant. This example is outlined to be an evaluation of health effects for either the general population or highly exposure populations, so there would be no exclusions based occupational exposures. And as for exposure, studies with less precise exposure data are unlikely to be excluded unless there it has been established that the data are fundamentally flawed or there was a large database of epidemiological studies that have really good measures of exposure and outcome. For PFOA and PFOS immunotoxicity, there is a relatively small database of human studies, and no exclusions would be established based on exposure methods or metrics. There are a number of wildlife studies of PFOA or PFOS that include immune effects. Therefore, one of the considerations for this topic would be whether or not to include wildlife. For this example, the wildlife studies were included and would be evaluated with the other animal data as a distinct group to reflect the observational study design, rather than controlled exposure experimental studies.

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Table 16.1 PubMed search strategy

Box 1: PFOA and PFOS exposure search terms
perfluoroalkyl*[tiab] OR perfluorocaprylic[tiab] OR perfluorocarbon*[tiab] OR perfluorocarboxyl*[tiab] OR perfluorochemical*[tiab] OR (perfluorinated[tiab] AND (C8[tiab] OR carboxylic[tiab] OR chemical*[tiab] OR compound*[tiab] OR octanoic[tiab])) OR PFAA*[tiab] OR "fluorinated polymer"[tiab] OR "fluorinated polymers"[tiab] OR (fluorinated[tiab] AND (polymer[tiab] OR polymers[tiab])) OR (fluorocarbon[tiab] AND (polymer[tiab] OR polymers[tiab])) OR Fluoropolymer*[tiab] OR (fluorinated[tiab] AND telomer*[tiab]) OR fluorotelomer*[tiab] OR fluoro-telomer*[tiab] OR fluorosurfactant*[tiab] OR "FC 143"[tiab] OR FC143[tiab] OR 335-67-1 [rn] OR Pentadecafluoroctanoate*[tiab] OR Pentadecafluorooctanoate*[tiab] OR pentadecafluoroctanoic[tiab] OR pentadecafluorooctanoic[tiab] OR "pentadecafluoro-1-octanoic"[tiab] OR "pentadecafluoro-n-octanoic"[tiab] OR "perfluoro-1-heptanecarboxylic"[tiab] OR perfluorocaprylic[tiab] OR perfluoroheptanecarboxylic[tiab] OR perfluoroctanoate[tiab] OR perfluorooctanoate[tiab] OR "perfluoro octanoate"[tiab] OR "perfluorooctanoic acid"[nm] OR perfluoroctanoic[tiab] OR perfluorooctanoic[tiab] OR "perfluoro octanoic"[tiab] OR "perfluoro-n-octanoic"[tiab] OR "perfluorooctanoyl chloride"[tiab] OR PFOA[tiab] OR APFO[tiab] OR 1763-23-1[rn] OR 307-35-7[rn] OR "1-octanesulfonic acid"[tiab] OR "1-perfluorooctanesulfonic"[tiab] OR "1-perfluoroctanesulfonic"[tiab] OR "heptadecafluoro-1-octanesulfonic"[tiab] OR "heptadecafluoro-1-octane sulfonic"[tiab] OR "heptadecafluorooctanesulfonic"[tiab] OR "heptadecafluorooctane sulfonic"[tiab] OR "heptadecafluoroctane sulfonic"[tiab] OR "perfluoroalkyl sulphonate"[tiab] OR perfluoroctanesulfonate[tiab] OR perfluorooctanesulfonate[tiab] OR "perfluoroctane sulfonate"[tiab] OR "perfluorooctane sulfonate"[tiab] OR "perfluoro-n-octanesulfonic"[tiab] OR perfluoroctanesulfonic[tiab] OR perfluorooctanesulfonic[tiab] OR "perfluorooctane sulfonic acid"[nm] OR "perfluoroctane sulfonic"[tiab] OR "perfluorooctane sulfonic"[tiab] OR perfluoroctanesulphonic[tiab] OR perfluorooctanesulphonic[tiab] OR "perfluoroctane sulphonic"[tiab] OR "perfluorooctane sulphonic"[tiab] OR perfluoroctylsulfonic[tiab] OR PFOS [tiab]

Table 16.1 (continued)

Box 2: Immune/immunotoxicology search terms
immunology[sh] OR immune[tiab] OR immunocomp*[tiab] OR immunogen*[tiab] OR immunolog*[tiab] OR immunotox*[tiab] OR immunotoxins[mh] OR immunity[tiab] OR autoimmun*[tiab] OR "host resistance"[tiab] OR immunocompetence[mh] OR "immune system"[mh] OR spleen[tiab] OR splenic[tiab] OR splenocyt*[tiab] OR thymus[tiab] OR thymic[tiab] OR thymocyt*[tiab] OR leukocyt*[tiab] OR granulocyt*[tiab] OR basophil*[tiab] OR eosinophil*[tiab] OR neutrophil*[tiab] OR lymph[tiab] OR lymphoid*[tiab] OR lymphocyt*[tiab] OR "b-lymphocyte"[tiab] OR "b-lymphocytes"[tiab] OR "t-lymphocyte"[tiab] OR "t-lymphocytes"[tiab] OR "killer cell"[tiab] OR "killer cells"[tiab] OR "NK cell"[tiab] OR "NK-cell"[tiab] OR "NK-cells"[tiab] OR macrophag*[tiab] OR "mast cell"[tiab] OR "mast cells"[tiab] OR monocyt*[tiab] OR phagocyt*[tiab] OR dendrit*[tiab] OR "t-cell"[tiab] OR "t cell"[tiab] OR "t cells"[tiab] OR "t-cells"[tiab] OR "T helper"[tiab] OR "T-helper"[tiab] OR "b-cell"[tiab] OR "b cell"[tiab] OR "b cells"[tiab] OR "b-cells"[tiab] OR antibod*[tiab] OR histamine*[tiab] OR histocompatib*[tiab] OR immunoglobulins[mh] OR immunoglobulin*[tiab] OR "immunoglobulin A"[tiab] OR IgA[tiab] OR "immunoglobulin D"[tiab] OR IgD[tiab] OR "immunoglobulin E"[tiab] OR IgE[tiab] OR "immunoglobulin G"[tiab] OR IgG[tiab] OR "immunoglobulin M"[tiab] OR IgM[tiab] OR "antigens, CD"[mh] OR CD3 [tiab] OR CD4 [tiab] OR CD8 [tiab] OR CD25 [tiab] OR CD27 [tiab] OR CD28 [tiab] OR CD29 [tiab] OR CD45*[tiab] OR cytokines[mh] OR cytokine*[tiab] OR chemokine*[tiab] OR inteferon*[tiab] OR interleukin*[tiab] OR "IL-6"[tiab] OR "IL-8"[tiab] OR lymphokine*[tiab] OR monokine*[tiab] OR ("tumor necrosis"[tiab] AND (factor[tiab] OR factors[tiab])) OR "TNF alpha"[tiab] OR "TNFalpha"[tiab] OR "immune system diseases"[mh] OR autoimmun*[tiab] OR addison[tiab] OR rheumatoid[tiab] OR glomerulonephritis[tiab] OR diabetes[tiab] OR graves[tiab] OR lupus[tiab] OR thyroiditis[tiab] OR hypersensitiv*[tiab] OR sensitization OR hyperresponsiv*[tiab] OR allergy[mh] OR allerg*[tiab] OR atopy[tiab] OR atopic[tiab] OR dermatitis[tiab] OR eczema[tiab] OR otitis[tiab] OR "ear infection"[tiab] OR "ear inflammation"[tiab] OR Respiratory tract infections [mh] OR (respiratory[tiab] AND infection*[tiab]) OR asthma[tiab] OR bronchitis[tiab] OR pneumonia[tiab] OR bronchiolitis[tiab] OR rhinitis[tiab] OR sinusitis[tiab] OR wheez*[tiab] OR crackle*[tiab] OR cough[mh] OR cough*[tiab] OR dyspnea[tiab] OR gastroenteritis[tiab] OR inflammation[mh] OR inflammat*[tiab] OR pro-inflammat*[tiab] OR anti-inflamm*[tiab] OR "inflammation mediators"[mh] OR autacoid*[tiab] OR eicosanoid*[tiab] OR prostaglandin*[tiab] OR immunomodulation[mh] OR immunomodul*[tiab] OR immunotherap*[tiab] OR vaccin*[tiab] OR immuniz*[tiab] OR immunosuppress*[tiab] OR desensitiz*[tiab] OR immunoproteins[mh] OR immunoprotein*[tiab] OR "c-reactive protein"[tiab] OR CRP[tiab] OR "complement component" [tiab] OR (complement[tiab] AND (C1 OR C2 OR C3 OR C4 OR C5 OR C6 OR C7 OR C8 OR C9))

The exposure × effects search strategy for PFOA- or PFOS-associated immune effects was developed by combining exposure terms in box #1 and immunotoxicology terms from box #2

Table 16.2 lists the immune outcomes considered relevant and categorizes them as more (primary) or less (secondary) predictive for immunotoxicity (i.e., how well do the assessed outcomes predict adverse immunological effects). Primary outcomes are considered to be the most direct, or applicable, to the project. Secondary outcomes are relevant, but less direct and can include upstream indicators, intermediate outcomes, or measures biologically-related to our primary outcomes.

For the evaluation of immunotoxicity, primary outcomes are those with more predictive value for immunotoxicity such as disease resistance assays and functional

Table 16.2 Eligibility table for inclusion criteria and directness of immune outcomes

Humans	Animals	*In vitro* assays
Primary outcomes	**Primary outcomes**	**Primary outcomes**
Immune-related diseases and measures of immune function	Disease resistance assay or measures of immune function	*Immune function assays following in vitro exposure to the test substance* (e.g., natural killer cell [NK] activity, phagocytosis or bacterial killing by monocytes, proliferation following anti-CD3 antibody stimulation of spleen cells or lymphocytes)
Immunosuppression (e.g., otitis, infections, or decreased vaccine antibody response);	*Disease resistance assays* (e.g., host resistance to influenza A or trichinella, changes in incidence or progression in animal models of autoimmune disease)	
Sensitization and allergic response (e.g., atopic dermatitis or asthma);	*Immune function assays following in vivo exposure to the test substance* (e.g., antibody response [T-cell dependent IgM antibody response (TDAR)], natural killer cell [NK] activity, delayed-type hypersensitivity [DTH] response, phagocytosis by monocytes, local lymph-node assay [LLNA])	
Autoimmunity (e.g., thyroiditis or systemic lupus erythematosus)		
Secondary outcomes	**Secondary outcomes**	**Secondary outcomes**
Immunostimulation[a] (e.g., unintended stimulation of humoral immune function)	*Observational immune endpoints* (e.g., lymphoid organ weight, lymphocyte counts or subpopulations, lymphocyte proliferation, cytokine production, serum antibody levels, serum or tissue autoantibody levels, or histopathological changes in immune organs)	*Observational immune endpoints following in vitro exposure to the test substance* (e.g., general mitogen-stimulated lymphocyte proliferation, cytokine production)
Observational immune endpoints (e.g., lymphocyte counts, lymphocyte proliferation, cytokine levels, serum antibody levels, or serum autoantibody levels)		

An outcome eligibility table defines the relevance and eligibility for screening references on an outcome basis. Later in the evaluation process, when rating confidence in bodies of evidence, this same table identifies the applicability or directness of outcomes. Primary outcomes are those with more predictive value for immunotoxicity such as disease resistance assays and would not be downgraded for indirectness. Secondary outcomes are those with less predictive value or observational parameters such as lymphoid cell counts that would be downgraded for indirectness

[a]Note that stimulation of the immune response is not adverse per se and most vaccine preparations include adjuvants to aid in stimulation of an immune response to microbes. It is generally agreed that stimulation of the immune system should not be disregarded (WHO 2012). Unintended immunostimulation will be considered for possible hazard in the context of potency and persistence of the elevated immune response. Because evaluation of immunostimulation is less well established for health assessment, outcomes that could be evaluated under autoimmunity or sensitization will be evaluated under these more established categories when possible

immune parameters. Secondary outcomes are those with less predictive value for immunotoxicity such as observational parameters including cell counts or cytokine levels. This dichotomy separating the more and less predictive measures of immunotoxicity is consistent with testing strategies that rely on more sensitive and predictive immune assays (see Luster et al. 1992; US EPA 1996a, b, 1998a) and the NTP and WHO methods to categorize the evidence of immune system toxicity. Under these systems, measures of immune function or the ability of the immune system to respond to a challenge are weighed more heavily than observational parameters (Germolec 2009; WHO 2012). For *in vitro* studies, we are interested in immune measures that may support the biological plausibility of observed immune outcomes. For example, *in vitro* stimulation of immunoglobulin E (IgE) production would support a functional measure of sensitization or allergic response, but it would not support suppression of the natural killer response.

The health effects are also defined in the context of current understanding of the biological relatedness of outcomes and effects are "grouped" for analyzing data on related effects to reflect the four main categories of immune response: immunosuppression, immunostimulation, sensitization and allergic response, and autoimmunity. Eligible publications must include an indicator of PFOA or PFOS exposure analyzed in relation to any one of the following primary or secondary outcomes listed in Table 16.2.

16.4.4 Extract Data from Studies

The published information relevant to the evaluation from included studies is captured in a database to facilitate critical evaluation of the results, including data summary and display using separate data collection forms for human, animal, and *in vitro* studies. Procedures specified in the protocol should address quality assurance procedures such as extraction in duplicate or individual extraction followed by review.

16.4.5 Assess Quality of Individual Studies

Study quality has long been considered within environmental health assessments as an important part of synthesizing the evidence to reach conclusions (WHO 1999). However, individual study quality has not been consistently or explicitly assessed. In fact, the definition of study quality varies widely across groups, and therefore an important aspect of systematic review is to be clear where and how study quality is assessed within an evaluation. Broadly speaking, study quality can includes:

- **Reporting quality** – how thoroughly the information about a study was reported.
- **Internal validity or risk of bias** – how credible are the findings based on study design and conduct.

- **External validity or directness and applicability** – how well a study addresses the topic under review.

Internal validity or risk of bias assessment of individual studies is considered critical and is the primary study quality assessment in the OHAT method. Reporting quality is considered as part of the risk of bias assessment, as studies that do not report sufficient detail to address a risk of bias question are given a higher risk of bias rating for that question. External validity is considered when rating confidence in the body of evidence. Assessment approaches that mix these different aspects of study quality or provide a single summary score are discouraged (Balshem et al. 2011; Higgins and Green 2011; Viswanathan et al. 2012). The OHAT framework avoids these issues and addresses study quality in multiple steps in an evaluation. When major limitations for internal validity or external validity are known in advance (e.g., unreliable methods to assess exposure or health outcome), the basis for excluding those studies can be outlined as an exclusion criteria in the protocol.

The OHAT risk-of-bias tool adapts and extends guidance and specific questions from the Agency for Healthcare Research and Quality (AHRQ) methods for systematic review (Viswanathan et al. 2012). There are a number of risk-of-bias tools to address human studies that differ in specifics, but all assess some common key issues such as whether there could be systematic differences in baseline characteristics between groups (e.g., Higgins et al. 2011; Johnson et al. 2014; Viswanathan et al. 2012). The AHRQ approach was selected because it included both the key risk of bias issues found across multiple other tools and provided a "parallel approach" to address experimental and observational studies with a single set of questions. OHAT used this parallel approach to extend a common set of risk of bias questions to also address experimental animal studies which have potential sources of bias that are conceptually similar to human trials. Individual risk-of-bias questions from the OHAT tool are designated as applicable only to certain types of study designs (e.g., human controlled trials, experimental animal studies, cohort studies, case-control studies, cross-sectional studies, case series or case reports), with a subset of the questions applying to each study design (Table 16.3).

All references are independently assessed for risk of bias by two reviewers who answer all of the applicable questions with one of four rating options (definitely low, probably low, probably high, or definitely high risk of bias) (CLARITY Group at McMaster University 2013). Disagreements are resolved by reaching agreement through discussion or consultation of subject matter experts. The guidance for answering each question, and criteria to discriminate among the four ratings is outlined in extensive detail in the protocol. This guidance is specific to study design, and an example is presented in Table 16.4 for experimental animal studies. Each relevant outcome or health effect within a study is evaluated separately. While most of the risk of bias ratings will likely be the same across different outcomes, two areas of risk of bias are likely to vary by outcome: (1) potential confounding, and (2) the outcome assessment method, including the relative impact that blinding or failing to blind outcome assessors to treatment group may have had on the recorded values (e.g., white blood cell count measured by an automated cell sorter vs.

Table 16.3 OHAT internal validity or risk-of-bias questions

	Experimental animal[a]	Human controlled trials[b]	Cohort	Case-control	Cross-sectional	Case series
Selection BIAS						
Was administered dose or exposure level adequately randomized?	X	X				
Randomization requires that each human subject or animal had an equal chance of being assigned to any study group including controls (e.g., use of random number table or computer generated randomization)						
Was allocation to study groups adequately concealed?	X	X				
Allocation concealment requires that research personnel do not know which administered dose or exposure level is assigned at the start of a study. Human studies also require that allocation be concealed from human subjects prior to entering the study						
Note: (1) a question under performance bias addresses blinding of personnel and human subjects to treatment during the study; (2) a question under detection bias addresses blinding of outcome assessors						
Were the comparison groups appropriate?			X	X	X	
Comparison group appropriateness refers to having similar baseline characteristics between the groups aside from the exposures and outcomes under study						
Confounding BIAS						
Did the study design or analysis account for important confounding and modifying variables?	X	X	X	X	X	X
Note: a parallel question under detection bias addresses reliability of the measurement of confounding variables						
Did researchers adjust or control for other exposures that are anticipated to bias results?	X	X	X	X	X	X

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Performance BIAS						
Were experimental conditions identical across study groups?	X					
Did researchers adhere to the study protocol?	X	X	X	X	X	X
Were the research personnel and human subjects blinded to the study group during the study?	X	X				
Blinding requires that study scientists do not know which administered dose or exposure level the human subject or animal is being given (i.e., study group). Human studies require blinding of the human subjects when possible						
Attrition/exclusion BIAS						
Were outcome data complete without attrition or exclusion from analysis?	X	X	X	X	X	
Attrition rates are required to be similar and uniformly low across groups with respect to withdrawal or exclusion from analysis						
Detection BIAS						
Were the outcome assessors blinded to study group or exposure level?	X	X	X	X	X	X
Blinding requires that outcome assessors do not know the study group or exposure level of the human subject or animal when the outcome was assessed						
Were confounding variables assessed consistently across groups using valid and reliable measures?	X	X	X	X	X	X
Consistent application of valid, reliable, and sensitive methods of assessing important confounding or modifying variables is required across study groups						
Note, a parallel question under selection bias addresses whether design or analysis account for confounding						
Can we be confident in the exposure characterization?	X	X	X	X	X	X
Confidence requires valid, reliable, and sensitive methods to measure exposure applied consistently across groups						

(continued)

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Table 16.3 (continued)

	Experimental animal[a]	Human controlled trials[b]	Cohort	Case-control	Cross-sectional	Case series
Can we be confident in the outcome assessment?	X	X	X	X	X	X
Confidence requires valid, reliable, and sensitive methods to assess the outcome and the methods should be applied consistently across groups						
Selective reporting BIAS						
Were all measured outcomes reported?	X	X	X	X	X	X
Other						
Were there no other potential threats to internal validity (e.g., statistical methods were appropriate)?						
On a project specific basis, additional questions for other potential threats to internal validity can be added and applied to study designs as appropriate						

The OHAT risk-of-bias questions are applied to evaluate the risk of bias of studies on an outcome basis. The study design determines which questions are applicable as indicated in the table by an "X" for each question that applies to a given study design. Risk-of-bias ratings are developed by answering each applicable question with one of four options (definitely low, probably low, probably high, or definitely high risk of bias)

[a]Experimental animal studies are controlled exposure studies. Non-human animal observational studies could be evaluated using the design features of observational human studies such as cross-sectional study design

[b]Human Controlled Trials (HCTs): studies in humans with a controlled exposure, including Randomized Controlled Trials (RCTs) and non-randomized experimental studies

[c]Cross-sectional studies include population surveys with individual data (e.g., National Health and Nutrition Examination Survey or NHANES) and population surveys with aggregate data (i.e., air pollution exposure estimated by zip code)

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Table 16.4 Example risk of bias guidance

Definitely low risk of bias
There is direct evidence that animals were allocated to any study group including controls using a method with a random component. Acceptable methods of randomization include: referring to a random number table, using a computer random number generator, coin tossing, shuffling cards or envelopes, throwing dice, or drawing of lots (Higgins and Green 2011). Restricted randomization (e.g., blocked randomization) to ensure particular allocation ratios will be considered low risk of bias. Similarly, stratified randomization and minimization approaches that attempt to minimize imbalance between groups on important factors prognostic factors (e.g., body weight) will be considered acceptable. This type of approach is used by NTP, i.e., random number generator with body weight as a covariate. Please note that investigator-selection of animals from a cage is not considered random allocation because animals may not have an equal chance of being selected, e.g., investigator selecting animals with this method may inadvertently choose healthier, easier to catch, or less aggressive animals. Use of a concurrent control group is required as an indication that randomization covered all study groups.
Probably low risk of bias
There is indirect evidence that animals were allocated to any study group including controls using a method with a random component (i.e., authors state that allocation was random, without description of the method used) **OR** it is deemed that allocation without a clearly random component during the study would not appreciably bias results. For example, approaches such as biased coin or urn randomization, replacement randomization, mixed randomization, and maximal randomization may require consultation with a statistician to determine risk-of-bias rating (Higgins and Green 2011). Use of a concurrent control group is required as an indication that randomization covered all study groups.
Probably high risk of bias
There is indirect evidence that animals were allocated to study groups using a method with a non-random component **OR** there is insufficient information provided about how subjects were allocated to study groups. Non-random allocation methods may be systematic, but have the potential to allow researchers to anticipate the allocation of animals to study groups (Higgins and Green 2011). Such "quasi-random" methods include investigator-selection of animals from a cage, alternation, assignment based on shipment receipt date, date of birth, or animal number. A study with indirect evidence that there was a lack of a concurrent control group is another indication that randomization to all study groups was not conducted.
Definitely high risk of bias
There is direct evidence that animals were allocated to study groups using a non-random method including judgment of the investigator, the results of a laboratory test or a series of tests (Higgins and Green 2011). A study reporting lack of a concurrent control group is another indication that randomization to all study groups was not conducted.

Risk of bias guidance specific for experimental animal studies is outlined below for the question "Was administered dose or exposure level adequately randomized?"

behavioral observations made by trained research personnel). There is currently active methods development for risk of bias tools to address the types of evidence typically considered in environmental health – observational human, experimental animal, and *in vitro* studies. While assessing risk of bias of individual studies is critical to an environmental health assessment, the specific approach used is less important than clear documentation of the method used, along with consistent application of that method.

16.4.5.1 Assessing Risk of Bias for PFOA/PFOS Immunotoxicity Studies

Exposure, confounders, and outcome-specific modifications are the three areas of the risk of bias assessment that are likely to be the most evaluation-specific. Acceptable exposure measurements will depend on the chemical under study and the known confounders will vary by chemical and outcome. Sex and age are important confounders for evaluating immune effects because age and sex-dependent changes in immune function or observational parameters such as circulating immunoglobulin levels are common (WHO 2012). Immune-specific outcome guidance should describe the best methods and potential problems for immune assays used to measure outcomes found in the dataset. It is helpful if these criteria are described in lists or tabular form so that it can be updated quickly and shared easily to ensure the guidance is applied consistently across all studies. Inclusion of both older and newer outcome and exposure assessment methods and synonymous terms will aide reviewers to reconcile and assess the breadth of methods in the published literature, so the guidance should not only cover current terminology and methods. An abbreviated example describing discriminating risk of bias ratings for assays of antibody function (i.e., outcome assessment) are outlined below (the full guidance used is available here: http://ntp.niehs.nih.gov/ntp/ohat/evaluationprocess/appendix_2_pfoa_pfos_riskofbias.pdf).

Example

Question: Can we be confident in the outcome assessment?

Information necessary to reach a "Definitely low risk of bias" rating

- Direct evidence that immunization antigen batch/lot is the same for all treatment groups
- Direct evidence that antigen batch/lot is the same for immunization and the plating/assay

Information necessary to reach a "Probably low risk of bias" rating

- Indirect evidence that antigens used for immunizations are from the same batch/lot for all treatment groups
- Indirect evidence that antigens used for plating/assay are from the same batch/lot for all treatment groups

Information necessary to reach a "Probably high risk of bias" rating

- Indirect evidence that immunization antigens differed across treatment groups
- Indirect evidence that plating/assay antigens differed across treatment groups

Information necessary to reach a "Definitely high risk of bias" rating

- Direct evidence that immunization antigens differed across treatment groups
- Direct evidence that plating/assay antigens differed across treatment groups

16.4.6 Rate Confidence in the Body of Evidence

Groups of studies with data on an outcome (or biologically related outcomes) comprise a body of evidence that proceeds together through the evaluation process. Confidence ratings are developed separately for the human and animal bodies of evidence by considering the strengths and weaknesses of collections of studies with similar design features (Fig. 16.3). Ratings reflect confidence that the study findings accurately represent the true association between exposure to a substance and an effect. The OHAT method for rating confidence is based on the Grading of Recommendations Assessment, Development, and Evaluation Working Group (GRADE) (Guyatt et al. 2011) and AHRQ approaches (Balshem et al. 2011; Lohr 2012). This methodology is largely consistent among authoritative systematic review groups, including the Cochrane Collaboration (Schünemann et al. 2012). In the OHAT Approach ratings are developed on a 4-point scale to indicate the level of confidence in the body of evidence (High, Moderate, Low, and Very Low) consistent with the recommendations of the CLARITY Group at McMasters University (2013).

16.4.6.1 Initial Confidence Rating

For each body of evidence, studies are given an initial confidence rating by the presence or absence of four key study design features and then studies that have the same initial rating are considered together as a subgroup. The design features

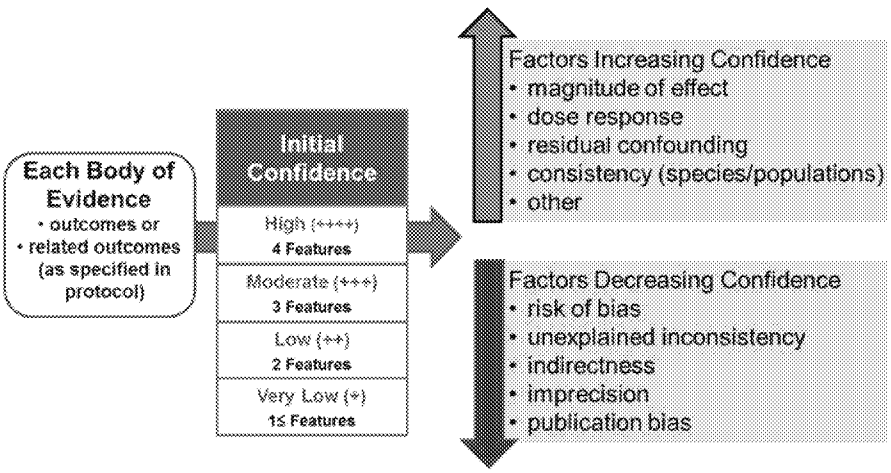

Fig. 16.3 Rating confidence in the body of evidence
Confidence ratings are developed for the human and animal bodies of evidence separately by considering the strengths and weaknesses of the collection of studies with similar design features

consider the ability of the study design to determine causality or assurance that exposure preceded and was associated with the outcome. Studies are differentiated based on whether or not: (1) exposure to the substance was controlled, (2) exposure occurred prior to the development of the outcome, (3) outcomes were assessed on the individual level not the population level, and (4) the study included a comparison group. Only experimental studies have controlled exposure, and therefore these studies will generally have all four features and be initially rated "High". Observational studies do not have controlled exposure and are differentiated by presence or absence of the three remaining study design features.

16.4.6.2 Downgrading and Upgrade Confidence Rating for Factors that Affect Confidence in the Results

The initial rating is downgraded for factors that decrease confidence or upgraded for factors that increase confidence in the results. Then, confidence across all available study designs and biologically related outcomes is assessed. The reasons for downgrading or upgrading confidence may not fit neatly into a single factor. If the decision to downgrade is borderline for two factors, the body of evidence is downgraded once to account for both partial concerns. Confidence should not be not downgraded twice for what is essentially the same limitation that may apply to more than one factor (or upgraded twice for the same asset). The protocol may specify severe factors that could downgrade confidence by two levels (typically it is moved only one level).

Five properties of the body of evidence (risk of bias, unexplained inconsistency, indirectness, imprecision, and publication bias) are considered to determine if the initial confidence rating should be downgraded. For each of the properties, a judgment is made and documented regarding whether or not there are issues that decrease the confidence rating for each property for the outcome.

- **Risk of bias of the body of evidence**: risk-of-bias assessments of individual studies developed earlier serve as the basis for an overall risk of bias conclusion for the body of evidence
- **Unexplained inconsistency**: large variability in the magnitude or direction of estimates of effect across studies, that cannot be explained by other factors (e.g., exposure assessment method, population characteristics, funding source)
- **Indirectness**: external validity (outcome, exposure, or population differs from that of the evaluation question [e.g., oral exposure studies may be downgraded as indirect evidence for an evaluation of effects from inhalation exposure]) or indirect measures of the health outcome
- **Imprecision**: lack of certainty for an estimate of effect for a specific outcome (often reflected in very wide confidence intervals around effect estimates)
- **Publication bias**: selective reporting or non-reporting of entire studies

Similarly, four properties of the body of evidence (large magnitude of effect, dose-response, residual confounding, and cross-species/population/study

consistency) are considered to determine if the confidence rating should be upgraded. Again, a judgment is made and documented regarding whether or not there are factors that increase the confidence rating for each property for the outcome.

- **Large magnitude of effect**: an observed effect that is sufficiently large such that it is unlikely to have occurred by chance despite possible unaccounted for confounding factors
- **Dose-response**: plausible dose-response relationship demonstrated between level of exposure and outcome
- **Residual confounding**: consideration of confounding factors, including the healthy worker effect or effect modification, that would bias the effect estimate towards the null – yet an effect is still seen
- **Cross-species/population/study consistency**: consistent results reported across multiple experimental animal models or species; or across populations that differ in factors such as time, location, and/or exposure levels; or studies with different design features.

16.4.6.3 Combine Confidence Conclusions for All Study Types and Multiple Outcomes

When considering evidence across study types and multiple outcomes, conclusions are based on the evidence with the highest confidence. While confidence ratings are initially set based on key design features of the available studies for a given outcome (e.g., for experimental studies separately from observational studies), only studies with the highest confidence rating form the basis for the final confidence conclusion. At this point, consistency of results across study designs should also be considered and could contribute to an upgraded confidence conclusion across the combined body of evidence.

If the only available body of evidence receives a "Very Low" confidence rating, then the evaluator should consider whether or not to move conclusions for those outcomes forward for hazard assessment. Effectively, "Very Low" confidence can be treated the same as having no data.

After confidence conclusions are developed for a given outcome, conclusions for multiple outcomes and the entire evaluation are developed. The project-specific definition of an outcome and the grouping of biologically related outcomes used in this step follow the approach defined in the protocol; any deviations are taken with care, justified, and documented. When outcomes are sufficiently biologically related that they may inform confidence on the overall health outcome, confidence conclusions may be developed in two steps. Each outcome would first be considered separately. Then, the related outcomes would be reconsidered together for properties that relate to downgrading and upgrading the body of evidence.

16.4.6.4 Rating Confidence for PFOA/PFOS Immunotoxicity Studies

Of the 80 animal studies on PFOA and PFOS immune effects that were identified through the literature search process (see Fig. 16.1) there were 5 PFOA studies and 8 PFOS studies that reported results on antibody response data. All of the PFOS animal studies (Dong et al. 2009, 2011; Keil et al. 2008; Lefebvre et al. 2008; Peden-Adams et al. 2008; Qazi et al. 2010; Zheng et al. 2009, 2011) would be given a high initial confidence based on having the four key study design features consistent with most experimental studies. Then this 8-study body of evidence would be evaluated for the five factors that may decrease confidence and four factors that may increase confidence that the study findings accurately represent the true association between PFOS exposure and the antibody response (independent of the presence or direction of a reported effect).

An assessment of "Indirectness" will be used as an example to show how the properties of the body of evidence would be considered for each factor. Indirectness reflects both external validity and indirect measures of the health outcome. The key questions, PECOTS statement, and eligibility criteria outlined in the protocol would state the population, exposure, outcome, comparator, timing, and settings of interest for the evaluation. A strict PECOTS statement and eligibility definition could essentially eliminate all indirect evidence; however, for most datasets the eligibility criteria define the directly relevant data as well as upstream or indirect data or populations. These factors are considered in more depth when determining if studies deviated from those of most interest to the evaluation. Experimental animals are considered directly relevant to the animal evidence stream and therefore would not be downgraded. The outcomes of interest would also be defined in the protocol. Table 16.2 outlines those outcomes and specifies that functional outcomes such as the antibody response are primary outcomes. Therefore, the antibody response data would not be downgraded as they are direct measures of an outcome of interest with good predictive value for the evaluation of immunotoxicity. The following summary outlines the decision not to downgrade for indirectness for this outcome.

Indirectness Rating for PFOS Animal Antibody Data:
Rating = "Not Serious," Therefore No Downgrade

- Exposure (PFOS) and model (experimental animal studies in mice and rats) directly relevant
- Antibody response is a primary outcome with good predictive value for immunotoxicity
- SRBC IgM response by PFC or ELISA are among the best measures of antibody response

An evidence profile should be developed to summarize each of the downgrade and upgrade decisions to support and communicate the scientific judgments made to reach a confidence rating for the body of evidence. Table 16.5 illustrates how a

Table 16.5 Example evidence profile

Body of evidence	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Publication bias	Magnitude	Dose response	Residual confounding	Consistency across species/ model	Final rating
Example of the type of information that should be in an evidence profile										
Human or animal	Serious or not serious	Serious or not serious	Serious or not serious	Serious or not serious	Detected or undetected	Large or not large	Yes or no	Yes or no	Yes or no	**Final rating**
(# Studies) initial rating	• Describe trend • Describe key questions • Describe issues	• Describe results in terms of consistency • Explain apparent inconsistency (if it can be explained)	• Discuss use of upstream indicators or populations with less relevance	• Discuss ability to disti nguish treatment from control • Describe confidence intervals	• Discuss factors that might indicate publication bias (e.g., funding, lag)	• Describe magnitude of response	• Outline evidence for or against dose response	• Address whether there is evidence that confounding would bias toward null	• Describe cross species, model, or population consistency	**High, moderate, or low**
Endpoint: functional antibody response (example "hypothetical" illustration for PFOS)										
Animal	Not serious	Not serious	Not serious	Not serious	Undetected	Not large	**Yes (increase)**	No	No	**High**
(8 PFOS studies) Initial Rating • High	• General low • Key question –Randomize= mixed low and probably high –Outcome=low • Probably high for allocation concealment	• Consistent suppression • Potential inconsistent response, but differed by: –Species (rat vs mouse), –Outcome (IgG vs IgM), –Antigen (SRBC vs KLH)	• SRBC IgM response by PFC or ELISA are among best measures of antibody response	• General small, confidence interval (CI) • Overlapping CIs between control and exposed	• No evidence of lag bias • Funding –Government –Universities –Industry	• Not sufficiently large to overcome potential bias	• Dose-response observed in multiple studies	• No evidence of confounding that would bias toward null	• All positive results from mice	Started high No serious downgrades Upgrade for dose-response Final rating would be High

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simple evidence profile can be constructed. This table provides examples of the type of information required to support the basis of scientific judgments as well as hypothetical conclusions for the PFOS antibody response body of evidence. An actual profile may have many lines of evidence depending on whether the data set has multiple bodies of evidence on a given outcome (e.g., wildlife observational studies and experimental animal studies), data streams (human and animal), and outcomes (functional antibody response data and observational data such as total IgG or IgM levels).

16.4.7 Translate Confidence into Level of Evidence (Toxicity or No Toxicity)

The step to translate confidence into level of evidence is a simple step that incorporates the direction of the effect (i.e., whether the data support toxicity or no toxicity) into the confidence conclusions developed previously. The strategy uses four terms that reflect both the confidence in the body of evidence for a given outcome and the direction of effect. If data support that exposure to the substance is associated with a heath effect, the three descriptors used ("High," "Moderate," or "Low") **level of evidence** directly translate from the confidence ratings ("High," "Moderate," or "Low") **confidence in the body** of evidence. If the data support that exposure is not associated with the health effect in question, then a separate descriptor ("Evidence of No Health Effect") is used to indicate confidence that the substance is not associated with a health effect. There is inherent difficulty in proving a negative, and as such a conclusion of evidence of no health effect is only reached when there is "High" confidence in the body of evidence. A "Low" or "Moderate" level of evidence results in a conclusion of inadequate evidence to reach a conclusion.

16.4.8 Integrate Evidence to Develop Hazard Identification Conclusions

The last step in evidence integration is to develop hazard identification conclusions from the level of evidence ratings by integrating the animal and human evidence with the additional consideration of the impact of mechanistic data (Fig. 16.4). For a given health effect, the highest level of evidence from each of the evidence streams is combined in the final step of the evidence assessment process. In the absence of either human or animal data, conclusions can be developed on the remaining evidence stream by treating the missing data as a "Low" level of evidence.

The five hazard identification conclusion categories used by OHAT are "Known," "Presumed," "Suspected," "Not classifiable," and "Not identified" to be a hazard to humans. Just as confidence conclusions can be developed on individual outcomes or

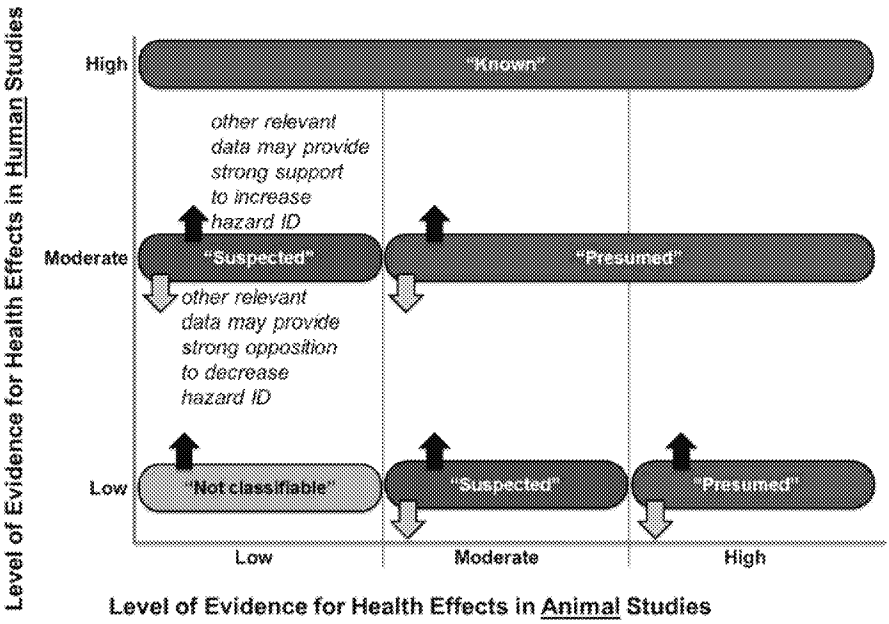

Fig. 16.4 Integrating evidence to develop hazard identification conclusions
Hazard identification conclusions reflect an integration of the level of evidence ratings from the human and animal evidence with the additional consideration of the impact of mechanistic data (Reproduced from Environmental Health Perspective: http://ehp.niehs.nih.gov/1307972/)

groups of biologically related outcomes as defined in the protocol, hazard identification conclusions may be developed as appropriate based on the objectives and the available data. The support for conclusions should be documented along with the rationale stating which outcomes were incorporated into each conclusion.

A schematic of how evidence is integrated into hazard identification conclusions is provided in Fig. 16.4. A "High" level of human evidence will result in a "Known" hazard identification conclusion, as animal and mechanistic data will not impact the decision if the human evidence is strong and consistent. Conversely, even if the human evidence has limitations and only has a "Moderate" level of evidence, a hazard identification conclusion of "Known" can be still be reached if there is both a "High" animal level of evidence and mechanistic evidence providing strong additional support for an association. In such case, additional experimental or longitudinal studies in humans are not necessary to identify a known hazard.

On the other end of the spectrum, it is anticipated that few systematic reviews would be initiated for topics where both the human and animal evidence is low and strong mechanistic evidence does not exist. As indicated in Fig. 16.4, such a review would result in a "Not classifiable" hazard identification conclusion.

When either the human or animal data support a conclusion of no health effect, the level-of-evidence conclusions for the two evidence streams are evaluated

together (human and animal) and the impact of mechanistic data is considered. If the human and animal level-of-evidence conclusions support no health effect, and this is not opposed by strong mechanistic data, the hazard identification conclusion is "not identified." While theoretically possible, we have not included in the schematic the hypothetical situation when the evidence streams are in direct conflict with one another (one has high confidence of no effect and the other shows evidence of an effect). Such a scenario, if possible, could be resolved by scoping and problem formulation to address this biologically implausible scenario.

When the levels of evidence are "Moderate" or "Low" for both human and animal evidence streams, mechanistic evidence has the greatest potential to influence the final hazard identification conclusions of "Presumed" or "Suspected." We anticipate that such hazard identification decisions will be accompanied by detailed description of the scientific considerations that support hazard identifications in this middle arena. Development of parallel methods to evaluate mechanistic evidence and incorporate predictive toxicology information is an area of active research.

16.4.8.1 Integrating Human, Animal and Mechanistic PFOA/PFOS Immunotoxicity Evidence

As previously indicated, only subsets of the available PFOA/PFOS immunotoxicity evidence were used in this illustration of the systematic review concepts, so no hazard identification conclusions have been developed. If the human level of evidence were to be "Moderate" or "Low" then addition mechanistic evidence would be considered. A walkthrough of the PFOS antibody response example will illustrate the process. Using the hypothetical animal data confidence conclusion of "High" confidence in the animal evidence for suppressed antibody response in Table 16.5, this data would support a "High" level of evidence for the animal data on humoral immunity because there is "high" confidence of toxicity or an effect. Using this framework, the potential hazard identification conclusions begin to emerge even before reviewing the human data. The animal data alone ("high" level-of-evidence) would support a conclusion of "suspected" to be a hazard to humans, and this would be the final hazard conclusion if there were no human data, or human data with "moderate" or "low" level-of-evidence. Human data with "high" level-of-evidence for antibody suppression would result in a hazard identification conclusion of "known."

A conclusion of "known" to be a hazard to humans could also be reached with "high" level of evidence from animal studies in the absence of human data if the mechanistic evidence provided strong support for the biological plausibility of the effect. In this case, the type of evidence providing that increased support would be *in vitro* or mechanistic studies indicating dose and temporal support for reductions in the antibody response. For example, if PFOS-associated reductions in antigen processing or presentation were consistently reported at or below the concentration of PFOA associated with antibody suppression. Consistent disruption in the antibody response across model systems, or multiple steps in the antibody response

such as interference with antigen presenting cells, as well as disruption of T-cell signaling, and reduced B-cell secretion of antibodies would provide stronger mechanistic evidence and support upgrading the hazard identification conclusions.

16.5 Summary

An approach to evaluate health effects of PFCs has been presented incorporating examples from a case study on PFOA/PFOS and immunotoxicity. The final goal of identifying the potential for hazard to human health can be reached by a systematic approach to evaluating the available literature. Interpretation of the final conclusion of such an evaluation is strengthened by using objective and reproducible methods and documenting scientific judgments in a transparent manner. The OHAT Approach to hazard identification, founded on systematic review methodology from clinical medicine, provides a scientifically rigorous approach to environmental health assessment and improves communication with the wider community of stakeholders. Future risk assessment of PFCs will also be strengthened by a clear hazard identification assessment, such as this example of PFOA/PFOS and immunotoxicity.

Acknowledgements We appreciate the helpful comments received during draft development from Dr. Kristina Thayer and Dr. Chad Blystone. We also acknowledge that this document was substantially informed by our experience with case studies developed to test the OHAT framework for systematic review and evidence integration. A case study evaluation of PFOA and PFOS immunotoxicity (http://ntp.niehs.nih.gov/go/36501) was used to provide input for refining the OHAT framework.

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Chapter 17
Toxicology Data for Alternative "Short-Chain" Fluorinated Substances

Robert C. Buck

Abstract Per- and poly-fluoroalkyl substances (PFASs) have been manufactured and widely used since the 1950s in numerous industrial and consumer applications. However, various perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), which have been manufactured and sold themselves and are impurities and breakdown products from numerous commercial per- and poly-fluoroalkyl surfactant and polymeric products, have been found widely in the environment. Concerns about the potential environmental impact of "long-chain" PFAAs has led to a substantial phase out of production of PFOS and PFOA and related compounds. Manufacturers have moved to production of alternative products which cannot be transformed in to long-chain PFAAs. This chapter provides an overview of available toxicology data for alternative fluorinated technologies including: short-chain fluorotelomer, short-chain electrochemical fluorination, perfluoropolyether, fluorinated oxetane, per- and poly-fluoroalkyl ether carboxylates and short-chain perfluoroalkyl acids.

Keywords Perfluoroalkyl • Polyfluoroalkyl • Acids • Toxicology • Alternative • Short-chain • Fluorotelomer • Electrochemical fluorination • Perfluoropolyether • Oxetane

17.1 Introduction

Per- and poly-fluoroalkyl substances (PFASs) have been manufactured and widely used since the 1950s in numerous industrial and consumer applications (Kissa 2001). The chemical and thermal stability of the carbon-fluorine bond in a perfluoroalkyl moiety and its hydrophobic and oleophobic properties render useful and unique properties to surfactants and polymers into which it is incorporated (Kissa

R.C. Buck (✉)
The Chemours Company FC, LLC, E. I. duPont de Nemours and Company, Inc., 974 Centre Road, CRP702-2211B, P.O. Box 2915, Wilmington, DE 19805, USA
e-mail: Robert.C.Buck@dupont.com

© Springer International Publishing Switzerland 2015

J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_17

ED_002330_00119699-00457

2001). Fluorinated surfactant products may provide unparalleled surface tension lowering in aqueous systems (Kissa 2001; Taylor 1999; Buck et al. 2011a, 2012). Polymeric products may provide oil and water repellency and stain release protection to textiles and carpet that cannot be achieved with current non-fluorinated alternatives (Kissa 2001; Rao and Baker 1994). However, various perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), which have been manufactured and sold themselves and are impurities and breakdown products from numerous commercial per- and poly-fluoroalkyl surfactant and polymeric products, have been found widely in the environment (Buck et al. 2011a). The global regulatory community has specifically designated as "long-chain" perfluoroalkane sulfonates ($C_nF_{2n+1}SO_3H$, $n \geq 6$, PFSAs, e.g., PFOS) and perfluorocarboxylic acids ($C_nF_{2n+1}CO_2H$, $n \geq 7$, PFCAs, e.g., PFOA) and precursor substances which can break down to form them. Concerns about the potential environmental impact of these "long-chain" PFAAs has led to a substantial phase out of production of PFOS and PFOA and related compounds. For example, major global fluorotelomer manufacturers have committed to replace historic long-chain products that contained perfluoroalkyl chains (C_nF_{2n+1}) with eight or more carbons ($n \geq 8$) that are potential precursors to long-chain perfluorocarboxylic acids (PFCAs), such as perfluorooctanoate (PFOA) (Ritter 2010). As a result, and to be able to continue to deliver the unique performance benefits of per- and poly-fluoroalkyl containing products, manufacturers have moved to production of alternative products which cannot be transformed in to long-chain PFAAs. The fluorinated alternatives generally have short-chain perfluoroalkyl moieties as a key functional component. These alternatives have more favorable environmental and biological properties and, most notably, they eliminate rapidly from living systems. The alternatives have been approved for manufacture, sale and use by regulators.

This chapter provides an overview of available toxicology data for a number of the alternative fluorinated technologies and presents data that addresses questions asking what is known about the chemistry and safety aspects of these alternatives (Wang et al. 2013). The following product chemistries will be discussed: short-chain fluorotelomer, short-chain electrochemical fluorination, perfluoropolyether, fluorinated oxetane, per- and poly-fluoroalkyl ether carboxylates and short-chain perfluoroalkyl acids. Readers seeking additional toxicology information or specific study details are encouraged to read the publications cited and contact product manufacturers.

17.2 Short-Chain Fluorotelomer-Based Products

A schematic of fluorotelomer-based products is shown in Fig. 17.1. Key manufacturing raw materials include 6:2 fluorotelomer alcohol, 6:2 fluorotelomer methacrylate and 6:2 fluorotelomer sulfonyl chloride. These are reacted to create surfactant and polymeric products (Kissa 2001; Taylor 1999; Buck et al. 2011a, 2012; Rao and Baker 1994). These products contain a six-carbon perfluoroalkyl moiety ($C_6F_{13}^-$).

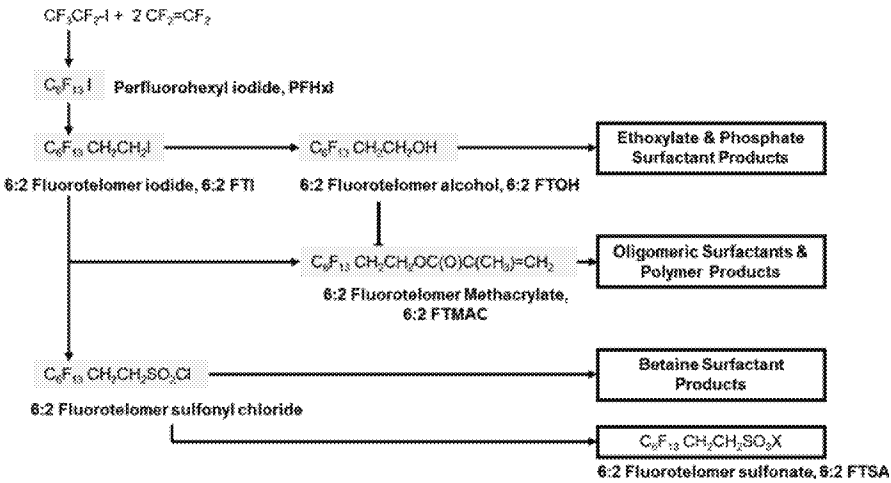

Fig. 17.1 Short-chain fluorotelomer-based raw materials and products

17.2.1 Fluorotelomer-Based Manufacturing Raw Materials

17.2.1.1 6:2 Fluorotelomer Alcohol, 6:2 FTOH

6:2 Fluorotelomer alcohol (6:2 FTOH; $C_6F_{13}CH_2CH_2OH$, CAS# 647-42-7) is a manufacturing raw material used to produce surfactant and polymeric products (Kissa 2001; Taylor 1999; Buck et al. 2011a, 2012; Rao and Baker 1994). It may also be present as an impurity in products (Larsen et al. 2006).

Acute and Subchronic

6:2 Fluorotelomer alcohol was assessed for acute, genetic, and subchronic toxicity using *in vitro* and *in vivo* methods (Serex et al. 2014). In rats, the oral LD_{50} was 1,750 mg/kg and the dermal LD_{50} was >5,000 mg/kg. In rabbits, 6:2 FTOH was not a primary skin or eye irritant, and did not produce a dermal sensitization response in mice. Additionally, 6:2 FTOH was not mutagenic in the bacterial reverse mutation test or in the mouse lymphoma assay and was not clastogenic in a chromosome aberration assay in human lymphocytes. In a 90-day subchronic oral study in rats (doses: 0, 5, 25, 125, 250 mg/kg/day), mortality was observed at 125 and 250 mg/kg/day. The NOAEL in the subchronic study was 5 mg/kg/day based on hematology and liver effects. Benchmark Dose Analysis was performed on the most sensitive endpoints from the 90-day oral gavage study and these levels were all above the study NOAEL of 5 mg/kg/day.

Development and Reproduction

6:2 FTOH was administered by oral gavage to rats at dosages of 5, 25, 125, or 250 mg/kg/day following OECD Test Guideline 414 for developmental toxicity and OECD Test Guideline 415 for one-generation reproductive toxicity (O'Connor et al. 2014). For the developmental toxicity study, adverse maternal toxicity observed at 250 mg/kg/day included reductions in body weight parameters and food consumption. Evidence of developmental toxicity was limited to increases in skeletal variations (ossification delays in the skull and rib alterations) at 250 mg/kg/day in the presence of maternal toxicity. There were no adverse maternal or developmental effects observed at 5, 25, or 125 mg/kg/day and there were no effects on reproductive outcome or quantitative litter data at any dose level. For the one-generation reproduction toxicity study, systemic parental and developmental toxicity were observed at 125 and 250 mg/kg/day. At 250 mg/kg/day, there was increased mortality among male and female parental rats, effects on body weight parameters, food consumption, and clinical signs, and there were effects on offspring survival indices and body weights. At 125 mg/kg/day, there was an increase in mortality in parental males only, and parental toxicity was limited to effects on body weight gain, food consumption (lactation), and clinical signs. Uterine weights were decreased at 125 and 250 mg/kg/day, although there were no corroborative histopathological changes. At 125 mg/kg/day, pup mortality was increased on lactation day 1, and body weights of the offspring were decreased during the second half of lactation paralleled by reduced food consumption of nursing females. There was no evidence of either parental or developmental toxicity at 5 or 25 mg/kg/day, and there were no effects on reproductive outcome at any dose level. Based on these data, 6:2 FTOH is not a selective reproductive or developmental toxicant. Effects occurred only at dosages that induced clear maternal/parental toxicity.

6:2 fluorotelomer alcohol (6:2 FTOH) was evaluated for potential reproductive toxicity in mice, in accordance with the OECD Test Guideline 415 (Mukerji et al. 2015). 6:2 FTOH was administered by oral gavage to CD-1 mice as a suspension in 0.5 % aqueous methylcellulose with 0.1 % Tween-80 at dosages of 1, 5, 25, or 100 mg/kg/day. The no-observed-adverse-effect level (NOAEL) for systemic toxicity was 25 mg/kg/day (males) and 5 mg/kg/day (females), based on effects at higher doses on mortality, clinical observations, body weight, nutritional parameters, hematology (red and white blood cell), clinical chemistry (liver-related), liver weights, and histopathology (liver, teeth, reproductive tract, and mammary gland). However, the test substance was not a selective reproductive toxicant. The NOAEL for reproductive toxicity was >100 mg/kg/day; no effects on reproductive outcome were observed at any dosage. The NOAEL for viability and growth of the offspring was 25 mg/kg/day, based on clinical signs of delayed maturation in pups, and reductions in pup survival and pup body weight during lactation at 100 mg/kg/day. Compared with previous studies of 6:2 FTOH in rats, mice were generally more sensitive with respect to systemic toxicity, and systemic toxicity of the parental animals, particularly the dams, was likely responsible for the more severe effects

observed in the offspring (i.e., effects on pup growth and viability). The liver appeared to be the primary target organ in both species, evidenced by increased liver size, trophic and toxic histopathological effects, and elevations in clinical chemistry parameters that were consistent with hepatotoxicity. Mortality, clinical abnormalities, red blood cell changes, and reductions in male body weights were consistent across species, but the reduction in P1 female body weight during lactation was markedly greater in mice than in rats. Correspondingly, changes in the female reproductive tract and mammary gland secondary to body weight decrements were observed only in mice, and the magnitude of the reductions in lactation index and pup body weight were greater in mice than in rats. While the severity of the effects was generally greater in mice than rats, the overall NOAELs for systemic toxicity and offspring viability/growth were identical across species, 5 mg/kg/day. 6:2 FTOH was not a selective reproductive toxicant in either species; no effects on reproductive outcome occurred at any dose level, and any effects observed in offspring occurred at dose levels that induced mortality and severe toxicity in maternal animals. This study in mice corroborated the NOAEL and Benchmark Dose of 5 mg/kg/day previously reported for 6:2 FTOH in rats (Serex et al. 2014). Together, these two studies provide an evaluation of 6:2 FTOH that encompasses the action of the parent chemical, 6:2 FTOH, and all metabolites formed from its biotransformation. No effects on reproductive organs such as the testes or ovaries were observed in the rat reproduction study or the mouse developmental toxicity study that would suggest the potential for endocrine effects from 6:2 FTOH.

Inhalation

In a 4-weeks vapor-phase inhalation study, four groups of male and female Crl:CD(SD) rats were exposed whole body 6 h/day, 5 days/week to 0, 1.0, 10 or 100 ppm 6:2 FTOH for a total of 22 exposures (Serex et al. 2012). Exposure to 6:2 FTOH demonstrated no adverse effects on body weight, body weight gains, food consumption or food efficiency at any dose. No test substance-related adverse clinical signs of toxicity were observed over the course of this study. Male and female rats exposed to 100 ppm 6:2 FTOH demonstrated increased mean serum bilirubin levels when compared to the control group and several females at this exposure level exhibited increased alanine aminotransferase (ALT). All animals exposed to 100 ppm 6:2 FTOH demonstrated increased mean absolute liver weights as well as increased mean liver weights relative to body and brain weight. Male rats exposed to 100 ppm 6:2 FTOH also demonstrated decreased motor activity during the 4th week of exposure. Changes in bilirubin, ALT, liver weights and motoractivity in animals exposed to 100 ppm resolved following a 4-week recovery period. Microscopic findings were limited to the incisor teeth of male and female rats exposed to 100 ppm 6:2 FTOH observed as basophilic striations within the inner dentin. This change is a common finding in rats following exposure to and metabolism of fluorine-containing compounds, was not associated with changes in

odontogenic epithelium and was considered non-adverse. Therefore, under the conditions of this study, the NOAEL for 6:2 FTOH was 10 ppm. These findings correlate with the target organ effects observed in oral studies with 6:2 FTOH (Serex et al. 2014; Mukerji et al. 2015) and indicate a lack of a route of entry effect. Therefore, route-to-route extrapolation for systemic effects is appropriate for risk assessment of 6:2 FTOH.

Metabolism and Kinetics

The *in-vitro* metabolism of 6:2 FTOH and selected metabolites by rat, mouse, and human hepatocytes to determine metabolic pathways was investigated (Gannon et al. 2010). 6:2 FTOH clearance in hepatocytes from rodent species was more rapid than in humans. Major metabolic pathways for 6:2 FTOH in hepatocytes from all three species included either the formation of glutathione, glucuronic acid, or sulfate conjugates of the parent. Formation of the glutathione conjugate was by far the most significant metabolic pathway in all three species. Another important metabolic pathway resulted in the formation of 6:2 fluorotelomer aldehyde (6:2 FTAL) leading sequentially to 6:2 fluorotelomer unsaturated aldehyde (6:2 FTUAL, $C_6F_{13}CH_2CHO$), 5:3 UAcid ($C_6F_{13}CH{=}CHCOOH$) and 5:3 Acid ($C_5F_{11}CH_2CH_2COOH$). The 6:2 FTAL also led to production of 6:2 FTCA (6:2 fluorotelomer carboxylic acid, $C_6F_{13}CH_2COOH$), 5:3 beta-keto aldehyde ($C_5F_{11}C(O)CH_2CHO$), perfluorohexanoic acid (PFHxA), and perfluoropentanoic acid (PFPeA). The metabolic pathways were qualitatively similar between rat, mouse, and human hepatocytes, however, there were differences in metabolic flux leading to differences in the relative amounts of metabolites produced by each species.

Male and female rats were dosed daily via gavage with 6:2 FTOH for 90 days with 0, 5, 25, 125, or 250 mg/kg/day followed by a recovery phase of 1 or 3 months (Serex et al. 2014). Liver, fat, and blood were collected from this study for toxicokinetic evaluation at days 91, 120 and 180 (Gannon et al. 2012). 6:2 FTOH was eliminated rapidly, principally in urine as parent conjugates. In plasma, 5:3 Acid was the major metabolite observed in the main study, 1 month recovery, and 3 month recovery samples. The quantifiable levels of 5:3 Acid present in the plasma at the 3 month recovery samples represented 0.02–0.05 mol% of the applied cumulative 6:2 FTOH dose. 4:3 Acid, $C_4F_9CH_2CH_2COOH$, was also observed in plasma at a concentration nearly two orders of magnitude less than 5:3 Acid. These two metabolites were also the most abundant in fat and liver. Concentrations of these two metabolites in liver and fat were less than or equal to the concentrations in plasma indicating that these metabolites are not preferentially accumulating in these tissues. In those instances where a tissue:plasma ratio could be calculated there was no indication of preferential retention.

Himmelstein et al. (2012) investigated the profile of 6:2 FTOH and its metabolites in plasma following controlled inhalation exposure to provide a basis for com-

parison of plasma metabolites and dosimetry between inhalation and oral dosing of 6:2 FTOH. Male and female rats were exposed to 6:2 FTOH at targeted vapor concentrations of 0.5 or 5 ppm for a single 6-h exposure. Blood was collected at 1, 3 & 6 h during nose-only exposure and at 6 & 18 h after the end of exposure. In two additional studies, samples were collected at 18–22 h after the end of either 5 or 23 days of whole body exposure to 1, 10, or 100 ppm (6 h/day), respectively. Plasma samples were analyzed by LC/MS/MS for 6:2 FTOH, carboxylic acids (PFBA, PFPeA, PFHxA, PFHpA), and polyfluorinated acids (e.g., 4:3 Acid; 5:3 Acid; 6:2 FTCA, fluorotelomer carboxylic acid; 6:2 FTUCA, fluorotelomer unsaturated carboxylic acid). 6:2 FTOH, barely detected in plasma during exposure to 0.5 ppm (LOQ = 80 nM), increased by threefold at 5 ppm (300 nM), and was only quantifiable 22 h after the highest repeated exposure of 100 ppm (~150 nM). From the single exposure, the ranking of the metabolites (as mol% of dose in plasma at C_{max}) in male rats was 6:2 FTCA (0.9–2.2 %) >5:3 Acid (1.1–1.8 %) > PFHxA (0.2–0.5 %) ~ PFHpA (0.1–0.5 %) >6:2 FTUCA (0.13–0.32 %) > PFBA (0.21–0.26 %). A similar pattern was observed in female rats except the perfluorocarboxylic acids were as much as 10× lower than males. The majority (~75–90 %) of the plasma burden was eliminated between C_{max} and 18 h after the end of exposure. The predominant metabolite in plasma of both sexes after repeated exposure was 5:3 Acid. 5:3 Acid increased in direct proportion with CxT (ppm-h) exposure suggesting minimal day-to-day accumulation. The findings are consistent with repeated oral dose plasma data (Gannon et al. 2012).

The toxicokinetics of 6:2 fluorotelomer alcohol (6:2 FTOH) and its terminal perfluoroalkyl and polyfluoroalkyl acid metabolites (PFBA, PFHxA, PFHpA and 5:3 Acid) have been calculated from laboratory studies of in rats and from a biomonitoring study of humans (Russell et al. 2015). In vitro studies with mouse, rat and human hepatocytes indicate qualitatively similar metabolic pathways of 6:2 FTOH. In a 1-day inhalation study of 6:2 FTOH in rats, PFBA, PFHxA, PFHpA and 5:3 Acid were determined to be the major metabolites in plasma with calculated elimination half-lives ranging between 1.3 and 15.4 h and metabolic yields of up to 2.7 mol%. In 5-day and 23-day inhalation studies and a 90-day oral study of 6:2 FTOH, the plasma or serum concentration profile of 5:3 Acid was several-fold higher than concentrations observed in the single day study, resulting in an estimated elimination half-life of 20–30 days. In contrast, the concentrations of PFBA, PFHxA and PFHpA showed little or no concentration increase with increased duration of exposure. Elimination half-lives of PFHxA, PFHpA and 5:3 Acid in humans were estimated from a study of professional ski wax technicians who experienced a unique occupational exposure to aerosolized and volatilized components of fluorinated glide wax. The resulting elimination half-life values of PFHxA, PFHpA and 5:3 Acid in humans were estimated to be 32, 70 and 43 days, respectively. Due to potential ongoing co-exposure in the skiwax technicians mean values are referred as "apparent" values and could be somewhat lower as well. Nilsson et al. (2013) considered from the same data the PFHxA half-life in human blood as less than 1 month.

Based on a one compartment toxicokinetic model, current environmental air concentrations of 6:2 FTOH were estimated to result in plasma concentrations of PFHxA, PFHpA and 5:3 Acid less than or equal to typical LOQ values, in agreement with extant biomonitoring results.

Risk Characterization

A study was conducted to (1) compare the oral and inhalation repeated-exposure toxicity data to confirm systemic toxicity, target organs, and lack of an exposure route effect, (2) confirm similar metabolic and toxicokinetic profiles via both exposure routes, and (3) conduct an inhalation risk assessment for reported ambient air concentrations (Serex et al. 2013). In an inhalation range-finder (5-days) and a 28-day inhalation toxicity study, the profile of 6:2 FTOH and its metabolites in plasma under controlled inhalation exposure was investigated as well as the systemic toxicity and target organs (Serex et al. 2012; Himmelstein et al. 2012). These studies provided a basis for toxicity comparison, plasma metabolites, and dosimetry between inhalation and oral dosing. Similar toxicity, metabolic and toxicokinetic profiles via both exposure routes was confirmed. Benchmark Dose Analysis (BMD) was conducted on the subchronic toxicity endpoints to determine the most sensitive effect and the corresponding BMD associated with this effect. Based on this analysis, the corresponding human equivalent dose (HED) was calculated to be 1.4 mg/ kg bw/day. An additional assessment factor of 2 was applied to extrapolate from the subchronic exposure to a chronic exposure and resulted in a final HED of 0.7 mg/kg bw/day. An equivalent air concentration was determined using an allometric scaling factor to arrive at a human equivalent concentration (HEC) of 2.5 mg/m^3. This HEC was then divided by the reported indoor and outdoor air concentrations to arrive at a margin of exposure (MOE). MOEs calculated for inhalation exposure to indoor or outdoor air ranged from 1.1E+05 to 2.5E+07. This assessment indicates there is no human health risk expected even at the highest ambient air concentrations of 6:2 FTOH reported.

Aquatic (Environ International Corporation 2014)

6:2 FTOH has a water solubility of 18.8 mg/L (Liu and Lee 2007). In a chronic study on *Daphnia magna*, the NOEC for effects on reproduction was 2.16 mg/L. The same study determined the LC_{50} for *Daphnia magna* to be 3.87 mg/L. The most sensitive NOEC observed, 0.623 mg/L, was from a chronic study on algal (*Pseudokirchneriella subcapitata*) growth inhibition (measured as biomass). Acute studies on *Daphnia magna* determined a range of EC_{50} values for immobility from 7.84 to 8.3 mg/L. Additional acute studies on fathead minnow, rainbow trout and medaka determined a range of LC_{50} values from 4.48 to 9.0 mg/L, showing low toxicity to fish species. Overall, 6:2 FTOH has low toxicity to aquatic organisms.

Bioconcentration (Environ International Corporation 2014)

Two studies measured the aquatic bioconcentration factor (BCF) for 6:2 FTOH in carp (*Cyprinus carpio*). Both studies used a 28-day, flow-through exposure of carp to nominal concentrations of 1 μg/L and 10 μg/L 6:2 FTOH. BCFs in the first study were less than 36 L/kg for the 1 μg/L and 46 L/kg in the 10 μg/L exposure groups. BCFs from the second study ranged from 8.4 L/kg to 58 L/kg in the 1 μg/L exposure group, and from 24 L/kg to 99 L/kg in the 10 μg/L group.

17.2.1.2 6:2 Fluorotelomer Methacrylate, 6:2 FTMAC

6:2 Fluorotelomer methacrylate (6:2 FTMAC, $C_6F_{13}CH_2CH_2OC(=O)C(CH_3)=CH_2$, CAS# 2144-53-8) is a manufacturing raw material used to produce surfactant and polymeric products (Kissa 2001; Taylor 1999; Buck et al. 2011a, 2012; Rao and Baker 1994). It may also be present as an impurity in products (Larsen et al. 2006).

Acute and Subchronic (Anand et al. 2012a)

6:2 FTMAC had low acute oral and dermal toxicity (LD_{50} >5,000 mg/kg), was not a skin or eye irritant, and did not demonstrate skin sensitization potential in a local lymph node assay (LLNA). 6:2 FTMAC was not mutagenic in the bacterial reverse mutation (Ames) test or in the mouse lymphoma assay. 6:2 FTMAC was administered at 0, 100, 500 and 1,000 mg/kg/day via oral gavage to male and female SD rats for 14 days. No test substance-related effects on mortality, clinical signs, body weights, nutritional parameters, or clinical pathology were observed at any dose. Statistically significant increases in liver weights at all dose levels (except 100 mg/kg/day males) and thyroid and kidney weights in 500 and 1,000 mg/kg/day males were noted, but there were not associated histopathological or clinical pathology changes. The changes noted in teeth (altered mineralization; retention of basophilic material) and femur (increased mineralization) in all treated groups were not associated with clinical signs or microscopic changes and were likely related to free fluoride formed from 6:2 FTMAC metabolism. Plasma (three to fourfold) and urine (30- to 50-fold) fluoride was higher in treated groups versus controls. Therefore, the changes noted in organ weights, teeth, femur, plasma or urine were not considered adverse. In the repeated dose toxicity study, the no-observed-adverse-effect-level (NOAEL) was 1,000 mg/kg/day.

Metabolism

6:2 FTMAC metabolism was evaluated *in vitro* in mammalian hepatocytes and *in vivo* in rats (DuPont-17784-1388 2014; DuPont-17784-1599 2014). *In vitro*, 6:2 FTMAC was rapidly metabolized in live rat and mouse hepatocytes compared to heat

inactivated controls (DuPont-17784-1599 report as cited in 6:2 FTMAC REACH Dossier 2014). The major metabolite was 6:2 uFTOH-glutathione (6:2 unsaturated fluorotelomer alcohol glutathione conjugate). In rat hepatocytes, the half-life was reported as less than 3 min. An actual rate was not attainable due to the fact that there was <5 % of the parent compound remaining 5 min after incubation and no parent compound remaining after 15 min of incubation. Significant metabolism was also observed in male mice hepatocytes, but half- life and clearance rates could not be calculated due to the shape of the data curve. The steep portion of the curve between 0 and 3 min indicated rapid metabolism. From the *in* vivo study, where rats were dosed with a single oral dose of 300 mg/kg, blood, fat and liver samples were analyzed for 6:2 FTMAC to provide an estimate of the tissue-plasma ratio. 6:2 FTMAC was rapidly metabolized and detected only in very small amounts in plasma (<40.0 ng/mL at the 1-, 2- and 4-h post-dosing time points), liver (<20.0 ng/mL at the 6-, 12-, and 24-h post-dosing time points), and fat (<20.0–99.9 ng/g at the 6-, 12-, 24-, 48-, 72-, and 96-h post-dosing time points) (DuPont-17784-1388 report as cited in 6:2 FTMAC REACH Dossier 2014). The very small amounts of 6:2 FTMAC detected precluded determination of plasma clearance or tissue/plasma ratios.

Aquatic (Anand et al. 2012a)

6:2 FTMAC has a water solubility of approximately 0.4 mg/L. Based on mean measured concentrations, the 96-h LC_{50} in fathead minnow was >14.5 mg/L and the 72-h EC_{50} in *Pseudokirchneriella subcapitata* was >24.6 mg/L, while the 48-h EC_{50} in *Daphnia magna*, based on nominal concentrations, was >120 mg/L.

17.2.2 Fluorotelomer-Based Products

17.2.2.1 Acrylic Polymer (Fig. 17.2)

6:2 Fluorotelomer methacrylate copolymers are generally synthesized by aqueous emulsion polymerization of 6:2 FTMAC and co-monomers to create an aqueous dispersion of polymeric particles (Kissa 2001; Rao and Baker 1994). The polymers are applied to a variety of surfaces to provide water and oil repellency and stain and soil resistance.

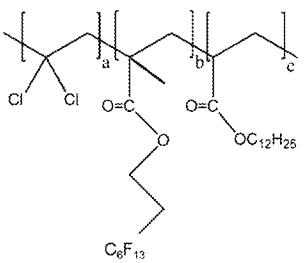

Fig. 17.2 Schematic of a 6:2 fluorotelomer methacrylate polymer

Mono-phosphate	$C_6F_{13}CH_2CH_2O\text{-}P(=O)(OH)_2$	6:2 PAP
Bis-phosphate	$[C_6F_{13}CH_2CH_2O]_2\text{-}P(=O)(OH)$	6:2 di-PAP
Tris-phosphate	$[C_6F_{13}CH_2CH_2O]_3\text{-}P(=O)$	
Pyrophosphate	$[C_6F_{13}CH_2CH_2O]_2\text{-}P(=O)\text{-}O\text{-}P(=O)\text{-}_2[OCH_2CH_2C_6F_{13}]$	

Fig. 17.3 6:2 fluorotelomer alcohol phosphate product components

A 6:2 fluorotelomer methacrylate polymer aqueous dispersion was evaluated in one-generation reproduction and repeated-dose toxicity studies (Singh et al. 2012). In the repeated-dose study, four groups of young adult male and female Crl:CD(SD) rats were daily administered at 0, 100, 500 or 1,000 mg/kg/day by oral gavage for approximately 90 days. No primary test substance-related effects on mortality, clinical signs, neurobehavioral assessment, body weight, nutritional parameters or clinical pathology were observed at any dose. Treatment related adverse nasal lesions consistent with regurgitation rhinitis were observed in males and females at ≥100 mg/kg/day. Test substance was observed in the nasal cavity. The underlying cause of the apparent reflux is uncertain and relevance of these findings for humans is questionable. In the one generation reproductive study, rats were administered test substance (25/sex/group) daily at 0, 20, 100, 500, or 1,000 mg/kg/day for at least 109 days. No effects on reproductive endpoints at any dose were observed at any dose. The test substance was not a selective reproductive toxicant.

17.2.2.2 Fluorotelomer Alcohol Phosphate

Fluorotelomer phosphate is used as a surfactant at levels between 100 and 500 ppm in aqueous coatings systems to lower aqueous surface tension and enhance wetting and spreading (Kissa 2001; Taylor 1999).

The toxicity profile of a 6:2 fluorotelomer alcohol phosphate mixture (Fig. 17.3) was evaluated (Anand et al. 2012b). The test substance had low acute oral and dermal toxicity (LD_{50} >5,000 mg/kg), was not an irritant to the eye or skin, and did not cause skin sensitization in the LLNA assay. It was not mutagenic in the Ames test or in the in vitro chromosomal aberration assay. The repeated dose toxicity potential was assessed by administering 0, 5, 25, and 125 mg/kg/day via oral gavage to male and female SD rats for 28 days. There were no test substance-related adverse effects on in-life parameters, clinical pathology, gross observations, organ weights, and microscopic findings. The increase in liver (males and females) and kidney (males) weights at 125 mg/kg/day as compared to controls noted at the end of exposure period was not considered adverse as there were no correlative microscopic histopathological findings. There were no organ weight changes following a 30 day recovery period after dosing was terminated. At the end of exposure period, statistically significant increases in plasma (25 and 125 mg/kg/day) and urine (all doses) fluoride levels were observed in males and females indicating parent metabolism yielding fluoride. While fluoride plasma levels were similar to controls, urine fluoride levels at 125 mg/kg/day were

statistically significantly higher than controls following the recovery period. The NOAEL for the study was 125 mg/kg/day. In an assessment of acute aquatic toxicity, the 96-h LC50 in fathead minnows was >120 mg/L, 96-h LC50 in zebra fish was >150 mg/L, 48-h EC50 in *Daphnia magna* was 16.2 mg/L, and 72-h EC50 in algae was >120 mg/L. In a chronic study, the 21-day no-observed-effect-concentration (NOEC) in *Daphnia magna* was 0.047 mg/L.

17.2.2.3 6:2 Fluorotelomer Sulfonate, 6:2 FTSA

6:2 Fluorotelomer sulfonate (6:2 FTSA) is a fluorinated surfactant that may be used as a polymer processing aid in the synthesis of fluoropolymers and is a biodegradation product of fluorotelomer thiol and fluorotelomer sulfonyl chemistry (Buck et al. 2011a).

The potassium salt of 6:2 fluorotelomer sulfonate ($C_6F_{13}CH_2CH_2SO_3^-K^+$, CAS# 59587-38-1) had an oral LD_{50} = 2,000 mg/kg (rat), was not irritating to skin (rabbit) and was negative in LLNA assay therefore not a skin sensitizer (Buck et al. 2011b). 6:2 FTSA was not mutagenic in the bacterial reverse mutation test with *Salmonella typhimurium*, negative in the Unscheduled DNA Synthesis (UDS) test in mammalian cells and negative for clastogenicity in micronucleus and chromosome aberration assays (Buck et al. 2011b).

The acute aquatic toxicity to fish, invertebrates and algae for 6:2 FTSA has been determined (Buck et al. 2011b). In rainbow trout, the 96 h LC_{50} was >107 mg/L, the 48 h EC_{50} in *Daphnia magna* was >109 mg/L and in *Pseudokirchneriella sucapitata* (green algae) the 72 h EC_{50} was >96 mg/L. In a 90-day early life-stage rainbow trout study (Hoke et al. 2009), the NOEC was 2.62 mg/L based on mean, measured concentrations and first day of hatching. The LOEC (lowest observed effect concentration) and MATC (maximum acceptable toxicant concentration) for the same endpoint were 4.85 and 3.56 mg/L, respectively. A guideline study (OECD TG 305) that included the addition of a dietary exposure conducted under GLP was conducted to evaluate the bioconcentration and bioaccumulation potential 6:2 FTSA. Exposure conditions included a dilution water control, 1 ug/L and 10 ug/L aqueous exposures and a 10 ug/kg dietary exposure with a 56 day uptake phase followed by a 28 day depuration phase. Tissue residues of the test substance in whole fish were evaluated at multiple time points during both study phases. The steady-state bioconcentration factors (BCF) for the nominal 1 and 10 μg/L aqueous test substance concentrations were 22 and 3, respectively. The kinetic bioconcentration factors (BCFs) were 36 and 3, respectively, for the nominal 1 and 10 μg/L test substance concentrations. The dietary assimilation efficiency, the growth corrected half-life and the dietary biomagnification factor (BMF) for the dietary exposure were 0.435, 23.5 days and 0.295, respectively. The test results indicated that 6:2 FTSA is not bioaccumulative in aquatic ecosystems according to regulatory criteria.

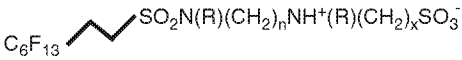

Fig. 17.4 6:2 fluorotelomer betaine

17.2.2.4 6:2 Fluorotelomer Betaine (Fig. 17.4)

6:2 Fluorotelomer betaine is a fluorinated surfactant used as an ingredient in aqueous fire-fighting foam formulations (Pabon and Corpart 2002).

An amphoteric 6:2 fluorotelomer-based betaine surfactant in glycol solvent mixture and water, was evaluated in a 28-day oral gavage study (OECD 407) with a 28-day recovery subset and a one-generation reproduction study subset (OECD 422) (O'Connor et al. 2013). Groups of 20 Crl:CD(SD) rats were dosed by gavage with vehicle (deionized water) containing 0, 10, 50, or 200 mg/kg/day test substance. Dams were allowed to deliver and rear their offspring until postnatal day (PND) 4. Litter examinations were determined at birth and on PND 4. For the subchronic and recovery evaluations, gross postmortem examinations were performed on selected rats and selected organs were weighed and/or retained for histopathological examination. There were no test substance related deaths or clinical observations, no effects on body weight or nutritional parameters, no effects on neurobehavioral endpoints, clinical pathology, reproductive performance, or on offspring at any dose. Test substance-related changes occurred at ≥50 mg/kg/day in the kidneys of male rats. Increased hyaline droplets consistent with $alpha_{2u}$ globulin were noted at >50 mg/kg/day in the cortical tubules of males after 28 days of administration, and were also observed in the P1 males after 45 days of test substance administration. Increased hyaline droplet accumulation was not present in the recovery males. The systemic toxicity NOAEL was 10 mg/kg/day in male rats based on histopathologic effects observed in the kidneys. The NOAEL for reproductive toxicity and effects on offspring was 200 mg/kg/day, the highest dose tested. Nasal olfactory epithelium effects observed in males at 200 mg/kg/day and females at ≥50 mg/kg/day have been recently reassessed (DuPont, 2014, Unpublished results) and appear to be associated with regurgitation rhinitis of the test substance and not related to systemic effects (Damsch et al. 2011).

17.3 Short-Chain Perfluorobutane Sulfonyl Products via Electrochemical Fluorination

A schematic of perfluorobutane sulfonyl-based products is shown in Fig. 17.5. Key manufacturing raw materials include N-methyl perfluorobutane sulfonamido alcohol, MeFBSE, and N-methyl perfluorobutane sulfonamidoethyl acrylate, MeFBSMAC. These are reacted to create surfactant and polymeric products (Kissa 2001; Taylor 1999; Buck et al. 2011a, 2012; Rao and Baker 1994). These products contain a four-carbon perfluoroalkyl moiety ($C_4F_9^-$).

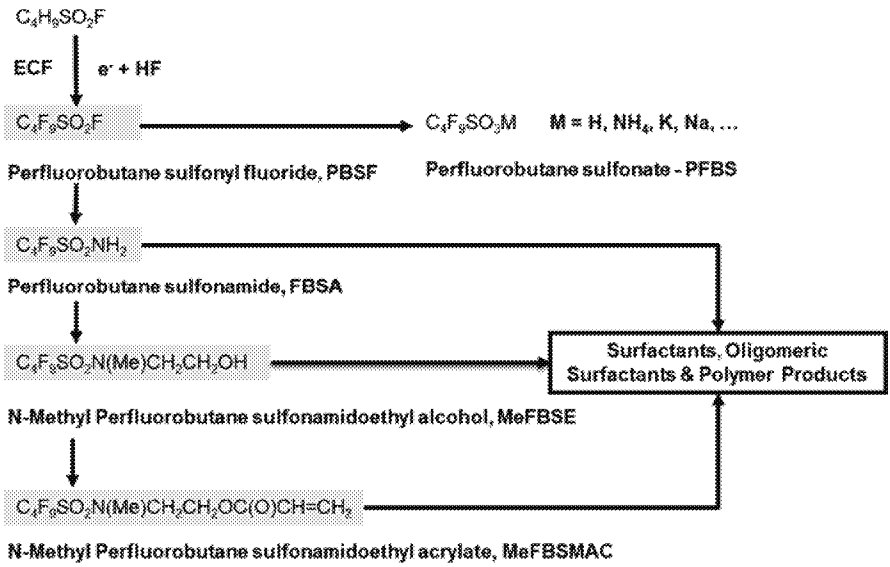

Fig. 17.5 Perfluorobutane sulfonyl-based manufacturing raw materials and products

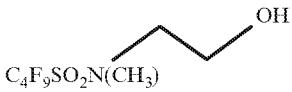

Fig. 17.6 N-methyl perfluorobutane sulfonamido ethanol, Me-FBSE

17.3.1 Manufacturing Raw Materials

17.3.1.1 N-Methyl Perfluorobutane Sulfonamidoethanol, Me-FBSE (Fig. 17.6)

n-Methyl perfluorobutane sulfonamido ethanol (1-Butanesulfonamide, 1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-N-methyl-, $C_4F_9SO_2N(Me)CH_2CH_2OH$, CAS# 34454-97-2) is a manufacturing raw material used to produce surfactant and polymeric products (Kissa 2001). It may also be present as an impurity in products.

From the available data found (3M™ Stain Resistant Additive SRC-220 Safety Data Sheet (U.S.); 3M™ Novec® Fluorosurfactant FC-4432 Safety Data Sheet (U.K.); 3M™ Novec® Fluorosurfactant FC-4432 Safety Data Sheet (U.S.)), MeFBSE has an oral LD50 >2,000 mg/kg, was not a skin irritant and a mild eye irritant in rabbit and negative in guinea pig skin sensitization. It was not mutagenic, negative *in-vitro* germ cell mutagenicity. In a 28-day repeat dose reproductive/developmental toxicity screening study in rats with MeFBSE, reductions in the number of live born pups, neonatal survival, and pup body weights, as well as increases in the number of stillborn pups were observed at the 250 mg/kg dose level. The no observed adverse effect level (NOAEL) for viability and growth of offspring

was 50 mg/kg. The NOAEL for effects on mating, fertility, and estrous cycling was >250 mg/kg (no effects were observed at any level). Additional study details and, or data may be available from the manufacturer.

17.3.1.2 N-Methyl Perfluorobutane Sulfonamidoethyl Acrylate, MeFBSAC (Fig. 17.7)

n-Methyl perfluorobutane sulfonamido ethyl acrylate (MeFBSAC, $C_4F_9SO_2N(CH_3)CH_2CH_2$-C(=O)CH=$CH_2$, 2-Propenoic acid, 2-[methyl[(1,1,2,2,3,3,4,4,4-nonafluorobutyl)sulfonyl]amino]ethyl ester, CAS# 67584-55-8) is a manufacturing raw material used to produce surfactant and polymeric products. From the available data found (3M™ Novec® Fluorosurfactant FC-4432 Safety Data Sheet (U.K.)), the oral LD_{50} is >2,000 mg, it is not a skin irritant, is a moderate eye irritant, was determined to be skin sensitizing in a guinea pig assay and was not mutagenic in-vitro. Additional study details and, or data may be available from the manufacturer.

17.3.2 Products

17.3.2.1 Product CAS# 1017237-78-3

2-Propenoic acid, 2-[methyl[(1,1,2,2,3,3,4,4,4-nonafluorobutyl)sulfonyl]amino] ethyl ester, telomer with 3-mercapto-1,2-propanediol, 2-methyloxirane polymer with oxirane di-2-propenoate, and 2-methyloxirane polymer with oxirane mono-2-propenoate, tert-Bu 2-ethylhexaneperoxoate-initiated, is a non-ionic polymeric fluorinated surfactant that provides wetting, levelling and flow in coatings systems.

According to a product safety data sheet (3M™ Novec® Fluorosurfactant FC-4432 Safety Data Sheet (U.K.)), the oral LD_{50} is >5,000 mg, dermal LC_{50} is >5,000 mg and in a 28 day oral toxicity study the NOEL was 1,000 mg/kg/day. In aquatic studies in Fathead Minnow the 96 h LC50 was 768 mg/L, in *Daphnia magna* the 48 h EC50 was 99 mg/L and in green algae the 96 h EC50 was 763 mg/L. Additional study details and, or data may be available from the manufacturer.

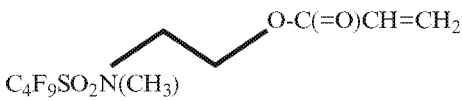

Fig. 17.7 N-methyl perfluorobutane sulfonamidoethyl acrylate, MeFBSAC

17.4 Per- and Poly-Fluoroalkyl Ether Carboxylates Used as Polymer Processing Aids in the Manufacture of Fluoropolymers

Per- and poly-fluorinated ether carboxylates have been developed as alternative polymer processing aids for the aqueous emulsion polymerization of tetrafluoroethylene (TFE) and other fluorinated monomers in the synthesis of fluoropolymers. They have replaced ammonium perfluorooctanoate which was historically used for this purpose (Buck et al. 2011a).

17.4.1 Ammonium 4,8-Dioxa-3H-Perfluorononanoate

Ammonium 4,8-dioxa-3H-perfluorononanoate, $CF_3OCF_2CF_2CF_2OCHFCF_2CO_2H{\bullet}NH_3$ (CAS# 958445-44-8, Propanoic acid, 2,2,3-trifluoro-3-[1,1,2,2,3,3-hexafluoro-3-(trifluoromethoxy)propoxy]-, ammonium salt (1:1)), was evaluated for acute and repeat-dose toxicity, eye and skin irritation, dermal sensitization, genotoxicity, and developmental toxicity (Gordon 2011). The substance was also evaluated as a peroxisome proliferator-activated receptor alpha agonist in rats. The substance was moderately toxic orally and practically non-toxic dermally in acute studies in rats, a mild skin irritant and a moderate to severe eye irritant in rabbits. It was a weak dermal sensitizer in local lymph node assays in mice. It was not genotoxic. Developmental toxicity in rats was not observed at doses where there was no maternal toxicity observed. Peroxisome proliferation-activated receptor alpha was evident in male rats. NOAELs in 28- and 90-day oral repeated-dose studies in rats were 10 mg/kg/day for males and 100 mg/kg/day for females. The liver was the primary target organ in male rats and the kidney was the primary target organ in female rats.

17.4.2 Ammonium, 2,3,3,3-Tetrafluoro-2-(Heptafluoropropoxy)-Propanoate

Ammonium, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate, $CF_3CF_2CF_2OCF(CF_3)COOH{\bullet}NH_3$ (CAS# 62037-80-3) was evaluated for acute and repeated-dose subchronic and chronic toxicity, and genotoxicity in mammals, acute and chronic exposure in aquatic species and in an avian reproduction study (European Chemicals Agency). In the acute studies the oral LD_{50} (rat) was 1,750 mg/kg, the dermal LD_{50} was >5,000 mg/kg, the inhalation 4 h acute lethal concentration (ALC) was >3.6 mg/L vapor. The genetic toxicity was determined to be negative based on a battery of tests including Ames and chromosome aberration in-vitro studies and mouse micronucleus, mouse bone marrow and rat UDS in-vivo studies.

In an oral 90-day sub-chronic study in rats the NOAEL was 10 mg/kg/day. In an oral 90-day sub-chronic in mice the NOAEL was 0.5 mg/kg/day. The test substance was evaluated in a developmental toxicity study in rats and an enhanced one-generation reproduction study in mice. As in other studies, liver toxicity was present in maternal animals. Effects on offspring were mostly limited to body weight decrements at maternally toxic doses. Therefore, the substance was not uniquely toxic to the offspring. The substance was not embryolethal or teratogenic in rats and produced no effects on fertility or prenatal development in mice at any of the doses tested, including the maternally toxic doses.

The chronic toxicity and carcinogenicity of the test substance was evaluated in a 2-year oral study in male and female rats. The NOAELs were 1 and 50 mg/kg/day in males and females, respectively. Effects were limited to the highest doses tested (50 and 500 mg/kg/day in males and females, respectively) and included tumor findings characteristic of the rodent-specific response to peroxisome proliferation PPARα antagonists. Non-neoplastic effects following chronic exposure to rats were similar to those seen in shorter duration studies but also included microscopic and clinical pathology findings consistent with liver toxicity and, in females, evidence of irritation of the gastrointestinal tract and kidney.

In acute aquatic studies, based on measured concentrations the fish 96 h LC_{50} for multiple species was >100 mg/L, the *Daphnia magna* 48 h EC_{50} was >102 mg/L and the algal 72 h EC_{50} was >106 mg/L. Three chronic aquatic studies were conducted: in a fish 90-day early life stage study the NOEC was 8.9 mg/L; in a *Daphnia magna* 21-day life-cycle NOEC was 4.2 mg/L; and in an algae 72 h study the NOECr was 106 mg/L. Bioconcentration testing in the common carp, *Cyprinus carpio*, with the acid form of the substance indicated that the bioconcentration factor (BCF) was ≤30.

In a 20-week bobwhite quail dietary reproduction study, the NOEC was >1,000 ppm (84.5 mg/kg/day) and no treatment-related mortalities, overt signs of toxicity, effects on feed or body weight consumption, or effects on reproductive performance were observed at any test concentration. The dietary bioaccumulation factors based on residues in blood, liver, egg membrane, egg yolk, egg albumin, and egg shell were ≤0.01 for all test concentrations.

17.5 Perfluoropolyether (PFPE) Products

Perfluoropolyethers (PFPEs) are a class of fluorinated oligomers whose backbone consists of perfluorinated carbon units such as $-CF_2-$, $-CF_2CF_2-$, and possibly $-CF(CF_3)CF_2-$ separated by oxygen atoms (Sianesi et al. 1994). A proprietary process, involving UV-photo oxidation of TFE with oxygen obtains this fluorinated backbone where the fluorinated carbon units are generally distributed randomly rather than in blocks. A general representation of perfluoropolyether derivatives is: $X-CF_2O-(CF_2CF_2O)_m-(CF_2O)_n-CF_2-X$, where X is a generic functional group. The backbone has a high content of oxygen atoms in its structure, is di-functional and the molecular weight is tunable with an average MW of about 1,500. Since the

repeating units contain only two or three perfluorinated carbon atoms per oxygen atom, their degradation cannot lead to the formation of long-chain substances. Products are marketed as surface treatments for natural stone, metal, glass, plastic, textiles, leather, and paper and paperboard treatment for food-contact applications.

17.5.1 Manufacturing Raw Material

17.5.1.1 PFPE Diol

PFPE diol, $HOCH_2CF_2O(CF_2O)_m(CF_2CF_2O)_nCF_2CH_2OH$, is a manufacturing raw material used to make products (Solvay Fluorolink® and Solvera® products; Solvay Fluorolink® D-10, PFPE Diol, Safety Data Sheet). In acute studies, the oral and dermal LD_{50} were >2,000 mg in rats. The diol was not a skin irritant or an eye irritant in rabbits and was not a skin sensitizer in the guinea pig. The plasma elimination half lives following a single oral dose and single intravenous dose in the rat were 60 and 45 h respectively. In acute aquatic toxicity studies the fish LC_{50} was 100 mg/L, Algae ErC_{50} >100 mg/L and *Daphnia magna* EC50 was >100 mg/L. Additional study details and, or data may be available from the manufacturer.

17.5.2 Product

17.5.2.1 PFPE Phosphate, Ammonium Salt (Solvay Solvera® PT5045, Phosphate ammonium salt, Safety Data Sheet)

The phosphate product, $(HO)_2(O)POCH_2CF_2O(CF_2O)_m(CF_2CF_2O)_nCF_2CH_2OP(O)(OH)_2$ is used in paper applications and has an oral LD_{50} in rat >2,000 mg/kg and a dermal LD_{50} in rat >2,000 mg/kg. The phosphate was not a dermal irritant or eye irritant in rabbits and was not a skin sensitizer in the guinea pig. In addition, the phosphate was negative in the Ames test.

17.6 Fluorinated Oxetane Products

Fluorinated oxetane polymers (oligomers) are found in products such as floor polishes, overprint varnishes, wood stains, automotive clear coats, ink jet inks, electronics coatings, powder/pigment dispersions, adhesives and household cleaners. They are hydrocarbon polyether polyols with fluorinated side chains of controlled chain length. They may be manufactured by substituting a fluorinated

alcohol onto a halogenated methyl oxetane and undergoing ring opening polymerization (Kausch et al. 2002, 2003; Thomas 2006; Omnova 2014). Both the degree of polymerization (molecular weight) and the length of fluorinated side chains can be controlled precisely. They have reactive sites at each end of the molecule and a wide range of end group chemistries have been commercially practiced, including sulfates, alcohols, acids and acrylates. In addition, copolymers with PEO (polyethylene oxide) and PEO-PPO (polyethylene-polypropylene oxide) have been produced to influence solubility and other properties. Current commercially available products grades contain either $-CF_3$ or $-C_2F_5$ side chains. Studies of biodegradation show products are extremely resistant to degradation (Omnova 2014). Additional study details and, or data may be available from the manufacturer.

17.6.1 Manufacturing Raw Material

17.6.1.1 Pentafluoroethyl ($-C_2F_5$) Functionalized Oxetane Diol (Fig. 17.8)

A polyether diol containing $-C_2F_5$ side chains with degree of polymerization (n) of 6 and molecular weight of 2,815 had an oral LD_{50} >2,000 mg in the rat was a minor eye irritant and minor skin irritant (Polyfox™ PF-151N Fluorosurfactant 2003). The product safety data sheet indicated the substance "may cause gastrointestinal irritation." Additional study details and, or data may be available from the manufacturer.

17.7 Short-Chain Perfluoroalkyl Acids (PFAAs)

The family of short-chain perfluoroalkyl acids (PFAAs) includes perfluoroalkyl carboxylic acids (PFCAs) with seven or less carbons ($C_nF_{2n-1}OOH$, $n \leq 7$, e.g., PFHxA n=6) and perfluoroalkyl sulfonic acids (PFSAs) with five or less carbons ($C_nF_{2n+1}SO3H$, $n \leq 5$, e.g., PFBS n=4) (Buck et al. 2011a). In this section, two of the most prominent PFAAs, perfluorohexanoic acid and perfluorobutane sulfonic acid are discussed.

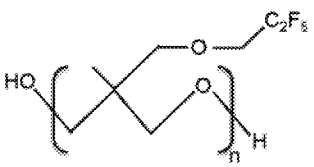

Fig. 17.8 A fluorinated oxetane polymer

17.7.1 Perfluorohexanoic Acid, PFHxA, $C_5F_{11}COOH$

Perfluorohexanoic acid (PFHxA, CASN 307-24-4) is both a degradation product and potential impurity in fluorotelomer-based products and in perfluoroalkane sulfonyl-based electrochemical fluorination products. PFHxA is not generally manufactured and used itself for commercial purposes. PFCAs such as PFHxA were released directly into the environment during the historical manufacture and use per- and poly-fluoroalkyl substances (Prevedouros et al. 2006; Wang et al. 2014a, b). PFHxA is water soluble and persistent in the environment.

17.7.1.1 Acute and Sub-chronic

In an acute oral toxicity study of sodium perfluorohexanoate all rats dosed with 175 or 550 mg/kg survived, whereas one of four rats dosed with 1,750 mg/kg died on the day of dosing, and all three rats dosed with 5,000 mg/kg died on the day of dosing (Loveless et al. 2009). Clinical signs of systemic toxicity were observed in most rats receiving doses from 175 to 5,000 mg/kg.

Several studies have investigated the potential mutagenicity of PFHxA. PFHxA did not generate reactive oxygen species or cause DNA damage in human HepG2 cells (Eriksen et al. 2010), and was found not to be genotoxic based on negative results from both the bacterial reverse mutation assay and the in vitro chromosomal aberration assay (Loveless et al. 2009).

In a 90 day repeated-dose in rats at doses of 0, 20, 100, or 500 mg/kg using sodium perfluorohexanoate, NaPFHx was a moderate inducer of hepatic peroxisomal β-oxidation elevated hepatic β -oxidation levels observed following 1-month recovery in male and female rats at 500 mg/kg/day (Loveless et al. 2009). In another 90-day repeated dose study in rats using perfluorohexanoic acid at doses of 0, 10, 50, and 200 mg/kg/day (Chengelis et al. 2009), a NOAEL of 50 mg/kg/day and 200 mg/kg/day were noted for male and female rats, respectively, based on liver weight increases and hepatocellular hypertrophy (Chengelis et al. 2009). Reversible clinical pathology changes including liver histopathology and weight changes were observed at doses of at least 100 mg/kg/day (Loveless et al. 2009; Chengelis et al. 2009).

17.7.1.2 Development and Reproduction

In a developmental study in rats at doses of 0, 20, 100, or 500 mg/kg using sodium perfluorohexanoate, NaPFHx (Loveless et al. 2009), decreases in maternal and fetal rat weights following 500 mg/kg/day were observed, but no effects on other developmental indices such as fetal, visceral, or skeletal variations were observed at any dose. Reproductive effects were limited to decreases in maternal body weight gains

and F1 pup weights at 500 mg/kg/day. No mortality or effects were observed at any dose on any reproductive indices including mating, fertility, gestation length, number of implantation sites, estrous cyclicity, sperm parameters, litter size, sex ratio, and pup clinical observations. A maternal and developmental NOAEL for developmental toxicity of 100 mg/kg/day was identified. For the reproduction study, the P1 adult rat NOAEL was 20 mg/kg/day, based on reduced body weight parameters, whereas the NOAEL for reproductive toxicity was 100 mg/kg/day, based on reduced F1 pup weights.

The reproductive toxicity potential of ammonium perfluorohexanoate (NH_4PFHx) in mice was investigated (Iwai et al. 2014). Twenty females/group were administered the test substance or vehicle once daily from gestation day 6 through 18. Phase 1 doses: 0, 100, 350, and 500 mg/kg/day; phase 2: 0, 7, 35, and 175 mg/kg/day. Parameters evaluated include mortality, viability, body weights, clinical signs, abortions, premature deliveries, pregnancy and fertility, litter observations, maternal behavior, and sexual maturity in the F1 generation. The level of perfluorohexanoate (PFHx) was measured in the liver of F0 and F1 mice. At doses of 350 and 500 mg/kg/day maternal mortalities, excess salivation and changes in body weight gains occurred. Pup body weights were reduced on postpartum day (PPD) 0 in all the dosage groups, but persisted only in the 350 and 500 mg/kg/day groups. Additional effects at 300 and 500 mg/kg/day included stillbirths, reductions in viability indices, and delays in physical development. PFHx levels in the livers of the 100 mg/kg/day dams were all below the lower limit of quantization (0.02 mg/mL); in the 350 mg/kg/day group, three of the eight samples had quantifiable analytical results. In phase 2, no PFHx was found in the liver. Adverse effects occurred only in the 175 mg/kg/day group and consisted of increased stillborn pups, pups dying on PPD 1, and reduced pup weights on PPD 1. Based on these data, the maternal and reproductive no observable adverse effect level of NH_4PFHx was 100 mg/kg/day.

17.7.1.3 Chronic

A 24-month chronic toxicity study was carried out on rats that received daily (7 days per week) oral doses of 2.5, 15, and 100 mg/kg/day for males and 5, 30, and 200 mg/kg/day for females of perfluorohexanoic acid for 104 weeks (Klaunig et al. 2014). There were no PFHxA-related effects on body weight, food consumption, functional observational battery, hematology, serum chemistry, or hormone parameters. Systemic and local toxicity was observed at the highest dose in males and females. There was no evidence that PFHxA induced tumorigenesis in the 24-month oral gavage study in male or female rats. PFHxA was not carcinogenic at dosages that included maximally tolerated levels. Overall study NOAELs were 15 mg/kg/day for males and 30 mg/kg/day for females.

17.7.1.4 Kinetics

Gannon and colleagues (Gannon et al. 2011) reported that essentially 100 % of the NaPFHx dose was eliminated in urine within 24 h, and that the route and extent of elimination was unchanged after 14 days of daily dosing. PFHx was also not quantifiable in all tissues except skin at time points ranging from 0.5 to 24 h following dosing. The authors noted that though PFHx is present in the environment at levels similar to other PFOA and PFOS, human blood monitoring data indicates much lower (often undetectable) amounts of PFHx. The authors concluded that this finding strongly suggests that humans rapidly eliminate PFHx similarly to rats and mice.

Russell et al. (2013) analyzed biomonitoring data in a cohort of professional ski wax technicians to estimate the apparent half-life of PFHxA in humans. Comparisons were also made with the kinetic studies of PFHxA elimination from mice, rats and monkeys. The apparent elimination half-life of PFHxA in mice, rats, monkeys and humans were shown to be proportional to body weight with no differences observed between genders, indicating similar volumes of distribution and similar elimination mechanisms among mammalian species. The half-lives of PFHxA in mice, rats, monkeys and humans were shown to be proportional to body weight with no differences observed between genders, indicating similar volumes of distribution and similar elimination mechanisms among mammalian species. The authors concluded that the study suggests that results obtained from animal models toxicology studies are therefore suitable for establishment of PFHxA benchmark dose and reference dose hazard endpoints that may be used in human risk assessment.

17.7.1.5 Aquatic

PFHxA has low acute aquatic toxicity (International Corporation 2014; Hoke 2012). In rainbow trout, the 96 h LC_{50} was >99.2 mg/L, the 48 h LC_{50} in *Daphnia* magna was >100 mg/L and the 72 h Eb_{50} in algae was >100 mg/L. In a 28-day, chronic study of effects on early life stages of rainbow trout the chronic NOEC was greater than 10.1 mg/L – the highest tested concentration in the study (Iwai et al. 2012).

17.7.1.6 Bioaccumulation

Numerous studies indicate that PFHxA is not bioaccumulative, does not bioconcentrate or biomagnify in either aquatic ecosystems or air-breathing mammals. Several controlled laboratory and field bioaccumulation experiments with aquatic species indicate that PFHxA is not bioaccumulative (Martin et al. 2003a, b; Conder et al. 2008; Rayne et al. 2009; Yeung and Mabury 2013). Recent studies discussed toxicokinetics in mammals showing rapid elimination in rats, mice, monkeys and humans with elimination half-lives proportional to body weight with no difference between genders (Russell et al. 2013).

17.7.2 Perfluorobutane Sulfonate, PFBS

Potassium perfluorobutane sulfonate (K-PFBS, CAS 29420-49-3) is a commercial product. In addition, PFBS is a degradation product and potential impurity in perfluoroalkane sulfonyl-based electrochemical fluorination products (Buck et al. 2011a). PFSAs such as PFBS were released directly into the environment during the historical manufacture and use per- and poly-fluoroalkyl substances (Buck et al. 2011a).

A comprehensive summary of PFBS physical-chemical property and hazard data is available (Australian Government Department of Health and Aging (NICNAS) 2005). The reader is directed toward this compilation as well as more recent publications (Olsen et al. 2009; Lieder et al. 2009a, b; Newsted et al. 2008; Giesy et al. 2010) for detailed information. A summary is provided here.

PFBS is highly water soluble (42 g/L) and exists in its anionic form in aqueous medium. PFBS is persistent. PFBS has low acute mammalian oral and dermal toxicity, is not irritating to the skin, is irritating to the eye and was not a skin sensitizer. Pharmacokinetic studies in rats, monkeys and humans show PFBS rapid elimination (Olsen et al. 2009). In a 90 day oral gavage study (Lieder et al. 2009b), male and female rats were dose at 0, 60, 200 and 600 mg/kg/day. The 200 and 600 mg/kg/day doses in male rats were associated with increased adverse clinical observations and reductions in red blood cells, hemoglobin concentration and hematocrit. The NOAEL for male rat was 60 mg/kg/day. The NOAEL for female rat was 600 mg/kg/ day.

In a two-generation reproduction study (Lieder et al. 2009a), parental-generation (P) rats were dosed orally by gavage with 0, 30, 100, 300 and 1,000 mg K-PFBS for 10 weeks prior to and through mating (males and females), as well as during gestation and lactation (females only). First generation (F1) pups were dosed similarly, beginning at weaning. Second generation (F2) pups were not directly dosed but potentially exposed to PFBS through placental transfer and nursing, and the study was terminated 3 weeks after their birth. The NOAEL in the parental generations (P and F1) was 100 mg/kg/day. In the 300 and 1,000 mg/kg/day dose group rats, there were (1) increased liver weight (absolute or relative) and corresponding increased incidence of adaptive hepatocellular hypertrophy (male only) and (2) increased incidence of minimal to mild microscopic findings in the medulla and papilla of the kidneys (male and female). There were no K+PFBS treatment-related effects on fertility or reproduction among the P or the F1 rats. There were no microscopic changes in male or female reproductive organs, and no biologically relevant effects on sperm parameters, mating, estrous cycles, pregnancy, and natural delivery in the P- or F1-generations. There were no K+PFBS treatment-related effects on survival of pups in the two-generation study. Litter size and average pup birth weight per litter were not statistically significantly different from controls in any dose group. In the F1-generation, terminal body weight was reduced in males at 1,000 mg/kg/day. Preputial separation was slightly delayed (approximately 2 days) at this dose, a finding consistent with the body weight reduction. Essentially no effects were observed

in the F1 females. F2 pups had normal bodyweights. The reproductive NOAEL was >1,000 mg/kg/day in both generations.

PFBS is not bioaccumulative or toxic to aquatic organisms (Australian Government Department of Health and Aging (NICNAS) 2005; Giesy et al. 2010). In the bluegill, the 96 h LC_{50} was 6,452 mg/L, and the NOEC was 6,452 mg/L. In the fathead minnow, the 96 h LC_{50} was 1,938 mg/L, and the NOEC was 888 mg/L. For *Daphnia magna*, the 48 h LC_{50} was 2,183 mg/L, and the NOEC was 886 mg/L and in a 21 day study the chronic NOEC was 707 mg/L. These data indicate that PFBS is not very toxic to freshwater organisms, with effect levels only being observed at a concentration greater than 700 mg/L.

Additionally, PFBS acute and chronic toxicity studies in Mallard and Northern Bobwhite Quail have been reported (Newsted et al. 2008). In acute dietary studies with juvenile mallards and northern bobwhite quail, 10-day-old mallards and quail were exposed to 1,000, 1,780, 3,160, 5,620 or 10,000 mg/kg feed, wet weight (ww) for 5 days and the birds were then fed an untreated diet and observed for up to 17 days. No treatment-related mortalities were observed in the study up to 10,000 mg/kg, ww feed. Body weight gains of quail exposed to 5,620 or 10,000 mg/kg feed were statistically less than that of unexposed controls. Weight gain of mallards exposed to 10,000 mg PFBS/kg feed was statistically less than that of controls. There were no statistically significant effects on feed consumption of either species. The no observed adverse effect concentration (NOAEC) for mallards and quail were 5,620 and 3,160 mg/kg, ww feed, respectively. In a reproduction study, adult quail were exposed to nominal dietary concentrations of 100, 300, or 900 mg/kg, ww feed for up to 21 weeks. There were no treatment-related mortalities or effects on body weight, weight gain, feed consumption, histopathology measures, or reproductive parameters evaluated in the study when compared to the control group. The dietary NOAEC was 900 mg/kg, ww feed.

17.8 Summary and Future Prospects

Per- and polyfluoroalkyl substances have unique performance attributes and must be manufactured, handled, used and disposed of responsibly. The widespread presence of long-chain perfluoroalkyl acids has led to a move to alternative short-chain fluorinated substances which have more favorable environmental and biological properties, most notably rapid elimination from living systems. Data for some of these alternatives has been presented here. It is incumbent upon the manufacturers of alternatives, fluorinated and otherwise, to conduct appropriate studies to assess the hazards of their products according to how they are handled, used and disposed of in the product life-cycle. Moreover, with heightened awareness and concern from a wide array of stakeholders regarding what is known about alternatives to long-chain substances, manufacturers need to consider communicating the results of these studies not only in their product safety data sheets but also in the scientific literature and scientific forums.

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Chapter 18
Conclusions and Recommendations

Jamie C. DeWitt

Abstract The chapters in this book have addressed human and wildlife exposure/body burdens, reviews of metabolism and toxicological effects by organ system/developmental stage, and aspects of PFAS toxicity that are driving PFAS research and regulatory oversight. The intent of arranging this book by these three themes was to emphasize the challenges associated with determining exposure concentrations, the multiple systems and processes that have toxicological responses following exposure to PFASs, and complexities associated with their regulatory oversight. The goal of this brief, concluding chapter is to highlight some of the lingering issues related to PFAS research.

Keywords PFAS exposure • PFAS body burdens • PFAS toxicity • PFAS regulatory oversight

18.1 Exposure Considerations and Body Burdens

The first chapter of this book introduced the topic of PFAS toxicity and asked why these compounds have drawn such immense interest for investigation. As the author of this chapter so eloquently stated "novel discoveries often lead to additional queries that require further investigation." The relatively recent discovery of these compounds in environmental media, including tissues and fluids of humans and wildlife, prompted deeper research into their environmental distribution and their potential toxicological effects in exposed organisms. While we have learned a great deal about the toxicity of PFASs over the past decades, this introductory chapter concluded that much is left to uncover, including the reasons behind their persistence in humans, how to predict human effects from experimental animal models, and the potential toxicities of replacement compounds. The next four chapters

J.C. DeWitt (✉)
Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC 27834, USA
e-mail: DEWITTJ@ecu.edu

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0_18

ED_002330_00119699-00484

summarized exposure studies to determine concentrations in the general human population, highly exposed human populations, and wildlife species. A chapter on measurement of these compounds in biological tissues and fluids also was included to emphasize the challenges of detecting compounds that are pervasive in products used daily not only in residences and workplaces, but in the very scientific equipment used to evaluate their presence in biological samples. These chapters highlighted the value of biomonitoring efforts to estimate internal doses in humans and wildlife and to identify potentially susceptible subpopulations and correlating identifiable environmental and occupational exposures with potential health outcomes. All of these chapters concluded that additional efforts are necessary to fully appreciate the relationships between exposure and toxicological outcomes, not only of the high chain PFASs, but for the shorter chain compounds that are likely going to predominate as replacement compounds.

18.2 Toxicological Effects

The middle section of this book summarized the myriad toxicities that have been reported following exposure to PFASs, mainly in experimental animal models, but also in humans from higher exposed populations and the general population. These chapters covered metabolism of PFASs by biological organisms, how PFASs impact metabolic processes, development, and the three main controlling systems (endocrine, immune, and nervous systems). Also included were chapters on evidence of carcinogenesis and reported health effects obtained from epidemiological studies of various human populations. This section of the book illustrated that PFASs have the potential to disrupt homeostatic processes that underlie every system in the body, including the body as it is developing. Because PFASs can be classified as *multi-system toxicants* as well as developmental toxicants, this complexity increases the challenge to establishing appropriate health and environmental guidelines.

Perhaps the most salient issue associated with the toxicity of PFASs is that the basic mechanism of toxic action has not been identified with any certainty. Activation of the peroxisome proliferator activated receptor alpha (PPARα) is an accepted mechanism for some types of toxicities, notably those involved in the PFOA-induced "tumor triad" discussed in detail in the chapter on carcinogenic effects of PFASs and for the developmental toxicity of PFOA discussed in the developmental toxicity chapter. However, for other types of PFASs and for humans, the relevance of this mechanism is still not known with certainty. Other modes and mechanisms of toxicity have been hypothesized, including endocrine disruption, oxidative stress stemming from the generation of reactive oxygen species and associated mitochondrial dysfunction, and disruption of homeostatic pathways involving other receptors and signals. These other modes and mechanisms are discussed in detail in individual chapters, but the resounding message across chapters is that additional investigation is necessary to fully understand how PFASs exert their toxic effects. Until these

studies are performed and definitive data are produced, establishing appropriate health and environmental guidelines will remain challenging.

18.3 Drivers of Research and Regulatory Oversight

The final chapters of this book addressed some of the "bigger picture" issues associated with understanding the toxicity of PFASs, including relating tissue dosimetry to toxicological effects in that tissue, the applicability of the PFASs dataset to hazard identification, how to assess the risks of PFASs, and ultimately, the ability of the industrial community to create suitable replacement compounds. These final chapters demonstrate that while the toxicological database of PFASs has grown exponentially (see Fig. 18.1) over the past several decades, much uncertainty still surrounds our understanding of these compounds and how to reconcile their use with their potential toxicities. Of particular concerns for evaluating the potential health risks of PFASs is determining (a) What systems are most vulnerable to exposures and at what concentrations? (b) Does the vulnerability of systems change when exposure occurs during development? (c) Do system and life stage vulnerability differ dramatically among species and/or strains? (d) can we develop appropriate dose metrics to understand pharmacokinetic elimination kinetics among species and

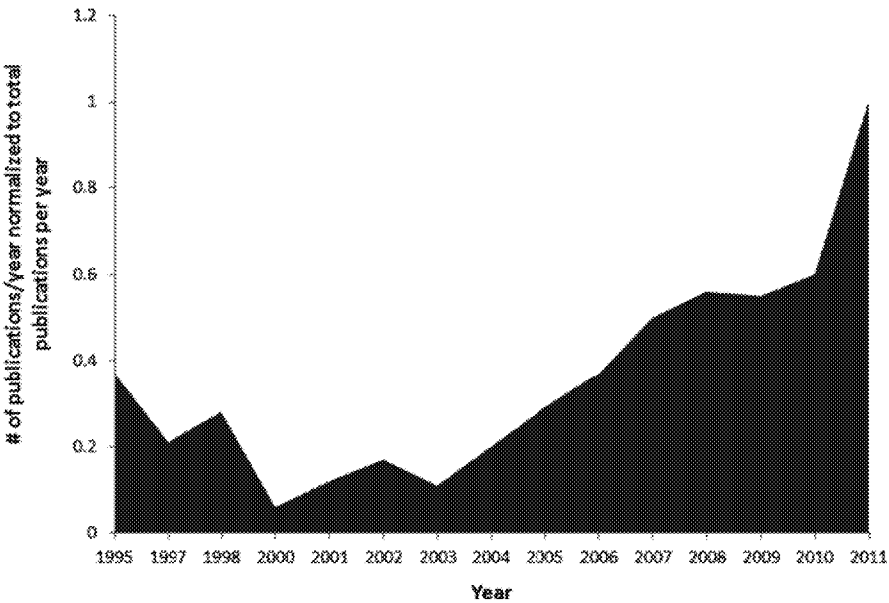

Fig. 18.1 Number of publications (normalized to the last full publication year for all results) related to the risk assessment of PFASs (Data from eTBLAST (eTBLAST: a text-similarity based search engine; http://etest.vbi.vt.edu/etblast3/)

to understand the relatively long half life in humans? (e) What toxicological endpoints are most appropriate for protection of public health and what point of departure should be used for extrapolation to lower doses? And perhaps most importantly, as pointed out in the chapter on the toxicity of alternatives, the manufacturers of PFASs are responsible for conducting appropriate studies to assess the hazards of their products according to how they are handled, used and disposed of in the product life-cycle and for communicating the results of these studies to both the general public and scientific communities.

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J.C. DeWitt (ed.), *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology,
DOI 10.1007/978-3-319-15518-0

ED_002330_00119699-00488

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