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REVIEW

Perfluoroalkyl Acids: A Review of Monitoring and Toxicological Findings

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In recent years, human and wildlife monitoring studies have identified perfluoroalkyl acids (PFAA) worldwide. This has led to efforts to better understand the hazards that may be inherent in these compounds, as well as the global distribution of the PFAAs. Much attention has focused on understanding the toxicology of the two most widely known PFAAs, perfluorooctanoic acid, and perfluorooctane sulfate. More recently, research was extended to other PFAAs. There has been substantial progress in understanding additional aspects of the toxicology of these compounds, particularly related to the developmental toxicity, immunotoxicity, hepatotoxicity, and the potential modes of action. This review provides an overview of the recent advances in the toxicology and mode of action for PFAAs, and of the monitoring data now available for the environment, wildlife, and humans. Several avenues of research are proposed that would further our understanding of this class of compounds.

Key Words: **Perfluoroalkyl acids; PFOS; biomonitoring.**

The perfluoroalkyl acids (PFAA) are a family of perfluorinated chemicals that consist of a carbon backbone typically 4–14 in length and a charged functional moiety (primarily carboxylate, sulfonate, or phosphonate). The two most widely known PFAAs contain an eight-carbon backbone and include perfluorooctanoic acid (PFOA) and perfluorooctane sulfate (PFOS). It should be noted that PFAAs are different from another class of perfluorocarbons, the perfluoroalkanes, which are primarily used clinically for oxygenation and respiratory ventilation. PFAAs are relatively contemporary chemicals, being in use only in the past half century, and until recently, have been considered biologically inactive.

In recent years there has been a great deal of research to understand the toxicological effects, and distribution of this class of compounds in the environment, wildlife, and humans. Several reviews have been written on the general toxicology of PFOA (Kennedy *et al.*, 2004) and the developmental toxicity of PFAAs (Lau *et al.*, 2004). However, there has been substantial progress in understanding additional aspects of the toxicology of these compounds, particularly related to the potential modes of action. The purpose of this review is to provide an overview of the monitoring data available for the environment, wildlife, and humans, as well as recent advances in the toxicology and mode of action for this class of compounds.

GENERAL BACKGROUND

Carbon-fluorine bonds are among the strongest in organic chemistry. Fully fluorinated hydrocarbons are stable in air at high temperatures (in excess of 150°C), nonflammable, not readily degraded by strong acids, alkalis, or oxidizing agents, and are not subject to photolysis; although recent evidence indicated that PFAAs might be decomposed by zero-valent iron in subcritical water (Hori *et al.*, 2006), or by irradiation and use of persulfate (Chen and Zhang, 2006). Nonetheless, the stability of these chemicals renders them practically non-biodegradable and persistent in the environment (Key *et al.*, 1997, 1998; Presher *et al.*, 1985). The fluorine moiety of PFAAs provides extremely low surface tension and contributes to their unique hydrophobic and oleophobic nature.

Naturally occurring fluorinated organic compounds are rare. PFOA, PFOS, and other PFAAs are man-made chemicals (Lehmler, 2005) that are primarily produced by two methods. The Simons Electrochemical Fluorination process is based on the reaction between an organic feedstock, such as

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1-octanesulfonyl fluoride ($C_8H_{17}SO_2F$) for PFOS or 1-heptanecarbonyl fluoride for PFOA, and anhydrous hydrogen fluoride. These reactions are fueled by an electrical current causing all the hydrogen atoms on the carbon backbone to be replaced by fluorine atoms. Resultant products, such as perfluorooctane sulfonyl fluoride (POSF, $C_8F_{17}SO_2F$), are formed. During this process, fragmentation and rearrangement of the carbon skeleton can occur producing fluorinated molecules of various carbon chain lengths and a mixture of linear, branched, and cyclic isomers. A lack of isomeric purity is a signature of this production process. An alternative production method involves telomerization of tetrafluoroethylene units that always yields straight-chain alcohols ($F(CF_2CF_2)_nCH_2CH_2OH$) that can be converted into final products for commercial application. Thus, PFAAs found in the environment are composed of a family of target compounds as well as by-products of various chain lengths and isomers.

The physical properties of PFAAs render these chemicals ideal surfactants (Kissa, 2001). Although all PFAAs share some surfactant properties, the eight-carbon chemicals are most effective. POSF and telomer-related products are found in over 200 industrial and consumer applications, ranging from water-, soil-, and stain-resistant coatings for clothing fabrics, leather, upholstery, and carpets, to oil-resistant coatings for paper products approved for food contact, electroplating, electronic etching bath surfactants, photographic emulsifiers, aviation hydraulic fluids, fire-fighting foams, paints, adhesives, waxes, and polishes (Renner, 2001; Seacat *et al.*, 2002). PFOA is used as an emulsifier in the production of polytetrafluoroethylene as well as other fluoropolymers and fluoroelastomers.

Historically, the production and use of PFOS (3500 metric tons in 2000) dwarfed those of PFOA (estimated at 500 metric tons). However, because the major manufacturer of PFOS, 3M, phased out production in 2002, the global production of this chemical dropped precipitously to 175 metric tons by 2003 (3M Company, 2003). In contrast, the global production of PFOA escalated to 1200 metric tons per year by 2004 and has presumably become the most common PFAA in commerce. In 2006, the U.S. Environmental Protection Agency initiated the PFOA Stewardship Program in which the eight major companies in the industry have committed to reduce facility emissions and product contents of PFOA and related chemicals on a global basis by 95% no later than 2010. A further goal of this program is to work toward eliminating emissions and product content of these chemicals by 2015 (U.S. EPA, 2006). However, it is anticipated that other PFAA products will be developed to fill the commercial void.

MONITORING STUDIES

Extensive amount of data have recently become available describing concentrations of PFAAs in environmental media, wildlife, and human tissues in many different geographic locations throughout the world. In general, the sample sizes reported have been small, but the number of reports is ever-increasing. In addition, various laboratory techniques have been used to analyze the PFAA samples. It is difficult to compare or interpret PFAA concentrations in biological matrices across the globe without internationally representative samples and standardized analytical methods. In some cases, wide ranges of values have been reported. It is impossible to determine whether this variability is due to different analytical techniques or truly marked exposure differences.

Since data quality improvement was needed in the analysis of perfluorinated compounds, the first worldwide interlaboratory study was conducted (Martin *et al.*, 2004a; Van Leeuwen *et al.*, 2006). The participants included 38 laboratories from 13 countries, and each laboratory analyzed 13 PFAAs in three environmental and two human samples. There was approximately 65% concordance among laboratories for PFOS and PFOA in both human blood and plasma; however, other PFAAs did not fare as well. Most laboratories underestimated PFAA concentrations in fish extracts due to matrix effects. The results for the fish tissue and water were also poor. It was concluded that additional work is needed to improve the analytical techniques for all matrices. A second interlaboratory study is currently underway.

PFAAs in the Environment

Although some uncertainty in the reliability of the reported levels remains, PFOA, along with PFOS and other PFAAs, have been detected in a variety of environmental matrices from around the globe. These include surface waters, air, sludge, soils, sediments, and ice caps. For example, PFOA and PFOS have been detected in the Tennessee River downstream from a fluorochemical manufacturing plant, in drinking water sources near production plants in West Virginia and Germany, in the Great Lakes, in rain water from an urban center in Canada, in coastal waters in south China, Japan, and Korea, and in river water samples collected from tributaries of the Yangtze and Pearl Rivers in China (Boulanger *et al.*, 2004; Emmett *et al.*, 2006a; Hansen *et al.*, 2002; Loewen *et al.*, 2005; Saito *et al.*, 2004; Skutlarek *et al.*, 2006; So *et al.*, 2004, in press). PFOS, perfluorohexane sulfonate (PFHS), and perfluorobutane sulfonate (PFBS) have been detected in surface sea water and fresh water samples from a number of cities in Japan (Taniyasu *et al.*, 2003). Typically, the levels of PFOA were in the parts per trillion range, although concentrations found in West Virginia tended to be higher, at about 3.5 parts per billion (ppb) (Emmett *et al.*, 2006a). Even higher levels of PFOS, PFOA, and PFHS (ranging from ppb to parts per million [ppm]) were reported after an accidental release of fire-fighting foam that subsequently entered Etobicoke Creek, a tributary of Lake Ontario (Moody *et al.*, 2002).

In ambient air, particulates containing PFOA have been detected, ranging from 0.07–0.9 ng/m^3 among different Japanese

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cities (Harada *et al.*, 2006b), to 0.12–0.9 μg/m^3 in a sampling area near a fluoropolymer manufacturing facility in the United States (Barton *et al.*, 2006). Two PFOS precursors, N-ethyl-perfluorooctane-sulfonamido ethanol (N-EtFOSE) and N-methyl-perfluorooctane-sulfonamido ethanol, have been measured in the air in Canada (Martin *et al.*, 2002). In addition, PFOA, PFOS, and PFHS have been detected in indoor dust samples from Canadian homes, averaging about 100, 450, and 400 ppb (ng/g), respectively (Shoeib *et al.*, 2005).

In soils, sediment, waste water, and sludge, PFOS and PFOA have been detected in various countries (3M Company, 2001, 2003; Boulanger *et al.*, 2005; DuPont, 2003a, 2005; Higgins *et al.*, 2005; Schultz *et al.*, 2006; So *et al.*, 2004; Wang *et al.*, 2005a; Yamishita *et al.*, 2004). Dry soil measurements near a fluorochemical manufacturing plant in West Virginia revealed PFOA at levels ranging from below the detection limit of 0.017–700 ng/g. PFOA was also detected in soils at 2.87 ng/g 2 months after fire-fighting foams were used to extinguish fires at major oil storage facilities following an earthquake in southern Japan. PFOA, PFOS, and many other PFAA intermediates have been found in San Francisco Bay area sediments and sludge in the ng/g range. Certain Nordic countries, the Netherlands, and the United States have all reported measurements of PFOA in sediment samples in the ng/g range. PFOA has also been detected in sewage sludge and effluent near a manufacturing plant in the United States, in sites far from manufacturing sites in the Nordic countries, at a water disposal site in Japan, at an urban-industrial wastewater treatment plant in Spain, and in waste water from various manufacturing plants in Austria (3M Company, 2001; Alzaga and Bayone, 2004). In remote regions of the High Arctic ice caps, PFOA, and several other PFAA intermediates have been detected in concentrations ranging from the low- to mid-pg/l range (Young *et al.*, 2007).

PFOS and PFOA are the final degradation products of a host of precursor perfluorinated chemicals and several of the precursors are considered volatile. Available evidence suggests the transformation or biodegradation of precursor molecules occurs by both abiotic and biotic biodegradation pathways (Dinglasan *et al.*, 2004; Ellis *et al.*, 2001; Lange, 2002; Wang *et al.*, 2005a,b). Water is expected to be the primary environmental compartment where PFOS and PFOA are found. Adsorption to soils, sediments, and sludge is uncertain, but expected to be limited based on their physical–chemical properties. PFOS or PFOA found in these matrices is probably associated with the water phase and/or weakly adsorbed by electrostatic interactions (Gannon *et al.*, 2006). Nonetheless, Higgins *et al.* (2005) have recently provided a quantitative assessment of PFAAs in sediments and domestic sludge in the United States, and found them to be in the ng/g range.

Our understanding of the environmental fate of PFOS, PFOA, and PFAA intermediates, including their sources, potential for biotransformation, as well as transport and distribution mechanisms is only in its infancy. Because of the popular uses of PFOA and PFOS in consumer and industrial products, it is not surprising to find these pollutants in urban areas. On the other hand, the detection of these perfluorochemicals in remote regions of the world is quite unexpected. Recently, two theories have emerged for the fate and transport of these chemicals around the world (Prevedouros *et al.*, 2006). The first is long range transport by oceanic currents (Yamashita *et al.*, 2005) which is supported by the finding of parts per quadrillion (pg/l) of PFAAs in the surface water of the Atlantic and Pacific Oceans, South China Sea, Sulu Sea, and the Labrador Sea, with PFOA being the major PFAA detected followed by PFOS. In this study, concentrations of PFAA were reported to decrease by two to four orders of magnitude from coastal waters to offshore, with traces of PFOA and PFOS detected in deep sea water. The second theory involves atmospheric transport and transformation of precursor chemicals (D'Eon *et al.*, 2006; Ellis *et al.*, 2003, 2004; Martin *et al.*, 2006; Stock *et al.*, 2004; Young *et al.*, 2007). While the volatility of PFOA and PFOS is nominal, that of their precursors and derivatives is high at normal temperature and pressure. According to one model, perfluorinated telomer alcohols (TA) have been found in the North American troposphere (11–165 pg/m^3), and their estimated atmospheric lifetime of 10–20 days is sufficient to account for the widespread hemispheric distribution (Wallington *et al.*, 2006). These precursors of PFOA and PFOS can be oxidized by hydroxyl radicals in the atmosphere, thereby providing an explanation for the presence of these anthropogenic chemicals in remote locales (3M Company, 2000; Ellis *et al.*, 2004; Young *et al.*, 2007). Of the precursors that comprise the fluorotelomer-based polymers, the 8–2 TA ($F(CF_2CF_2)_4CH_2CH_2OH$) has been the most studied (Dinglasan *et al.*, 2004; Ellis *et al.*, 2004; Hurley *et al.*, 2004; Lange, 2002; Martin *et al.*, 2004b; Wang *et al.*, 2005a,b). Longer-term abiotic and biotic degradation studies for fluorotelomer-based polymeric products in soils, sediments, and sludge are currently being undertaken to better understand the fate of these compounds in the environment.

Transport of PFOA from a point source was recently investigated at a fluoropolymer manufacturing site in the West Virginia and Ohio border areas (Davis *et al.*, 2007). Environmental media including air, water, and soil were monitored, and ground water flow and air dispersion models were applied. Available data suggested that PFOA emitted in air from the production site was transported by wind to the nearby well fields, deposited onto the surface soils, and then migrated downward with precipitation into the underlying aquifer. Extension of such modeling and surveying efforts to other PFAAs will be instrumental to our understanding of the geographic distributions of these chemicals.

PFAAs in Wildlife

A summary of the concentrations of PFAAs in serum or plasma of wildlife is presented in Table 1. As mentioned above,

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there are uncertainties associated with these levels due to variations in analytical techniques. In addition, multiple uncertainties exist since most of the samples were collected for other purposes and stored frozen for up to 30 years with little standardization in analytical procedures, statistics, and reporting.

Giesy and Kannan (2001) reported global distribution of PFOS in frozen wildlife liver and blood samples. Since that time, thousands of samples of biota have been analyzed for PFOS, PFOA, and other PFAAs. Recent reviews include Gannon *et al.* (2006), and Houde *et al.* (2006a, PFAAs globally); Environment Canada (2006, PFOS and related chemicals in Canada); and Kallenborn *et al.* (2004, PFAAs in the Nordic environment). In general, the highest concentrations of PFAAs have been found in the livers of fish-eating animals living near more industrialized areas. In North America, surveys found highest liver concentrations in mink, bottle-nosed dolphins, polar bear, ringed seal, and Brandt's cormorant (Bossi *et al.*, 2005a,b; De Silva and Mabury, 2004; Giesy and Kannan, 2001; Kannan *et al.*, 2001a,b, 2002a). In Europe, highest liver concentrations were reported for ringed seal, eels, and cod (Falandysz *et al.*, 2006; Hoof *et al.*, 2005b; Kannan *et al.*, 2002b). In Asia, highest liver concentrations were reported for the common cormorant (Kannan *et al.*, 2002c). Extremely high liver concentrations of PFOS were reported for a population of field mice living near a fluorochemical plant in Belgium (Hoff *et al.*, 2004). PFAAs have also been detected in organisms from the remote areas of the Arctic (De Silva and Mabury, 2004; Kannan *et al.*, 2001b; Martin *et al.*, 2004b,c; Smithwick *et al.*, 2005; Tomy *et al.*, 2004) and Antarctic (Giesy and Kannan, 2001; Tao *et al.*, 2006).

The pattern of contamination in biota is complex and varies among species and locations suggesting multiple emission sources. When analyses of multiple PFAAs are performed, PFOS is usually the highest concentration, except in some urban or industrial areas where PFOA may have local sources (Houde *et al.*, 2005). There are some reports providing evidence of bioaccumulation/biomagnification in top fish-eating predators (Houde *et al.*, 2006b; Tomy *et al.*, 2004; Verreault *et al.*, 2005). Others reported a pattern of temporal changes (both increases and decreases), mainly in liver concentrations (Bossi *et al.*, 2005b; Holmström *et al.*, 2004; Smithwick *et al.*, 2006). However, there is no clear pattern of increased PFAA tissue concentration with age of the organism (Houde *et al.*, 2005; Smithwick *et al.*, 2005, Van de Vijver *et al.*, 2005).

PFAAs in Humans

Human biomonitoring of the general population for PFAAs in various countries around the world began in 2000, while occupational populations have been monitored for much longer periods of time. In most cases, workers occupationally exposed have serum levels of both PFOA and PFOS approximately one order of magnitude higher than those reported in the general population. Most peer-reviewed literature contains reports of PFAAs detected in blood (whole blood, plasma and serum), from individual samples, although, some pooled samples were used. A limited number of papers have also reported PFAAs detected in breast milk, liver, seminal plasma, and umbilical cord blood (Apelberg *et al.*, 2007; Guruge *et al.*, 2005; Inoue *et al.*, 2004; Kärrman *et al.*, 2007; Kuklenyik *et al.*, 2004; Midasch *et al.*, 2007; Olsen *et al.*, 2003c; So *et al.*, 2006). Samples have been collected in countries worldwide including the United States, Japan, Canada, Peru, Colombia, Brazil, Italy, Poland, Germany, Belgium, Sweden, India, Malaysia, Korea, China, and Australia. In most cases, the analytes most often detected in human matrices, and usually in the highest concentrations, were PFOS, PFOA, and PFHS. Tables 2 and 3 present the concentrations of these chemicals in human populations in North America and worldwide, respectively. These tables are expanded from Butenhoff *et al.* (2006).

Other PFAAs detected in human tissue include perfluorooctane sulfonamide (PFOSA), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH or PFOSAA), perfluoroheptanoic acid, perfluorononanoate (PFNA), perfluorodecanoic acid (PFDeA or PFDA), perfluoroundecanoic acid (PFUA), perfluorododecanoic acid, perfluoropentanoic acid, perfluorohexanoic acid, and PFBS. Table 4 summarizes published concentrations of some of these analytes.

Various researchers have reported their results for PFAAs in whole blood, plasma, and serum. Most of these studies have assumed a 1:1 ratio between serum and plasma concentrations and have converted whole blood measurements to serum by doubling whole blood concentrations (Kannan *et al.*, 2004; Kuklenyik *et al.*, 2004). Two recent studies have compared the blood ratios in the various matrices and reported different results (Ehresman *et al.*, 2007a; Karrman *et al.*, 2006a). Ehresman *et al.* (2007a) reported mean serum or plasma to whole blood ratios that were 2.3–2.5 for PFHS, 2.2–2.4 for PFOS, and 2.0–2.1 for PFOA, whereas, mean plasma to whole blood ratios for PFOS, PFOA, PFHS, and PFNA in another study were described as 1.2, 1.4, 1.2, and 1.1, respectively (Karrman *et al.*, 2006a). Resolution of this issue will thus require additional work.

Based on the limited data currently available, it is clear that there are a small number of individuals who have been exposed at levels much higher than the majority of the population. Recent data indicate that individuals living near a U.S. facility that uses PFOA may have much higher PFOA serum concentrations than those currently reported for the general population (Calafat *et al.*, 2007; Emmett *et al.*, 2006a). Very little data are available on trends of PFAAs in human populations. Data from National Health and Nutrition Examination Survey of 1999–2000 have recently become available

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TABLE 1
PFOS, PFOA and PFHS Serum or Plasma Concentrations (ng/ml)—Ranges in Wildlife

Species	PFOS range	PFOA range	PFHS range	References
North America				
Ringed Seals	< 3–12			Giesy and Kannan, 2001
Gray Seals	11–49			Giesy and Kannan, 2001; Kannan *et al.*, 2001b
Northern Fur Seal	< 6–12			Kannan *et al.*, 2001b
Stellar Sea Lion	< 6			Kannan *et al.*, 2001b
Polar Bear	26–52			Kannan *et al.*, 2001b
Kemp's Ridley Sea Turtle	13.8–60.2	2.77–4.25	0.12–0.38	Keller *et al.*, 2005
Loggerhead Sea Turtle	1.4–96.8	0.5–8.14	< 0.05–1.08	Keller *et al.*, 2005
Snapping Turtle	< 1–170	< 2.5	< 1	Giesy and Kannan, 2001; Kannan *et al.*,. 2005a
Bald Eagles	< 1–2570			Giesy and Kannan, 2001; Kannan *et al.*,. 2001a
Herring Gull	66–450			Giesy and Kannan, 2001; Kannan *et al.*,. 2001a
Double Crested Cormorant	1–430			Giesy and Kannan, 2001; Kannan *et al.*,. 2001a
Europe				
Ringed Seals	5–242			Giesy and Kannan, 2001; Kannan *et al.*, 2001b
Gray Seals	14–76			Giesy and Kannan, 2001; Kannan *et al.*, 2001b
Eider Duck	12–38[a]	0.06–0.1[a]	0.4–2.9[a]	Falandysz *et al.*, 2006
Great Tit	24–1625[a]			Dauwe *et al.*, 2007
Cod	6.1–52[a]	0.05–0.7[a]	0.05–0.8[a]	Falandysz *et al.*, 2006
Asia and Pacific Ocean				
Black-tailed Gull	2–12			Giesy and Kannan, 2001
Laysan and Black-footed Albatross	0.3–39	< 0.1–0.3		Giesy and Kannan, 2001; Kannan *et al.*, 2001a; Tao *et al.*, 2006
Carrion Crow	11–150[a]		< 1[a]	Taniyasu *et al.*, 2003
Mallard	130[a]		9[a]	Taniyasu *et al.*, 2003
Pintail Duck	84–167[a]		6–20[a]	Taniyasu *et al.*, 2003
Common Sea Bass	30–146[a]		3.6–4[a]	Taniyasu *et al.*, 2003
Conger Eel	489[a]		121[a]	Taniyasu *et al.*, 2003
Flatfish	74–194[a]		28–38[a]	Taniyasu *et al.*, 2003
Japanese Stingfish	2–488[a]		< 2–4[a]	Taniyasu *et al.*, 2003
Rockfish	63–176[a]		2–5[a]	Taniyasu *et al.*, 2003
Black Seabream	29–31[a]		< 3–< 5.5[a]	Taniyasu *et al.*, 2003
Japanese Scad	7–238[a]		< 2.7–1[a]	Taniyasu *et al.*, 2003
White Croaker	33–50[a]		< 2.8[a]	Taniyasu *et al.*, 2003
Black Scraper	31–35[a]		< 5.5[a]	Taniyasu *et al.*, 2003
Filefish	33–66[a]		< 5.5[a]	Taniyasu *et al.*, 2003
Gizzard Shad	23[a]		< 5.5[a]	Taniyasu *et al.*, 2003
Redlip Mullet	23–47[a]		1[a]	Taniyasu *et al.*, 2003
Red Seabream	17–41[a]		< 5.5[a]	Taniyasu *et al.*, 2003
Yellowfin Seabream	27[a]		< 5.5[a]	Taniyasu *et al.*, 2003
Lefteye Flounder	7–50[a]		< 0.7–11[a]	Taniyasu *et al.*, 2003
Giant Trevally	15–24[a]		< 1.9[a]	Taniyasu *et al.*, 2003
Ornate Jobfish	4–21[a]			Taniyasu *et al.*, 2003
Perth Herring	1[a]		< 4.4[a]	Taniyasu *et al.*, 2003
Yellowfin Tuna	1[a]		< 2.2[a]	Taniyasu *et al.*, 2003
Blue Gill	455–834[a]		< 4.3[a]	Taniyasu *et al.*, 2003
Largemouth Bass	317–322[a]		< 4.3[a]	Taniyasu *et al.*, 2003
Carp	68–77[a]		< 4.3[a]	Taniyasu *et al.*, 2003
Antarctica				
Elephant Seal	< 0.08–3.5[a]			Tao *et al.*, 2006
Adelie Penguin	< 0.1[a]			Tao *et al.*, 2006
Polar Skua	< 1–1.4			Giesy and Kannan, 2001; Tao *et al.*, 2006

[a]Whole blood only. Taniyasu *et al.*, 2003; Falandysz *et al.*, 2006; Tao *et al.*, 2006; Dauwe *et al.*, 2007.

and will provide the baseline data from which future trends can be measured in the United States (Calafat *et al.*, 2007). These data generally confirm the results reported from smaller studies done in the United States (see Table 2). However, the results also indicate that males have higher average concentrations of PFOS, PFOA, and PFHS than females in the United States and that Mexican Americans have lower mean serum concentrations for these three compounds than non-Hispanic whites

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TABLE 2
PFOS, PFOA and PFHS Concentrations (ng/ml) in North American Human Populations

Location	Demographic	Sample type	Year of collection	Number	PFOS mean[a]	PFOS range	PFOA mean[a]	PFOA range	PFHS mean[a]	PFHS range	References
Los Angeles, CA	Adults	Serum	2001	125	40.4	6.6–205.0	4.1	2.1–34.1	1.9	< 2.1–12.4	Olsen *et al.*, 2003b
Boston, MA	Adults	Serum	2001	109	28	4.3–87.2	5.4	1.5–13.9	1.9	< 1.4–12.6	Olsen *et al.*, 2003b
Mpls–St Paul, MN	Adults	Serum	2001	100	33.1	7.7–207.0	4.5	1.9–20.0	1.5	< 1.4–15.2	Olsen *et al.*, 2003b
Charlotte, NC	Adults	Serum	2001	96	51.5	19.3–166.0	6.3	2.1–29.0	2.8	< 1.4–22.4	Olsen *et al.*, 2003b
Portland, OR	Adults	Serum	2001	107	27	6.0–1656	3.6	2.1–16.7	1.6	< 2.1–16.7	Olsen *et al.*, 2003b
Hagerstown, MD	Adults	Serum	2001	108	35.3	7.6–226.0	4.2	2.1–52.3	2.1	< 2.1–66.3	Olsen *et al.*, 2003b
Seattle, WA	Elderly adults	Serum	2001	238	31	3.4–175.0	4.2	1.4–16.7	2.2	< 1.4–40.3	Olsen *et al.*, 2004a
23 US States	Children (2–12 years)	Serum	1994–1995	598	37.5	6.7–515	4.9	1.9–56.1	4.5	< 1.4–711.7	Olsen *et al.*, 2004b
Washington Co., MD	Adults	Serum	1974	178	30.1	NR	2.1	NR	1.5	NR	Olsen *et al.*, 2005b
Washington Co., MD	Adults	Plasma	1989	178	33.3	NR	5.5	NR	2.5	NR	Olsen *et al.*, 2005b
Kentucky	Adult females	Serum	2000	46	32.5*	< 1.3–91.7	4.7*	< 3–7.3	3.6	< 1.3–13.2	Kannan *et al.*, 2004
Michigan	Adult males	Serum	2000	29	32.9*	< 1.3–124	5.7*	< 3–14.7	NR	NR	Kannan *et al.*, 2004
Kentucky	Adult females	Whole blood	2002	11	66*	11–130	23*	15–39	NR	NR	Kannan *et al.*, 2004
Kentucky	Adult males	Whole blood	2002	19	73.2*	19–164	41.6*	11.0–88	NR	NR	Kannan *et al.*, 2004
New York City	Adults	Plasma	2002	70	42.8*	16–83	27.5*	14–56	NR	NR	Kannan *et al.*, 2004
Atlanta, GA	Adult females	Serum	2003	10	54	3.6–164.0	4.2*	0.2–10.0	3.0*	0.4–5.6	Kuklenyik *et al.*, 2004
Atlanta, GA	Adult males	Serum	2003	10	57.97*	20.4–94.0	5.56*	2.8–10.4	4.88*	1.1–11.2	Kuklenyik *et al.*, 2004
Atlanta, GA	Adult females	Breast milk	2003	2							Kuklenyik *et al.*, 2004
United States	NHW	Serum	1999–2000	529	32.0	NR	5.6	NR	2.3	NR	Calafat *et al.*, 2007
United States	NHB	Serum	1999–2000	309	33.0	NR	4.8	NR	2.2	NR	Calafat *et al.*, 2007
United States	MA	Serum	1999–2000	584	22.7	NR	3.9	NR	1.5	NR	Calafat *et al.*, 2007
United States	NHWF	Serum	2001–2002	13 pooled	24.0*	NR	4.0*	NR	4.3*	NR	Calafat *et al.*, 2006a
United States	NHBF	Serum	2001–2002	6 pooled	17.9*	NR	2.9*	NR	2.4*	NR	Calafat *et al.*, 2006a
United States	MAF	Serum	2001–2002	8 pooled	10	NR	2.1*	NR	1.8*	NR	Calafat *et al.*, 2006a
United States	NHWM	Serum	2001–2002	13 pooled	40.2*	NR	7.0*	NR	4.3*	NR	Calafat *et al.*, 2006a
United States	NHBM	Serum	2001–2002	6 pooled	18.3*	NR	3.6*	NR	2.4*	NR	Calafat *et al.*, 2006a
United States	MAM	Serum	2001–2002	7 pooled	13.7*	NR	2.9*	NR	1.8*	NR	Calafat *et al.*, 2006a
United States	Adults	Serum	1990–2002	23 pooled	30	13.8–56.5	9.6	2.8–23.7	1.6	< 0.3–3.1	Calafat *et al.*, 2006b
St Paul, MN	Adult females	Plasma	2005	20	13.3*	6.7–29.9	2.3*	0.7–4.7	NR	NR	Olsen *et al.*, 2006, 2007
St Paul, MN	Adult males	Plasma	2005	20	19.2*	10.0–36.4	2.6*	0.7–4.2	NR	NR	Olsen *et al.*, 2006, 2007
St Paul, MN	Adult females	Serum	2000	50	39.2*	7.7–207.0	5.1*	1.4–20.0	NR	NR	Olsen *et al.*, 2006, 2007
Southeastern OH	Adults and children	Serum	2005	371	NR	NR	354*	NR	NR	NR	Emmett *et al.*, 2006a
Ottawa, Gatineau, Canada	Adult females	Serum	2002	21	29.7*	9.5–62.3	3.08*	< 1.2–6.1	NR	NR	Kubwabo *et al.*, 2004
Ottawa, Gatineau, Canada	Adult males	Serum	2002	35	28.3*	3.7–65.1	3.6*	< 1.2–7.2	NR	NR	Kubwabo *et al.*, 2004

Note. NHWF, non-Hispanic white females; NHWM, non-Hispanic white males; NHBF, non-Hispanic black females; NHBM, non-Hispanic black males MAF, Mexican American females; MAM, Mexican American males; NHW, non-Hispanic whites; NHB, non-Hispanic blacks; MA, Mexican Americans.

[a]Geometric means presented—those with * are arithmetic means.

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TABLE 3
Ranges of PFOS, PFOA, and PFHS Concentrations (ng/ml) in Adult Populations Worldwide

Location	Sample type	Year of collection	Number	PFOS range	PFOA range	PFHxS range	References
Cartagena, Columbia	Whole blood	2003	56	4.6–14	3.7–12.2	< 0.4–0.9	Kannan *et al.*, 2004
Rio Grande, Brazil	Whole blood	2003	27	4.3–35	< 20	< 0.6–15.3	Kannan *et al.*, 2004
Siena, Italy	Serum	2001	50	< 1–10.3	< 3	< 1–2.1	Kannan *et al.*, 2004
Gdansk, Poland	Whole blood	2003	25	16–116	9.7–40	0.5–2.6	Kannan *et al.*, 2004
Flanders, Wallonia, Belgium	Plasma	1998, 2000	20	4.5–27	< 1–13	< 1–1.4	Kannan *et al.*, 2004
Coimbatore, India	Serum	2000	45	< 1–3.1	< 3–3.5	< 1–2.9	Kannan *et al.*, 2004
Kuala Lumpur, Malaysia	Whole blood	2004	23	6.2–18.8	< 10	1.2–6.8	Kannan *et al.*, 2004
Daegu, Korea	Whole blood	2003	50	3.0–92	< 15–256	0.9–20	Kannan *et al.*, 2004
Yokohama, Tsukuba, Japan	Serum	2002	38	4.1–40.3	< 6.8–12.3	< 2.6–7.6	Kannan *et al.*, 2004
Hokkaido, Japan	Whole blood[a]	2003	15	4.9–17.6	0.5–2.3	NR	Inoue *et al.*, 2004
Various cities, Japan	Serum	2003–2004	200	3.4–92.2	0.4–25.5	NR	Harada *et al.*, 2006a
Tokoyo Bay area, Japan	Whole blood	2002	11	2.4–14	NR	< 1–1	Taniyasu *et al.*, 2003
Tokoyo Bay area, Japan	Serum	2002	3	19–41	NR	< 2.7	Taniyasu *et al.*, 2003
Sweden	Whole blood	1997–2000	66	1.7–37.0	0.5–12.4	0.4–28.4	Kärrman *et al.*, 2006b
Australia	Serum	2002–2003	3802[b]	12.7–29.5	5.0–9.9	2.7–19.0	Kärrman *et al.*, 2006a
Shenyang, China	Whole blood[c]	2003	85	10.6–142	NR	NR	Yeung *et al.*, 2006
Zhoushan, China	Breast milk	2004	19	0.05–0.36	0.05–0.21	0.004–0.100	So *et al.*, 2006
Uppsala, Sweden	Breast milk	2004	12	0.06 –0.47	< 0.21–0.49[d]	0.03–0.17	Kärrman *et al.*, 2007
Germany	Plasma	2003–2004	105[e]	6.2–130.7	1.7–39.3	NR	Midasch *et al.*, 2006
Bavaria, Germany	Plasma	2005	356	2.1–55.0	0.5–19.1	NR	Fromme *et al.*, in press
Catalonia, Spain	Whole blood	2006	48	0.8–16.2	0.8–3.1	0.65–20.0	Ericson *et al.*, 2007

Note. NR, not reported.
[a]Pregnant women.
[b]40 pooled samples.
[c]Whole blood converted to serum measurements (multiplied by a factor of 2).
[d]Only 1 sample above the detection limit (0.01 ng/ml)—blank was higher than 50% of other detected concentrations.
[e]Some children in sample.

and non-Hispanic blacks. In addition, concentrations of PFOS and PFOA were higher in more highly educated cohort members. A U.S. and a Japanese study have also reported on the historical trends of PFAAs in small samples of the population. In one study, blood samples collected in western Maryland in 1974 and 1989 ($n = 178$ for each time period) indicate that median concentrations of PFOS, PFOA, PFHS, and PFOSAA increased during this time period (Olsen *et al.*, 2005). However, additional samples from different study participants ($n = 108$) collected in the same region in 2001 did not indicate any significant difference between 1989 and 2001. Another U.S. pilot study suggests that blood levels of PFOS and PFOA declined from 2000 to 2005. It is noteworthy that these data are preliminary, cross-sectional, based on small sample sizes ($n = 40$ in 2005), and are derived from different matrices (plasma vs. serum) (Olsen *et al.*, 2006, 2007a). A Japanese study, on the other hand, reported that both PFOS and PFOA have increased steadily in Japanese residents from 1977 to 2003 (Harada *et al.*, 2004). This study also reported higher PFOS and PFOA serum levels in male participants than females and geographical differences within the country. Another study of 200 Japanese residents monitored from 1983 to 2004 showed a fourfold increase in PFOA serum levels over this time period, while those of PFOS leveled off in the late 1980s (Harada *et al.*, 2006a). Additional biomonitoring studies will better characterize the trends of human exposure to these fluorochemicals. The routes of human exposure to PFAAs are currently being investigated. Possible exposure pathways that are being examined include drinking water, dust in homes, and food or migration from food packaging and cookware (Begley *et al.*, 2005; D'eon and Mabury, in press; Emmett *et al.*, 2006a; Falandysz *et al.*, 2006; Kubwabo *et al.*, 2005; Moriwaki *et al.*, 2003; Powley *et al.*, 2005; Shoeib *et al.*, 2005; Sinclair *et al.*, in press; Tittlemier *et al.*, 2006, 2007).

PHARMACOKINETICS

The pharmacokinetic properties of PFOS and PFOA have been studied in some detail. Animal studies of both compounds have shown that they are well absorbed orally, but poorly eliminated; they are not metabolized, and undergo extensive uptake from enterohepatic circulation (Johnson *et al.*, 1984; Kemper and Nabb, 2005; Kuslikis *et al.*, 1992; Ophaug and Singer, 1980; Vanden Heuvel *et al.*, 1991). Both compounds

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are distributed mainly to the serum, kidney, and liver, with liver concentrations being several times higher than serum concentrations (Hundley *et al.*, 2006; Johnson *et al.*, 1979a; Seacat *et al.*, 2002, 2003). The volume of distribution at steady state of PFOS is approximately 200 ml/kg in the cynomolgus monkey, suggesting that distribution is mainly extracellular (Noker and Gorman, 2003). PFOS and PFOA have affinity for binding to β-lipoproteins (Jones *et al.*, 2003; Kerstner-Wood *et al.*, 2003) as well as albumin and liver fatty acid–binding protein (L-FABP) (Luebker *et al.*, 2002.). In humans, studies have reported detectable levels of PFOS and PFOA, as well as certain other PFAAs, in umbilical cord blood, indicating that these chemicals cross the placenta (Apelberg *et al.*, 2007; Inoue *et al.*, 2004).

The elimination half-lives of several PFAAs are summarized in Table 5. In general, the rate of elimination is enhanced with decreasing carbon chain length. Thus, the elimination half-lives of PFBS and PFBA in the cynomolgus monkey are shorter than those of PFOS and PFOA. This is also true in humans, but interestingly, the elimination half-life of PFHS in humans is longer than that of PFBS or PFOS (Table 5). The most notable feature of PFAA pharmacokinetics is the tremendous species differences in elimination. For PFOS, this ranges from 100 days in rats (Johnson *et al.*, 1979b) to 5.4 years in humans (Olsen *et al.*, 2007b). A similar trend is seen with PFOA, but there are also gender differences in the elimination of PFOA. Thus, the elimination half-life of PFOA in adult female rats is 2–4 h, but is 4–6 days in adult male rats (Kemper, 2003). This gender difference in elimination is developmentally regulated and the ability of female rats to rapidly excrete PFOA develops between 3 and 5 weeks of age (Hinderliter *et al.*, 2006). Gender differences have also been observed in other animal species, but the elimination is not always faster in females. For instance, male hamsters excrete PFOA more rapidly than female hamsters. In dogs, the half-life of PFOA is 20–30 days in males and 8–13 days in females (Hanhijarvi *et al.*, 1988). In cynomolgus monkeys, the half-life of PFOA is 30 days in females and 21 days in males (Butenhoff *et al.*, 2004c). In contrast, gender differences are not observed in mice or rabbits (Hundley *et al.*, 2006; Lau *et al.*, 2006) and none has been noted in humans, although uncertainty exists in the human data due to sample size (Olsen *et al.*, 2007b).

The reason for species and gender differences in elimination of PFOA is not well understood. In adult rats, the elimination of PFOA is downregulated by testosterone in both female and castrated male rats (Kudo *et al.*, 2001, 2002; Vanden Heuvel *et al.*, 1992) and upregulated by estradiol in male rats (Ylinen *et al.*, 1989). These differences 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 and Klaassen, 2004; Buist *et al.*, 2002; Kudo *et al.*, 2002). Some of these differences develop during the period of sexual maturation (Buist *et al.*, 2002).

Owing to the gender and species differences in elimination of PFAAs, comparisons of toxicological effects must utilize some measure of body burden rather than administered dose. Work in this area is in its infancy. To date, a one compartment model has been used for PFOA (U.S. EPA, 2005) and a pharmacokinetic model of PFOS has been explored in monkeys (Anderson *et al.*, 2006).

EPIDEMIOLOGY

Epidemiological and medical surveillance studies have been conducted primarily in the United States on workers occupationally exposed to POSF-based fluorochemicals. These include mortality and cancer incidence (Alexander, 2001a, b, 2004; Alexander *et al.*, 2003; DuPont, 2003b, 2006; Gilliland and Mandel, 1993; Karns and Fayerweather, 1991; Walrath and Burke, 1989), a study examining potential endocrine effects (Olsen *et al.*, 1998), an "episodes-of-care" study evaluating worker insurance claims data (Olsen *et al.*, 2004c), and worker surveillance studies examining associations between primarily PFOS and/or PFOA serum concentrations and hematology, hormonal and clinical chemistry parameters (Emmett *et al.*, 2006b; Gilliland and Mandel, 1996; Olsen *et al.*, 1999, 2000, 2003a). These studies that are discussed in detail elsewhere (U.S. EPA, 2005), specifically examined PFOS or PFOA exposures and possible adverse outcomes. In general, no consistent association between serum fluorochemical levels and adverse health effects has been observed.

A cohort study of PFOS-exposed workers in a fluorochemical manufacturing facility showed no statistically significant effect on mortality for most types of cancer and for nonmalignant causes (Alexander *et al.*, 2003). However, bladder cancer mortality was elevated among male workers who had worked in high PFOS exposure jobs for a minimum of one year. This finding was based on three cases. A follow-up worker health survey on the bladder cancer mortality confirmed an increased standardized mortality ratio (SMR) but analyses by duration worked showed no definitive trend (Alexander, 2004). Other cancer types and pregnancy history in female employees were ascertained via a self-administered questionnaire (Alexander and Grice, 2006). No significant associations between PFOS exposure and certain cancers or birth outcomes were observed.

Occupational studies on workers employed at various manufacturing sites of PFOA in the United States were mostly cross-sectional and focused primarily on males. One retrospective cohort study demonstrated a significant association between prostate cancer mortality and employment duration in the chemical facility of a plant that manufactures PFOA (Gilliland and Mandel, 1993). However, in an update to this study in which more specific exposure measures were used, this association was no longer observed (Alexander, 2001a, b). In a cancer incidence study undertaken at another plant,

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TABLE 4

Concentration (ng/ml) of Other PFC Analytes Measured in Human Populations Worldwide

Demographic	PFOSA mean	PFOSA range	Et-PFOSA-AcOH mean	Et-PFOSA-AcOH range	Me-PFOSA-AcOH mean	Me-PFOSA-AcOH range	PFNA mean	PFNA range	PFDeA mean	PFDeA range	PFUA mean	PFUA range	References
Atlanta, GA 2003 serum samples													
Adult females ($n = 10$)	0.26	< LOD–0.7	0.72	< LOD–1.4	1.46	0.4–3.1	2.24	1.6–3.8	0.37	< LOD–1.1	0.54	< LOD–1.3	Kuklenyik *et al.*, 2004
Adult males ($n = 10$)	0.25	< LOD–0.6	0.89	0.4–1.4	1.86	0.8–5.2	2.89	1.3–4.4	0.64	< LOD–1.4	0.82	< LOD–1.4	
Various locations in United States, 1999–2000 individual serum samples													
NHW	0.4*		0.7*		1.0*		0.6*						Calafat *et al.*, 2007
NHB	0.4*		0.5*		1.1*		0.8*						
MA	0.3*		0.6*		0.8*		0.3*						
Various locations in United States, 2001–2002 pooled serum samples													
NHWF	0.19*		0.43*		0.78*		0.51						Calafat *et al.*, 2006a
NHBF	0.19*		0.24*		0.55*		0.63						
MAF	0.19*		0.17*		0.35*		0.13						
NHWM	0.19*		0.43*		0.78*		1.1						
NHBM	0.19*		0.24*		0.55*		0.7						
MAM	0.19*		0.17*		0.35*		0.3						
Various locations in United States, 1990–2002, 23 pooled samples													
Adults		< 0.2–1.1	ND	< 0.4–2.7	ND	< 0.6–2.1	ND	< 0.1–0.5				< 0.3–3.6	Calafat *et al.*, 2006b
Sweden, 1997–2000, whole blood													
Adult females ($n = 26$)	2.6*	0.4–9.5					0.3*	< 0.1–1.0	0.1	< 0.1–0.6	0.1	< 0.1–0.7	Karrman *et al.*, 2006a
Adult males ($n = 40$)	3.2*	0.8–22.9					0.2*	< 0.1–1.9	0.1	< 0.1–0.5	0.1	< 0.1–0.6	
Sweden, 2004, paired serum and breast milk samples													
Adult females ($n = 12$) serum	0.24	< 0.10–0.49					0.80	0.4–2.5	0.53	0.27–1.8	0.40	0.20–1.5	Karrman *et al.*, 2007
Adult females breast milk	0.013[a]	< 0.007–0.030					0.017[b]	< 0.005–0.020					
Australia, 2002–2003, serum, 40 pooled samples													
Adult males and females	0.81	0.36–2.4					1.1	0.4–2.0					Karrman *et al.*, 2006b
Cartagena, Columbia, 2003, whole blood													
Adult females ($n = 25$)	1.4	< 0.4–3.8											Kannan *et al.*, 2004
Adult males ($n = 31$)	1.7	0.4–5.6											
Rio Grande, Brazil, 2003, whole blood													
Adult female	0.7	< 0.4–1											Kannan *et al.*, 2004
Adult male	1.5	< 0.4–2.3											
Siena, Italy, 2001, serum													
Adult females ($n = 8$)	1.7	< 1.3–1.7											Kannan *et al.*, 2004
Adult males ($n = 42$)	1.8	< 1.3–2.3											
Gdansk, Poland, 2003, whole blood													
Adult females ($n = 15$)	2.3	0.4–7.7											Kannan *et al.*, 2004
Adult males ($n = 10$)	1.7	< 0.4–4.4											
Flanders, Wallonia Belgium, 1998, 2000, plasma													
Adult females ($n = 4$)	< 3	< 3											Kannan *et al.*, 2004
Adult males ($n = 16$)	< 3	< 3											
Coimbatore, India, 2000, serum													
Adult females ($n = 11$)	< 3	< 3											Kannan *et al.*, 2004
Adult males ($n = 34$)	< 3	< 3											

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Kuala Lumpur, Malaysia, 2004, whole blood							Kannan *et al.*, 2004
Adult females ($n = 7$)	3.8	1.3–6.0					
Adult males ($n = 16$)	4.9	1.4–11					
Daegu, Korea, 2003, whole blood							Kannan *et al.*, 2004
Adult females ($n = 25$)	1.1	< 0.1–2.1					
Adult males ($n = 25$)	1.5	0.4–7.2					
Yokohama, Tsukuba, Japan, 2002, serum							Kannan *et al.*, 2004
Adult females ($n = 13$)	3.3	< 2.6–7.1					
Adult males ($n = 25$)	6.2	< 2.6–9.5					
Catalonia, Spain, 2006, whole blood							
Adults ($n = 48$)	0.42	ND–1.35	0.44	ND–1.5	0.30	ND–0.71	0.34 ND–0.84 Ericson *et al.*, 2007

Note. *Geometric mean—all others are arithmetic.
[a]Detected in eight samples.
[b]Detected in two samples.

standardized incidence ratios were elevated and significant for bladder and kidney cancer (DuPont, 2003b). Several SMRs were elevated in a retrospective cohort mortality study of over 6000 PFOA-exposed employees, including those for kidney, liver, and bladder cancer mortality in males, but only diabetes mellitus mortality was statistically significant (DuPont, 2006). Other mortality studies lacked adequate exposure data that could be linked to health outcomes.

A study examining hormone levels in workers reported an increase in serum estradiol levels among those individuals with the highest PFOA serum levels; however, these results may have been confounded by body mass index (Olsen *et al.*, 1998). In the same study, serum cholesterol and triglyceride levels were positively associated with PFOA exposure, which is inconsistent with hypolipidemic effects observed in rats (Olsen *et al.*, 2001a). A positive association was also reported between PFOA and triiodothyronine levels in workers but not for other thyroid hormones (Olsen *et al.*, 2001b).

MAMMALIAN TOXICOLOGY

The toxicology of PFOS and PFOA has been extensively reviewed (3M Company, 2003; Kennedy *et al.*, 2004; Lau *et al.*, 2004; OECD, 2002; U.S. EPA, 2005). Repeat-dose studies of PFOS in rodents and nonhuman primates have shown reduced body weight, increased liver weight, reduced cholesterol, and a steep dose–response curve for mortality (Goldenthal *et al.*, 1978a,b; Seacat *et al.*, 2002). A 2-year bioassay of PFOS in Sprague–Dawley rats showed an increase in hepatocellular adenomas at a high dose of 20 ppm in the diet (3M Company, 2002; Seacat *et al.*, 2003). In addition, one group was fed 20 ppm PFOS for one year and then monitored for an additional year. The male rats in the ''recovery'' group had an increase in thyroid follicular cell adenomas; the reason for this is unclear. Interestingly, thyroid follicular cell adenomas have also been noted in rats exposed to N-EtFOSE, a major precursor of PFOS (Thomford, 2001). A similar profile of effects has been described in repeat-dose studies of PFOA in rodents (Christopher and Marias, 1977; Goldenthal, 1978a; Metrick and Marias, 1977; Palazzolo, 1993) but studies in nonhuman primates have not shown a reduction in serum cholesterol (Butenhoff *et al.*, 2002). PFOA has been shown to induce hepatocellular adenomas, Leydig cell tumors, and pancreatic acinar cell tumors in male Sprague–Dawley rats (Biegel *et al.*, 2001; Cook *et al.*, 1992; Sibinski, 1987). Neither compound has been shown to be mutagenic in a variety of assays (3M Company, 2003; Kennedy *et al.*, 2004; U.S. EPA, 2005).

In the past 5 years, new data have become available to elucidate the mode of action for the liver toxicity in rodents, as well as to characterize the developmental effects, hormonal effects and immunotoxic potential of PFAAs. These recent findings are summarized accordingly.

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TABLE 5
Serum/Plasma Elimination $T_{1/2}$ of Various PFAAs

		PFHS			PFBA		PFOA		
Species	PFBS	Females	Males	PFOS	Females	Males	Females	Males	References
Rat				100 days	1.6–1.8 h	7–9 h	2–4 h	4–6 days	Chang *et al.*, 2007a; Johnson *et al.*, 1979b; Kemper and Jepson, 2003
Mouse					3 h	17 h	17 days	19 days	Chang *et al.*, 2007a; Lau *et al.*, 2005
Rabbit							7 h	5.5 h	Hundley *et al.*, 2006
Dog							8–13 days	20–30 days	Hanhijarvi *et al.*, 1982
Monkey	3.5–4 days	87 days	141 days	150 days	1.7 days		30 days	21 days	Butenhoff *et al.*, 2004b; Chang *et al.*, 2007a; Lieder *et al.*, 2006b; Noker and Gorman, 2003; Olsen *et al.*, 2005b; Seacat *et al.*, 2002
Human	1 month		8.5 years	5.4 years			3.8 years		Olsen *et al.*, 2005a, 2006

Hepatotoxicity

As noted above, PFOS and PFOA are associated with liver enlargement in rodents and nonhuman primates in addition to hepatocellular adenomas in rats. Agonism of the peroxisome proliferator–activated receptor-alpha (PPAR-α) has been suggested to be involved in tumor (primarily liver) induction by a number of nongenotoxic carcinogens in the rodents. Recently, the key events in the PPAR-α–agonist mode of action for rodent liver toxicity and hepatocarcinogenesis have been described, and include activation of PPAR-α followed by altered expression of genes involved in peroxisome proliferation, cell cycle control, and apoptosis (Klaunig *et al.*, 2003). A series of studies have been conducted to determine whether the PPAR-α–agonistic mode of action is involved in the hepatic toxicity and hepatocellular adenomas observed in rat bioassays with PFOS and PFOA. In addition, several studies have examined other PFAAs to determine the potential impact of the carbon chain length on hepatic toxicity and this specific mode of action.

A number of short-term studies in rats and mice have shown that PFOS and PFOA are capable of inducing peroxisome proliferation (3M Company, 2004; Berthiaume and Wallace, 2002; Ikeda *et al.*, 1985, 1987; Pastoor *et al.*, 1987; Sohlenius *et al.*, 1992, 1993). Accordingly, the first key event in this mode of action is activation of PPAR-α. Recent studies (Vanden Huevel *et al.*, 2006) using 3T3-L1 cells transfected with a luciferase reporter gene have demonstrated that mouse, rat, and human PPAR-α are activated by PFOA and PFOS. In comparison with naturally occurring fatty acids and the fibrate class of drugs, PFOA and PFOS are relatively weak ligands for PPAR-α. The human PPAR-α was most responsive to PFOA and PFOS, and the rat PPAR-α was the least responsive. Consistent with these findings, PFOS, as well as one of its precursors, PFOSA, were shown to activate mouse and human PPAR-α in a COS-1 cell-based luciferase reporter transactivation assay and in a rat liver cell model where the induction of endogenous PPAR-α target genes were monitored. Half maximal activation (EC_{50}) occurred at 13–15μM PFOS, with no significant difference in the responsiveness of mouse and human PPAR-α (Shipley *et al.*, 2004). Similarly, Maloney and Waxman (1999) demonstrated that PFOA activates PPAR-α using COS-1 cells transfected with a luciferase reporter gene. Maximal transcriptional activity with PFOA was seen at 10μM in mouse PPAR-α and at 20μM in human PPAR-α. N-EtFOSE did not activate mouse or human PPAR-α because of its insolubility in the culture medium (Shipley *et al.*, 2004). Results from a recent study by Takacs and Abbott (2007) using a different transfected cell system confirmed these findings.

While several perfluorinated compounds can activate PPAR-α, it should be noted that they may also induce peroxisome proliferation by perturbing lipid metabolism and transport. An *in vitro* study (Luebker *et al.*, 2002) has shown that PFOS, N-EtFOSE, PFOSA, and PFOA interfere with the binding of fatty acids or other endogeneous ligands to rat L-FABP. Since PPAR-α is activated by endogeneous cellular fatty acids (Maloney and Waxman, 1999), it was suggested that displacement of endogeneous ligands from L-FABP may be one mechanism by which PFOS induces peroxisome proliferation.

Although PFOS can activate PPAR-α, the data for the subsequent key events are not consistent with a PPAR-α–agonistic mode of action for hepatic toxicity or hepatocellular adenomas. Specifically, some inconsistencies exist in the dose–response data for PPAR-α activation and liver carcinogenicity of PFOS. Liver toxicity and carcinogenicity of PFOS are evident at doses lower than those (200–500 ppm) that induce peroxisome proliferation in short-term studies in rats. A cancer bioassay in which rats were given PFOS in the diet at concentrations of 0.5, 2, 5, or 20 ppm for 104 weeks, produced only an equivocal (< twofold) increase in hepatic palmitoyl-CoA activity at 4 weeks in the males at the high dose (20 ppm).

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Further, these observed mild increases in hepatic peroxisomal enzyme activity were not sustained, as no effects on palmitoyl-CoA oxidase activity and cell proliferation in the liver were observed at week 14 of the study, despite evidence for increased hepatocytic hypertrophy and vacuolation (Seacat *et al.*, 2003). The hepatic effects observed in PFOS-treated rats, therefore, do not appear to be related to peroxisome proliferation. It has been suggested that the induction of peroxisome proliferation by PFOS in rats may exhibit a threshold dose response in short-term assays that rapidly reach relatively high tissue concentrations of the fluorochemical. The cumulative doses after 4 or 14 weeks of dietary PFOS (20 ppm) may not have been achieved rapidly enough to produce the increases in peroxisome proliferation seen in the short-term studies. The presence of high cumulative tissue concentrations of PFOS in the cancer study without significant stimulation of peroxisome proliferation suggests that a mechanism may exist *in vivo* for an adaptive downregulation of the hepatic peroxisome proliferation response to PFOS treatment (Shipley *et al.*, 2004).

Hepatic lesions were also reported in Cynomolgus monkeys administered up to 0.75 mg/kg day PFOS for 6 months (Seacat *et al.*, 2002). As expected, there was no evidence of peroxisome proliferation and no increase in palmitoyl-CoA oxidase activity in the liver of the monkeys since monkeys appear to be refractory to peroxisome proliferative responses. The hepatic effects observed in the PFOS-treated monkeys, therefore, also do not appear to be related to peroxisome proliferation.

There is much stronger evidence to support PFOA-induced liver toxicity and adenomas via a PPAR-α–agonistic mode of action in the rodents. PFOA activates PPAR-α and the requisite dose–response and/or temporal associations between the subsequent key events have been characterized (Klaunig *et al.*, 2003). However, there is also some evidence that liver enlargement may be associated with a PPAR-α–independent mode of action; it is not known whether this alternate mode of action could also lead to hepatocellular adenomas. The activation of PPAR-α by PFOA is consistent with the finding that significant increases in liver weight were observed in wildtype (WT) mice exposed to dietary PFOA or WY-14,643, a classical peroxisome proliferator, at 0.02% and 0.125% (wt/wt), respectively, for 7 days (Yang *et al.*, 2002a,b). As expected, this response was absent upon treatment of PPAR-α null mice with WY-14,643 but surprisingly unaltered in null mice exposed to PFOA. These findings suggest that hepatomegaly may be induced by PFOA independently of PPAR-α in mice.

The hepatomegaly observed in PPAR-α null mice may be due to the accumulation of lipid droplets or the accumulation of PFOA in the liver. It is well known that PPAR-α is involved in the control of lipid metabolism and transport. Activation of PPAR-α has been shown to upregulate adipose differentiation-related protein which is responsible for the formation of lipid droplets in many cell types (Yang *et al.*, 2006). Studies of gene expression profiles in rat liver treated with PFOA show that the largest categories of induced genes are those involved in metabolism and transport of lipids, particularly fatty acids (Guruge *et al.*, 2006; Martin *et al.*, 2007; Rosen *et al.*, in press). Increased lipid droplets due to alteration in lipoprotein metabolism have been observed in PPAR-α null mice (Peters *et al.*, 1997; Yang *et al.*, 2006). The liver weight increase observed in the PFOA-exposed PPAR-α null mice may therefore be due to accumulation of lipid in the liver cells. A recent study has evaluated the microscopic and ultrastructural alterations of hepatocytes in PPAR-α knockout (KO) or WT mice given oral daily doses of PFOA at 1, 3, or 10 mg/kg or of WY-14,643 at 50 mg/kg. Interestingly, while the results showed increased hepatocyte hypertrophy in all mouse groups treated with PFOA or WY-14,643, a dose-dependent increase in hepatocyte vacuolation was only noted in KO mice treated at the highest dose of PFOA. The pathological significance of the vacuoles remains to be determined (Lau *et al.*, 2007). Preliminary results also suggest that PFOA exhibits the prototypical properties of a peroxisome proliferator but, in addition, possesses the properties of a mixed type enzyme-inducing agent as marked inductions of CYP2B2, CYP3A4, and CYP4A1 in liver microsomes have been observed. This profile of CYP induction suggests that PFOA interacts with multiple members of the nuclear hormone super family, particularly PPAR-α, constitutive androstane receptor (CAR), and pregnane X receptor (Elcombe *et al.*, 2007).

The effects of PFAA carbon chain length on the liver toxicity and peroxisome proliferation have been studied in rats and mice. In male Sprague–Dawley rats given 5 consecutive daily doses of PFBS, PFHS, or PFOS, liver weights and hepatic acyl CoA oxidase (ACOX) activities were significantly increased for all PFAAs tested. The doses in mmol/kg of PFBS required to produce similar increases in ACOX activity were about 50 times higher than those of PFOS or PFHS. However, liver concentrations of all three compounds were similar (Ehresman *et al.*, 2007b). Kudo *et al.* (2000) studied the induction of peroxisomal β-oxidation by PFHA, PFOA, PFNA, or PFDA in rat liver by administration of these PFAAs at doses ranging from 2.5 to 20 mg/kg/day for 5 days. In male rats, all compounds except PFHA induced the activity of peroxisomal β-oxidation. In female rats, however, only PFNA and PFDA effectively induced the activity. The induction of enzyme activity was dose dependent and there was a high correlation between the induction of peroxisomal β-oxidation and hepatic concentrations of PFAA. Hepatic concentrations of PFOA and PFNA were also markedly higher in male rats compared to females. Based on these results, it appeared that the difference in accumulation of these compounds in the rat liver was responsible for the different hepatic responses observed between PFAAs with different carbon chain length and between sexes.

Similar studies of PFAAs with six- to nine-carbon length chains have been conducted in mice (Kudo *et al.*, 2006). All compounds tested induced hepatomegaly and peroxisomal

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β-oxidase activity. The potency was in the order of PFNA > PFOA> perfluoroheptanoic acid >PFHA. The results indicated that the longer the perfluoroalkyl chain of these PFAAs, the higher accumulation of the compound in the mouse liver. In a study by Permadi *et al.* (1993), male C57B1/6 mice were fed a diet containing perfluoroacetic, -butyric, -octanoic, or -decanoic acids at different doses for varying periods of time, and liver weight, hepatic peroxisomal palmitoyl-CoA oxidase, and lauroyl-CoA oxidase were monitored. The greatest effects were observed with PFOA and PFDA, while the responses to PFBA were marginal and perfluoroacetic acid was inactive. However, the liver concentrations of these compounds were not examined.

Hepatomegaly has also been shown to be a prominent effect of PFBS or PFBA in subchronic toxicity studies in rats. A 28-day toxicity study in which rats were given PFBS at 100, 300, or 900 mg/kg resulted in significant increases in liver and kidney weights but these effects were seen only in highest dose group (3M Company, 2005). In a 28-day oral toxicity study, PFBA was given to rats at doses of 6, 30, and 150 mg/kg. Results from this study showed increased liver weight and decreased serum cholesterol in the mid- and high-dose males. $BMDL_{10}$ estimates for liver weight increase or cholesterol decrease were 10.5 and 3.4 mg/kg day, respectively (Lieder *et al.*, 2007).

Gap junctional intercellular communication (GJIC) is a process by which cells exchange ions, second messages, and other small molecules. In multicellular organisms, GJIC is important in the maintenance of tissue homeostasis and is involved in normal growth, development, and differentiation. It has been hypothesized that loss of GJIC may play a role in carcinogenesis (Trosko and Rush., 1998). Indeed, PFOS has been shown to inhibit GJIC in rats exposed orally (5 mg/kg) for either 3 days or 3 weeks, and, in a dose-dependent fashion in rat liver or dolphin kidney epithelial cell lines (Hu *et al.*, 2002). A number of fluorinated compounds structurally related to PFOS have also been demonstrated to inhibit GJIC *in vitro* and this effect is dependent on the length of the fluorinated carbon chains but not the nature of the functional group. PFAAs with carbon chain lengths of 7–10 were found to completely inhibit GJIC at concentrations of 50 μM while those with chain lengths less than five and more than 16 did not inhibit GJIC. The optimum chain length for the sulfonic acids was eight while that for the carboxylic acid was 10 (Hu *et al.*, 2002; Upham *et al.*, 1998). However, inhibition of GJIC is a widespread phenomenon. In these studies, its effect was neither species nor tissue specific and was generally reversible. Hence, the pathophysiological significance of GJIC inhibition in regard to the carcinogenic mode of action for PFOS and PFOA is currently unclear.

Developmental Toxicity

The adverse reproductive outcomes from exposure to PFAAs have been reviewed previously by Lau *et al.* (2004). Hence, this section will only update the previous review and primarily cover the research findings over the past five years. The teratological findings of PFOS and N-EtFOSE in rat, mouse, and rabbit are generally unremarkable when maternal toxicity is taken into consideration (Case *et al.*, 2001; Luebker *et al.*, 2005a,b; Thibodeaux *et al.*, 2003). Fetal weight reduction, cleft palate, delayed ossification of bone, and cardiac abnormalities were seen primarily at the highest doses where significant reductions of maternal weight gain were also noted. In contrast, when rats exposed to PFOS throughout pregnancy were allowed to give birth, dose-dependent deleterious effects were seen in newborns. In a study reported by Lau *et al.* (2003), all pups were born alive and active, however, in the high-dose group (10 mg/kg), newborns became pale, inactive, and moribund within 30–60 min. These pups died soon afterward. At 5 mg/kg PFOS, the neonates became moribund but survived for 8–12 h. However, over 95% of these offspring did not survive the first day of postnatal life and only a few pups reached puberty. Survival improved with lower PFOS exposure. The first week of postnatal life was also critical to the long-term survival of the neonates, as no mortality was detected thereafter. A similar scenario of neonatal mortality was observed by Luebker *et al.* (2005a), despite a very different PFOS exposure dose and regimen (see Table 6). Cross-fostering the PFOS-exposed rat pups with control nursing dams immediately after birth failed to improve survival of the neonates, thus ruling out a role for aberrant maternal behaviors associated with PFOS treatment (Case *et al.*, 2001, Lau *et al.*, 2003; Luebker *et al.*, 2005a). The morbidity and mortality of the newborn rats appeared to be correlated to their body burden of the fluorochemical (serum and liver). In fact, based on survival to postnatal day 8, a benchmark dose (BMD_5) was estimated at 1.07 mg/kg, with the lower limit of the 95% confidence interval ($BMDL_5$) estimated at 0.58 mg/kg (Lau *et al.*, 2003).

Because serum and liver levels of PFOS in the dams were measured at term (Thibodeaux *et al.*, 2003), one can extrapolate these benchmark treatment doses to body burdens by linear regression (Table 6). Thus, for the neonatal survival endpoint, the BMD_5, and $BMDL_5$ values correspond to 25 and 16 ppm, respectively, for serum, and 58 and 44 ppm, respectively, for liver. In the Luebker *et al.* (2005a,b) study, based on survival to postnatal day 5, these investigators provided BMD_5 and $BMDL_5$ estimates of 1.06 and 0.89 mg/kg, respectively. Using the same approach for extrapolation, these BMD values correspond to 67 and 59 ppm, respectively, for maternal serum, and 234 and 202 ppm, respectively, for maternal liver at term (Table 6). Estimates of BMD based on PFOS body burden in the latter study are generally higher than those reported by Thibodeaux *et al.* (2003) and Lau *et al.* (2003), thereby providing a BMD_5 range of 25–67 ppm for neonatal mortality in rat. A similar approach can be used to extrapolate BMDs for other developmental endpoints to body burdens.

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TABLE 6
Linear Regression Fits of Administered Doses and Mean Maternal Serum or Liver Levels at GD 21 (for rat) and GD 18 (for mouse) from Two Separate Studies of PFOS

Thibodeaux *et al.* study (rat)			Luebker *et al.* study (rat)			Thibodeaux *et al.* study (mouse)		
Treatment doses (mg/kg)	Maternal serum (ppm)	Maternal liver (ppm)	Treatment doses (mg/kg)	Maternal serum (ppm)	Maternal liver (ppm)	Treatment doses (mg/kg)	Maternal serum (ppm)	Maternal liver (ppm)
1	20 ± 2	45 ± 7	0.1	5 ± 1	29 ± 11	1	9	37
2	45 ± 2	82 ± 7	0.4	26 ± 16	107 ± 23	5	50	343
3	72 ± 7	139 ± 11	1.6	136 ± 87	388 ± 167	10	179	560
5	81 ± 3	156 ± 9	3.2	155 ± 39	610 ± 142	15	241	976
10	190 ± 7	312 ± 32				20	261	979
	$R^2 = 0.975$	$R^2 = 0.976$		$R^2 = 0.862$	$R^2 = 0.981$		$R^2 = 0.940$	$R^2 = 0.941$

Note. Thibodeaux *et al.* (2003) and Lau *et al.* (2003) treated the rats daily from GD 1 to GD 20, and the mice daily from GD 1 to GD 17; while Luebker *et al.* (2005b) treated the female rats daily for 42 days prior to mating, a maximum of 14 days during mating, and 20 days during gestation. Data represent means ± SE of 9–14 rat dams and six mouse dams in the Thibodeaux *et al.* (2003) study or 4–16 dams in the Luebker *et al.* (2005b) study, and are excerpted from primary studies that contained the experimental details. Goodness of fit for the linear regression is reflected by the R^2 value for each parameter. Based on postnatal survival of the rat offspring to day 8 (Lau *et al.*, 2003), a BMD_5 and a $BMDL_5$ were estimated at 1.07 and 0.58 mg/kg of treatment doses, respectively, that are extrapolated to maternal serum levels of 25 and 16 ppm, respectively, and maternal liver levels of 58 and 44 ppm, respectively. Based on postnatal survival of the rat offspring to day 5 (Luebker *et al.*, 2005b), a BMD_5 and a $BMDL_5$ were estimated at 1.06 and 0.89 mg/kg, respectively, that are extrapolated to maternal serum levels of 67 and 59 ppm, respectively, and maternal liver levels of 234 and 202 ppm, respectively. Based on postnatal survival of the mouse offspring to day 6 (Lau *et al.*, 2003), a BMD_5 and a $BMDL_5$ of 7.02 and 3.88 mg/kg, respectively, were reported, corresponding to maternal serum levels of 102 and 56 ppm, respectively, and maternal liver levels of 413 and 249 ppm, respectively.

An almost identical pattern of neonatal mortality was observed in mice exposed to PFOS during pregnancy (Lau *et al.*, 2003), although the treatment dose required for the effect was higher than that for the rat. Most offspring exposed to 15 or 20 mg/kg PFOS did not survive for 24 h after birth. The maternal dose corresponding to the BMD_5 and $BMDL_5$ for survival of mouse pups at postnatal day 6 was estimated at 7.02 and 3.88 mg/kg, respectively (about six to seven times higher than those for rat). These benchmark treatment doses correspond to 102 and 56 ppm, respectively, for maternal serum at term and 413 and 249 ppm, respectively, for maternal liver. Thus, while the benchmark treatment doses are six to seven times higher for the mouse than the rat, these differences can be narrowed considerably when based upon internal dose metrics. Hence, the use of internal dose (body burden) of PFOS will allow ready comparison of results from various studies that are derived from diverse dose and treatment schemes, as well as from different species. As more information about the presence of PFAAs in human populations (such as maternal and/or cord blood) becomes available (Inoue *et al.*, 2004; Midasch *et al.*, 2007), findings from animal studies can readily be extrapolated to evaluate the potential human health risks based on the "margin-of-exposure" paradigm.

The potential adverse effects of PFOS on reproductive outcome may not be limited to mammals. Molina *et al.* (2006) recently reported a dose-dependent reduction in the hatchability of white leghorn chicken eggs exposed to PFOS. Newsted *et al.* (2007) noted a statistically significant reduction of survival (72% vs. 87% in controls) in the 14-day-old Northern bobwhite quails when hens were fed a diet that contained 10 ppm of PFOS (corresponding to serum and liver PFOS levels of 8.7 and 4.9 ppm, respectively). In the same study, no adverse effects were seen in mallard ducks, even at serum and liver PFOS levels of 16.6 and 10.8 ppm, respectively. Ankley *et al.* (2004) reported that time to metamorphosis was delayed and growth was retarded in the Northern leopard frog exposed to 3 mg/l of PFOS. The same group demonstrated that exposure to PFOS led to reduced cumulative fecundity of fathead minnows at 0.1 mg/l or higher concentrations, although, no significant treatment-related effects on fertility were noted (Ankley *et al.*, 2005).

Postnatal growth of surviving rat pups has been shown to be adversely affected by *in utero* exposure to PFOS. In both Lau *et al.* (2003) and Luebker *et al.* (2005a), body weight gain of PFOS-exposed pups lagged significantly behind controls in a dose-dependent manner (10–20%) and this effect persisted past weaning. Development of these pups was also hindered as significant delays in eye opening, pinna unfolding, surface righting, and air righting were noted, although the onset of sexual maturation was not delayed. Reductions of total thyroxine (T4) and T3 in circulation were also detected, but the levels of free T4 (fT4) and thyroid stimulating hormone (TSH) remained unaffected (Lau *et al.*, 2003, Luebker *et al.*, 2005b). Because thyroid hormones are known to regulate brain development, the ontogeny of neurochemical and neuro-behavioral markers was evaluated in these studies. Prenatal exposure to PFOS produced only marginal deficits in choline acetyltransferase activity in the developing rat brain, an enzyme marker sensitive to thyroid hormone status, and did

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not affect learning and memory behaviors determined by T-maze delayed alternation, passive avoidance, or water maze. The lack of notable central nervous system changes in these studies is not consistent with the classical congenital hypothyroidism models, such as those produced by thyroidectomy or pharmacological manipulations. The physiological sequelae related to significant reduction of serum total T3 and T4 remain to be elucidated. Nonetheless, in a two-generation reproductive toxicity study (Luebker *et al.*, 2005a), the neonatal toxicity seen in the F_1 generation (mortality, growth, and developmental impairment) was largely undetected in the F_2 generation.

To characterize more fully the pathophysiological underpinnings of PFOS-induced neonatal mortality, Grasty *et al.* (2003) examined critical windows of exposure by treating rats with a high dose of PFOS (25 mg/kg/day) for a 4-day period during various stages of pregnancy. Neonatal mortality occurred after each treatment scheme but the incidence of neonatal death increased as the exposure period fell later during gestation, reaching 100% in the group treated on gestation days (GD) 17–20. One possible explanation for these findings could be related to the body burden of PFOS because as maternal weight increases during pregnancy, PFOS administered, based on body weight, also increases. This possibility was strengthened by the fact that maternal serum concentration determined on GD 21 was highest in rats receiving PFOS treatment during late gestation. In another experiment, exposure to 50 mg/kg/day of PFOS on GD 19 and 20 alone was sufficient to produce almost 100% mortality. Hence, the neonatal mortality resulting from PFOS administration to pregnant rats does not require chemical exposure before day 19 of gestation, suggesting that the critical period is late-gestational or perinatal. These results suggested that organ systems developing late in gestation may be targets for PFOS insult. This hypothesis is consistent with the relatively unremarkable teratological findings. Considering that PFOS-induced organ toxicity is incompatible with postnatal survival, maturation of the lung and pulmonary function is a plausible target of PFOS toxicity. Numerous chemicals have previously been shown to interfere with fetal lung development (Lau and Kavlock, 1994). Indeed, Grasty *et al.* (2003, 2005) described significant histological and morphometric differences between the control and PFOS-treated lungs in the newborns, suggesting that PFOS might inhibit or delay the perinatal lung development.

These findings prompted Grasty *et al.* (2005) to further investigate the effects of PFOS on lung maturation. As an indicator for pulmonary surfactant abnormalities and lung maturity, they examined the concentration and molecular speciation of phospholipids in the newborn rat lungs. They also evaluated the profile of gene expression in these lungs by microarray analysis. Furthermore, they attempted to ameliorate the adverse effects of PFOS by coadministration of dexamethasone or retinyl palmitate, agents that are known to promote lung maturation and pulmonary function. However, they found that PFOS did not affect lung phospholipids or alter the expression of marker genes for alveolar differentiation. The rescuing agents also failed to mitigate the extent of PFOS-induced neonatal mortality. These investigators surmised that the labored breathing and subsequent mortality observed in the PFOS-exposed newborns might not be related to immaturity of the lung *per se*. This contention, in fact, is consistent with recent findings reported by Lehmler *et al.* (2006). These researchers examined the mixing behavior of dipalmitoylphosphatidylcholine (DPPC), a major component of pulmonary surfactant, with PFOS by differential scanning calorimetry and fluorescence anisotropy measurements. They noted that PFOS had a high tendency to partition into lipid bilayers. Such PFOS–DPPC physical interactions might interfere with the normal physiological function of pulmonary surfactant. Similar findings with PFOA and hydrocarbon surfactant (octanesulfonic acid, OS) provided further support for this hypothesis (Xie *et al.*, 2007). In this study, the rank order of effectiveness for interacting with surfactant-DPPC was PFOS > PFOA $\gg$ OS. These results are in concert with those reported by Gordon *et al.* (2007). These researchers evaluated the surfactant surface tension using a captive bubble surfactometer and further demonstrated that a number of PFAAs (PFOS, PFOA, PFHS, PFBS) were capable of interfering directly with surfactant properties *in vitro* even at relatively low concentrations. The fact that PFOS has been detected in rat amniotic fluid (personal communications with John Butenhoff, 3M, and John Rogers, U.S. EPA) lends credence to this hypothesis. However, whether the apparent respiratory insufficiency in newborns results from interference with the function of pulmonary surfactant must await confirmatory results from additional *in vivo* experiments. Moreover, it must be noted that, even if the chemically induced respiratory distress may lead to neonatal mortality, this pathophysiological mechanism can only account for the death that occurred within minutes to hours postpartum. Additional biochemical/physiological mechanisms must be explored to explain the neonatal mortality that took place over the ensuing days.

A two-generation reproductive study of N-EtFOSE was conducted by Christian *et al.* (1999). Because N-EtFOSE is known to be metabolized to PFOS, it is not surprising to find the profile of N-EtFOSE developmental toxicity to resemble that of PFOS (Luebker *et al.*, 2005a). Accordingly, postnatal survival in the generation was significantly reduced, characterized by still birth and mortality within the first 3 days of life. In addition, impaired postnatal growth and retarded development were noted among the survivors. In contrast to PFOS, N-EtFOSE exposure also resulted in a slight, but statistically significant increase in still births and neonatal mortality in the F_2 generation.

A two-generation reproductive toxicity study was conducted in which PFOA (1–30 mg/kg) was given to rats for 70 days prior to mating, during mating, and during lactation (Butenhoff *et al.*, 2004b; Hinderliter *et al.*, 2005). The F_1-generation

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received similar treatment beginning at weaning, while the F_2-generation was monitored until weaning. The findings were generally unremarkable. Parental (P) and F_1-generation male rats showed decreased body weight along with increased liver and kidney weights at all doses. In contrast, female rats did not show similar changes and no reproductive endpoints were affected by PFOA treatment in either generation. A small body weight deficit was noted in F_1-generation pups in the highest dose group (30 mg/kg) where a slight delay in pubertal onset was also detected, although, these animals had normal reproductive performance. The relative lack of body and organ weight changes in females compared to males, and the normal reproductive outcomes in female rats exposed to PFOA may be related to the well documented gender difference in the elimination of this chemical in rats (Table 5). These dramatic variabilities thus complicate the extrapolation of findings from the rat model to humans for health risk assessment (Butenhoff *et al.*, 2004a; U.S. EPA, 2005).

On the other hand, results from studies by Uy-yu *et al.* (1990) and Sohlenius *et al.* (1992) provided insight that the mouse may serve as an alternative model for the evaluation of PFOA developmental toxicity. These investigators described similar PFOA related liver effects in male and female mice. Kudo *et al.* (2006) further added that no significant sex-related difference was observed in the mouse with regard to PFAA-induced hepatomegaly and the associated biochemical alterations. Lau *et al.* (2006) compared the serum levels of PFOA in rats and mice, and reported that profound sex-related differences were seen in rats but not in mice. Based on a ^{14}C-tracer analysis study, Hundley *et al.* (2006) provided confirmatory evidence that mice lacked a gender difference in the excretion of PFOA. Accordingly, Lau *et al.* (2006) carried out a reproductive toxicity study with PFOA in CD-1 mice using daily doses of 1–40 mg/kg throughout gestation. Full-litter resorptions were noted at 40 mg/kg. At 20 mg/kg, the percent of live fetuses and fetal weight were reduced and some structural abnormalities were seen in the fetuses. However, no significant increase in malformations was detected in the lower PFOA dose groups. The lack of significant teratological findings in mice was consistent with previous studies using rats and rabbits (Gortner, 1981, 1982; Staples *et al.*, 1984). However, when neonatal survival was evaluated in this study, a pattern of neonatal mortality mirroring that obtained with PFOS (Lau *et al.*, 2003) was observed. Postnatal survival was severely compromised at 10 or 20 mg/kg and moderately affected at 5 mg/kg. Postnatal growth impairment and developmental delays were noted among the survivors in these same dose groups. Interestingly, while the body weight deficits of the offspring recovered by 6.5 weeks of age, the PFOA-exposed mice continued to gain weight at a pace faster than the controls. In fact, recent results suggest that obesity and a number of organ specific abnormalities can be observed in these animals by 18 months of age (Fenton *et al.*, 2007). These findings require confirmation but future in-depth evaluations of the long-term effects of PFOA following developmental exposure may be warranted. BMD estimates for various parameters of PFOA developmental toxicity have been provided by Lau *et al.* (2006). Based on neonatal survival to weaning, a BMD_5 and a $BMDL_5$ were estimated at 2.84 and 1.09 mg/kg, respectively. These values are about 2.5 times lower than those for PFOS in the mouse. It would be interesting to see if such differences can be narrowed or eliminated when the results are expressed in terms of an internal dose metric such as serum PFOA.

Recently, Wolf *et al.* (2007) conducted studies to examine the critical windows of PFOA exposure in mice as well as the relevance of lactational PFOA exposure to neonatal viability. In this study, exposure to a high dose of PFOA (20 mg/kg) for 2 days late in gestation (GD 15–17) was sufficient to produce neonatal mortality and birth weight reduction, which are reminiscent of the findings reported by Grasty *et al.* (2003) using PFOS in the rat. At a lower dose (5 mg/kg), birth weight reduction, growth deficits, and developmental delays were seen only in mice exposed from GD 7–17 or GD 10–17 but not in those treated for shorter durations (GD 13–17, or GD 15–17). These findings likely reflect a critical body burden that the dams and fetuses must reach before adverse developmental effects are observed, although, differential sensitivity to PFOA during various developmental stages cannot be ruled out entirely. While maternal serum levels at parturition were not available, the serum levels of mouse dams assessed at postnatal day 22 may serve as surrogates, assuming that the rates of PFOA transfer through milk and excretion among all treatment groups are the same. Accordingly, the level of PFOA in the 20 mg/kg + GD 15–17 group, where neonatal mortality was seen, was twice of those found in the 5 mg/kg + GD 7–17 or GD 10–17 groups, where growth impairments were noted (54 ppm vs. 25–26 ppm). In the 5 mg/kg + GD 15–17 group, where no adverse developmental effects were found, maternal PFOA levels were 16 ppm. In the same study, pregnant mice were treated with 5 mg/kg of PFOA from GD 1–17 and newborns were cross-fostered to control or treated dams. While an increased incidence of neonatal mortality was seen in the *in utero* + lactational exposure group, *in utero* exposure to PFOA alone was found to be sufficient to produce postnatal growth deficits and developmental delays.

White *et al.* (2007) further investigated the effects and critical windows of PFOA exposure on mammary gland development in the nursing mouse dams and their offspring. In this study, daily PFOA treatment with 5 mg/kg from GD 1–17 did not affect the number of live pups born, but did impair postnatal growth similar to that described previously (Lau *et al.*, 2006; Wolf *et al.*, 2007). A significant reduction in mammary differentiation among dams exposed to PFOA from GD 1–17 or from GD 8–17 was evident on postnatal day 10; whereas delays in epithelial involution and alterations in milk protein gene expression were observed on postnatal day 20. These findings suggested that mammary gland abnormalities

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and lactational interferences may, in part, play a role in the early growth deficits detected in the PFOA-exposed mouse neonates. In addition, PFOA-exposed female pups displayed stunted mammary gland epithelial branching and growth at postnatal days 10 and 20, with no progression of duct epithelial growth evident over this period.

Since PFOA is a PPAR-α agonist, several studies have looked at the potential role of this pathway on mammary gland development and function. PPAR-α is not critical for mammary development in the neonate, as PPAR-α null mice exhibit normal mammary gland development and function (Lee *et al.*, 1995). Therefore, any effects of gestational exposure to PFOA on neonatal mammary tissue probably do not involve this pathway. In contrast, overexpression of PPAR-α causes impairment of normal differentiation of the mammary gland during pregnancy and lactation without significantly affecting milk production (Yang *et al.*, 2006). White *et al.* (2007) also noted little change in β-casein or α-lactalbumin following exposure to PFOA. Further, Qi *et al.* (2004) and Jia *et al.* (2005) suggested that the PPAR-binding protein (PBP) may be involved since PBP-deficient mammary glands exhibit retarded development during puberty (ductal branching, alveolar density), as well as differentiation during pregnancy and lactation. Further work is needed to fully understand the role of the PPAR pathway on the mammary gland. In addition, it will be imperative to ascertain the physiological significance of this developmental insult in future studies; for instance, would the findings reported by White *et al.* (2007) reflect a transgenerational effect of PFOA, as *in utero* exposure may lead to a disturbed lactation function in the F_1-generation and subsequent growth deficits in the F_2-generation? Alternatively, an extended period of mammary gland development may be important in the later susceptibility to mammary tumors.

The biochemical or physiological events underlying the PFOA-induced deficits in neonatal viability and development are not well understood. Despite being less effective than PFOS, the recent finding of a PFOA–DPPC interaction is intriguing and may lend support to the respiratory distress hypothesis (Xie *et al.*, 2007), although, these results must await confirmation with *in vivo* studies. Alternatively, results from Abbott *et al.* (in press) indicate that the PPAR-α molecular signaling pathway is a contributing factor. These investigators used a PPAR-α–KO mouse to determine if this molecular signal is required for PFOA-induced developmental toxicity. Similar to previous findings with CD-1 mice, *in utero* exposure of WT mice to PFOA led to neonatal mortality and deficits in postnatal developmental. However, survival of mouse pups till weaning was not affected by PFOA in the PPAR-α-null mouse. Maternal factors such as genetic background did not contribute to this difference in postnatal survival, as PFOA also caused neonatal death in heterozygous pups born to KO or WT dams. Taken together, these results suggest that PPAR-α signal is required for PFOA-induced postnatal lethality and expression of one copy of the gene is sufficient for this effect. Gene expression profiles from fetal mouse lung and liver further substantiated the involvement of PPAR-α signaling in PFOA developmental toxicity (Rosen *et al.*, unpublished data). In this study, RNA samples from fetal tissues were collected at term after pregnant dams were exposed to 0, 1, 3, 5, or 10 mg/kg/day of PFOA throughout pregnancy and evaluated by microarray analysis. Expression of genes related to fatty acid catabolism was altered in a robust and dose-dependent manner in both fetal liver and lung. These included genes associated with lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, retinol metabolism, proteosome activation, and inflammation. These changes are consistent with transactivation of PPAR-α, although, transactivation of other nuclear receptors such as CAR and farnesoid X receptor were suggested as well. By and large, the PFOA-induced changes in gene expression described in this study mirrored those reported previously with liver from adult male rats (Guruge *et al.*, 2006; Martin *et al.*, in press), thereby lending support to the intraspecies extrapolation with these biochemical endpoints.

The developmental toxicity of the 8–2 TA in the rat has been evaluated by Mylchreest *et al.* (2005). This TA is known to be metabolized (Fasano *et al.*, 2006; Kudo *et al.*, 2005) or biodegraded (Dinglasan *et al.*, 2004) to PFOA. Considering that little to no adverse reproductive outcomes were detected with PFOA in the rat (*vide supra*), it is not surprising to find only transient ill effects with the 8–2 TA. The no-observed-adverse-effect level (NOAEL) was estimated at 200 mg/kg/day. In light of more recent evidence (Fenton *et al.*, 2007; Lau *et al.*, 2006; White *et al.*, 2007; Wolf *et al.*, 2007), it would be interesting to see if the 8–2 TA will produce positive findings in the mouse model, in a fashion similar to N-EtFOSE. However, in such studies one must be cautious to interpret the reproductive toxicity data derived from the mouse. Fluorotelomer alcohols are known to be metabolized to a multitude of poly- and perfluorinated acids (Dinglasan *et al.*, 2004; Martin *et al.*, 2005). More recently, Henderson and Smith (2007) reported the presence of both PFOA and PFNA in maternal, fetal, and neonatal mouse serum following 8–2 TA treatment on GD 8. Hence, the resultant developmental toxicity will likely reflect the mixed or additive effects of PFOA and PFNA.

Information concerning the potential developmental effects of other PFAAs is less abundant and largely preliminary. A two-generation reproduction study with PFBS was conducted in the rat by Butenhoff and Lieder (2006) using a treatment scheme similar to that of PFOA (Butenhoff *et al.*, 2004b) and at doses ranging from 30 to 1000 mg/kg. PFBS treatment did not affect fertility or reproduction among the P or F_1-generation rats. Postnatal survival, growth, and development of pups in both F_1- and F_2-generations were not affected with the exception of a slight delay in the onset of puberty and weight gain for the F_1-males in the highest dose group. These findings are in concert with a general lack of overt toxicity of

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PFBS in the adult rats (Lieder and Butenhoff, 2006c) and may be related to the efficient elimination of this chemical in rats (Table 5). Another four-carbon chemical, PFBA has similar short half-life estimates (Table 5). Das *et al.* (2007) reported on the effects of PFBA exposure in mice during pregnancy. PFBA elevated maternal liver weight following full gestational exposures of 175 and 350 mg/kg in a manner similar to PFOS (Thibodeaux *et al.*, 2003) and PFOA (Lau *et al.*, 2006). However, in contrast to PFOS and PFOA, PFBA did not produce adverse effects on neonatal survival or postnatal growth. These findings are consistent with the relatively mild systemic toxicity reported in the adult rats which consisted chiefly of reduced body weight, increased liver weight, and decreased serum cholesterol (Lieder *et al.*, 2007).

Interestingly, among all the PFAAs evaluated thus far, the six-carbon chemical PFHS has the longest estimated half-life in humans and among the longest estimated in monkeys (Table 5). A reproductive and developmental toxicity study of PFHS was conducted in rats by York (2003). No treatment-related effect was reported on the fertility and reproductive outcomes or on viability and growth of the offspring. A NOAEL of 10 mg/kg/day was therefore estimated for the developmental effects of PFHS. The teratogenicity of the 10-carbon PFAA, PFDA, was assessed in mice (Harris and Birnbaum, 1989). Consistent with all other PFAA studies thus far, PFDA did not produce malformations and the developmental toxicity observed (increased fetal mortality and reduced body weight of live fetuses) was seen only at doses that were maternally toxic.

Immunotoxicity

Yang *et al.* (2000, 2001, 2002a, 2002b) were first to report the immunotoxic potential of PFOA in the mouse. PFOA given in the diet to male C57Bl/6 mice for 7–10 days led to decreased body weight, elevation of liver weight, and decreases in thymus and spleen weight (both absolute and relative weights). The time course of thymic and splenic atrophy was similar to that of PFOA-induced hepatomegaly and peroxisome proliferation. The numbers of thymocytes and splenocytes were decreased > 90% and about 50%, respectively, by PFOA treatment, and are likely related to an inhibition of cell proliferation. Concomitant with the effects on thymus and spleen, these investigators also observed a dramatic decrease in adipose tissue after exposure to PFOA, which reflected a loss of fat from the adipocytes (Xie *et al.*, 2002). The effects of PFOA were dose dependent. In addition to altering splenic cell density, PFOA was shown to be immunosuppressive in both *in vivo* and *ex vivo* systems (Yang *et al.*, 2002a). The primary humoral response to horse red blood cell immunization was prevented by PFOA pretreatment while *ex vivo* spleen cell proliferation in response to both T- and B-cell activation was attenuated by the fluorochemical. A subsequent study by DeWitt *et al.* (2007) largely confirmed these findings and were consistent with toxicogenomic studies and others that indicated suppression of inflammatory response by PFOA (Guruge *et al.*, 2006; Martin *et al.*, in press; Rosen *et al.*, unpublished data; Taylor *et al.*, 2002, 2005). In addition, Fairley *et al.* (in press) examined the effects of PFOA dermal exposure on the hypersensitivity response to ovalbumin (OVA) in mice and demonstrated increased IgE when PFOA and OVA were coadministered. OVA-specific airway hyperreactivity was increased significantly in these animals with an increased pleiotropic cell response characterized by eosinophilia and mucin production. These results thus suggested that PFOA is immunotoxic and its exposure may augment the IgE response to environmental allergens. The immunomodulating action of PFOA appeared to be mediated by the PPAR-α signaling pathway, as the reductions in thymus and spleen weight and the number of thymocytes and splenocytes caused by PFOA in the WT mice was not observed in the PPAR-α-null KO mice (Yang *et al.*, 2002b). Responses similar to those evoked by PFOA in the WT and KO mice were also observed after treatment with the classical PPAR-α agonist, WY-14,643, providing further evidence for a role of PPAR-α signaling pathway in the PFOA effects. As described in the previous section on liver toxicity, it is noteworthy that, unlike the effect of WY-14,643, PFOA-induced hepatomegaly was not abolished in the PPAR-α null mice. This finding raises the possibility of other (non-PPAR-α) biochemical signals that may mediate the hepatotoxic effects of PFOA. Indeed, results from a recent study by Wan and Badr (2006) have suggested that hepatocyte-specific retinoid X receptor-alpha plays a role in the anti-inflammatory response to PFOA.

Following withdrawal of PFOA from the diet, spleen and thymus weights return to normal within 5 and 10 days, respectively, in contrast to the more persistent effect on liver weight and hepatic peroxisome proliferation. The relatively prompt recovery of the spleen and thymus from PFOA treatment is rather surprising, considering that the half-life of this chemical in the mouse (CD-1) was estimated at 15–20 days (Lau *et al.*, 2005). In fact, the recovery in liver was more in line with the compound half-life. However, adipose tissue atrophy resulting from PFOA treatment, recovers at an even faster pace, beginning 2–5 days after chemical withdrawal (Xie *et al.*, 2003). Hence, additional studies to correlate the immunotoxic responses of PFOA with body burden of the chemical and to account for the uncoordinated processes responsible for the immunotoxic, hypolipidemic, and hepatotoxic effects of PFOA are warranted.

Hormonal Effects

Langley and Pilcher (1985) and Gutshall *et al.* (1988) provided the earliest reports on the effects of PFAA on thyroid hormones. They observed that a single dose of PFDA significantly reduced T4 and T3, lowered body temperature, and depressed heart rate in rats but T4 replacement failed to reverse the hypothermia produced by PFDA. Additional mechanistic studies revealed that PFDA decreased serum levels

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of thyroid hormones by reducing the responsiveness of the hypothalamic-pituitary-thyroid (HPT) axis and by displacing circulating hormone from their plasma protein binding sites (Gutshall *et al.*, 1989). Despite hormonal deficits in circulation, activity of the thyroid hormone-sensitive liver enzymes, glycerophosphate dehydrogenase and malic enzyme, were elevated by PFDA, leading the investigators to conclude that PFDA-treated rats were not functionally hypothyroid at the tissue level.

More recently, several studies (Lau *et al.*, 2003; Luebker *et al.*, 2005b, Seacat *et al.*, 2003; Thibodeaux *et al.*, 2003) have shown a depression of serum T4 and T3 in PFOS-exposed rats (adults, adults during pregnancy, and neonates), although a corresponding elevation of TSH through feedback stimulation of the HPT axis was absent. When measurement of free T4 (fT4) was carried out using an equilibrium dialysis step prior to the standard radioimmunoassay (ED-RIA), fT4 levels in PFOS-treated rats were found to be comparable to those of controls (Luebker *et al.*, 2005b). In a subsequent study, Chang *et al.* (in press) further elaborated on the merits of using ED-RIA to eliminate the negative bias of fT4 determination introduced by analog methods, primarily due to the high affinity for protein binding by PFOS. Moreover, in an acute exposure study, this group observed a decrease in total T4 (tT4), a transient increase in fT4, a transient decrease in TSH in circulation, and an increase in urinary excretion of labeled tracer from ^{125}I-T4 over the course of 24 h following a single dose of PFOS. These findings are consistent with the hypothesis advanced by Gutshall *et al.* (1989), suggesting that, similar to PFDA, PFOS may act by displacing thyroid hormones from their binding proteins in circulation. Based on a lack of response in serum fT4, TSH, and liver malic enzyme, Chang *et al.* (2007) surmised that short-term exposure (3 days to 3 weeks) to PFOS did not suppress the functional thyroid status in rats. However, these investigators also cautioned about the physiological significance of depressed tT4 and T3 produced by PFOS, particularly the long-term sequelae from chronic exposure to the chemical. On-going research to address these issues should provide a more definitive resolution in the near future.

While thyroid hormone imbalance has been reported in animal studies, corresponding findings have not been reported in humans in limited studies using PFOA (Emmett *et al.*, 2006b; Olsen *et al.*, 2003b) or PFOS (Inoue *et al.*, 2004). Continuing medical surveillance studies with repeated measures of thyroid hormone levels and additional biomonitoring studies in the future are needed.

In addition to thyroid hormone disruption, changes in sex steroid hormone biosynthesis by PFAA have been reported. Results from several studies (Biegel *et al.*, 1995; Bookstaff *et al.*, 1990; Cook *et al.*, 1992; Liu *et al.*, 1996) showed that administration of PFOA to adult male rats for 14 days led to a decrease in serum and testicular testosterone and an increase in serum estradiol levels. The latter was likely associated with increased hormone synthesis in the liver through induction of hepatic aromatase. Furthermore, these hormonal alterations have been implicated in the induction of Leydig cell adenomas seen in rats chronically exposed to PFOA (Biegel *et al.*, 2001). In a MCF-7 breast cancer cell system, Maras *et al.* (2006) recently described the estrogen-like properties of 6–2 and 8–2 TAs, but these potential "xenoestrogens" appeared to act through a mechanism different from that of 17β-estradiol, the classic reference for estrogenic action. The implication of these findings must await confirmation from *in vivo* studies in the future. Benninghoff *et al.* (2007) have described an estrogenic mechanism of PFOA to promote hepatocellular carcinoma in rainbow trout. In addition, PFNA, PFDA, and PFUA were shown to be estrogenic *in vivo*, based on a vitellogenin induction bioassay. Preliminary results thus indicated that these PFAAs might be weak xenoestrogens in the environment. Similar to thyroid hormones, no significant changes in reproductive hormones have been associated with serum levels of PFOA in humans (Olsen *et al.*, 1998).

Underlying Biochemical Effects

The causative biochemical events that lead to the adverse health outcomes after exposure to PFAA are largely undefined. Recent advances in genomics and bioinformatics have provided toxicologists with a powerful research tool to address these unknowns. Indeed, several papers have been published to examine the gene expression profiles in tissues (mostly liver) of rats and mice exposed to PFOS and PFOA (Guruge *et al.*, 2006; Hu *et al.*, 2005a,b; Martin *et al.*, in press; Rosen *et al.*, unpublished data). By and large, the findings from these diverse studies reached reasonable concordance. For instance, several categories of genes have been commonly altered by both PFAAs. These include peroxisome proliferation, fatty acid metabolism, lipid transport, cholesterol synthesis, proteosome activation and proteolysis, cell communication, and inflammation. Some of these alterations confirm previous findings. The agonistic properties of PFAAs on PPAR-α are well supported (Haughom and Ikeda *et al.*, 1985, 1987; Haughom and Spydevold, 1992; Intrasuksri *et al.*, 1998; Kudo *et al.*, 2000, 2005; Maloney and Waxman, 1999; Pastoor *et al.*, 1987; Sohlenius *et al.*, 1992; Van Raferghem *et al.*, 1988). As mentioned previously, a number of investigators have further compared the differential activation of PPAR isoforms (including human, mouse, and rat PPAR-α, PPAR-β, and PPAR-γ) by various perfluorinated chemicals with transient transfection cell assays (Shipley *et al.*, 2004; Takacs and Abbott, 2007; Vanden Huevel *et al.*, 2006). Thus far, results show that PPAR-α is the most likely target of PFOA and PFOS, with the former having more transactivity than the latter in both human and mouse isoforms. The transactivation of PPAR-γ or PPAR-β/δ was at a much lower extent. On-going research to provide a complete set of toxicological comparisons among all the relevant PFAAs will enhance the risk assessment capability for these chemicals

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and support the search for potential replacements of eight-carbon PFAAs in commerce.

The alteration of genes related to fatty acid metabolism by PFOA and PFOS correlates well with results from previous *in vivo* studies (Kudo *et al.*, 1999; Singer *et al.*, 1990; Sohlenius *et al.*, 1993; Van Raferghem *et al.*, 1988). Similarly, changes in genes responsible for mitochondrial metabolism by these fluorochemicals are supported by previous reports (Berthiaume and Wallace, 2002; Cai *et al.*, 1996; O'Brien and Wallace, 2004; Starkov and Wallace, 2002). In addition, results from studies that implicate interference with cell membrane function (Hu *et al.*, 2002, 2003), in part by inhibition of gap junction communication (Upham *et al.*, 1998), are also consistent with changes in gene expression in the liver induced by PFOS and PFOA.

FUTURE DIRECTIONS

There has been a great deal of progress in the last few years in understanding the toxicology and distribution of PFAAs in the environment, wildlife, and humans. However, there remain many questions. While monitoring studies have clearly shown the presence of PFAAs worldwide, the sources and pathways of exposure are unknown. In addition, more standardized analytical methods are needed in order to understand historical and future trends in exposure.

It is clear that there are vast species differences in the toxicokinetics of PFAAs. As summarized in Table 5, progress has been made in defining the elimination half-life for a variety of PFAAs in common laboratory animal models, as well as humans. However, there is a paucity of information for many PFAAs that have wide environmental distributions (i.e., PFNA and PFDA), as well as for most PFAAs in wildlife species. Further research is also needed in understanding the biological basis for the species differences in elimination. The putative involvement of multiple families of organic anion transporters is promising but much work is needed to confirm this. It is clear that an understanding of body burden is crucial for the interspecies extrapolation of toxicological effects. While some progress has been made in the development of pharmacokinetic models (e.g., Anderson *et al.*, 2006; U.S. EPA, 2005), additional investigation on this subject will be most warranted.

There have also been significant advances in descriptive toxicology for a variety of PFAAs as well as studies of the potential mode of action for some of the toxicological responses. Additional descriptive studies are needed to further characterize potential target organs, to elucidate the long-term consequences of prenatal exposures, and to elaborate on the role of chain length and functional group (perhaps by structure–activity relationship) in the toxicology of this class of compounds. In addition, further research is needed to understand the potential long-term consequences of the effects on thyroid hormone levels. Finally, it is critical to explore the possible modes of action of these compounds (beyond the apparent PPAR transactivation) and to determine whether any of these may be species-specific, thereby providing a sound basis for health risk assessment of these chemicals as a class.

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