Perfluorinated Compounds

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Abstract Perfluorinated compounds such as the perfluoroalkyl acids (PFAAs) and their derivatives are important man-made chemicals that have wide consumer and industrial applications. They are relatively contemporary chemicals, being in use only since the 1950s and until recently have been considered as biologically inactive. However, during the past decade, their global distribution, environmental persistence, presence in humans and wildlife, and adverse health effects in laboratory animals have come to light, generating scientific, regulatory, and public interest on an international scale. This chapter will provide a brief overview of recent advances in understanding environmental and human exposure, toxicology, and modes of action for this class of compounds in animal models, as well as a summary of epidemiological findings to date.

Keywords Perfluorinated compounds · Perfluoroalkyl acids · Perfluoroalkyl sulfonates · Perfluorooctane sulfonate · Perfluoroalkyl carboxylates · Perfluorooctanoic acid · Perfluoroalkyl phosphonates

Introduction

Perfluorinated compounds are organic chemicals in which all hydrogens of the carbon chain are substituted by fluorine atoms. Generally, there are two types of perfluorinated compounds, the perfluoroalkanes that are used primarily for

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oxygenation and respiratory ventilation clinically and the perfluoroalkyl acids (PFAAs) that are the subject of this chapter. Environmentally relevant PFAAs are a family of about 30 chemicals that consist of a carbon backbone typically 4–14 atoms in length and a charged functional group composed of either sulfonates, carboxylates, or phosphonates (and to a lesser extent, phosphinates). While many (>100) derivatives of PFAAs (such as alcohols, amides, esters, and acids) are used for industrial and consumer applications, they can be degraded or metabolized to PFAAs as end-stage products. Thus, PFAAs, rather than their intermediates or derivatives, have drawn the most public attention and research interest. The most widely known PFAAs are the eight-carbon (C8) sulfonate (perfluorooctane sulfonate, PFOS) and carboxylate (perfluorooctanoic acid, PFOA), although the C4 and C6 sulfonates, as well as the C4, C6, and C9 carboxylates, have also been used in commerce. The perfluoroalkyl phosphonates (PFPAs) are fairly new for this class of chemicals. They are typically used as leveling and wetting agents, and defoaming additives in the production of pesticides. They were considered biologically inert by the US Environmental Protection Agency until 2006. Mabury and coworkers [1] were the first to report the detection of PFPAs in the environment, and to date, only one additional paper has been published to describe the pharmacokinetics of PFPAs in the rat [2]. Discussion in this chapter will therefore focus on perfluoroalkyl sulfonates (PFSAs) and carboxylates (PFCAs), for which information is readily available. Indeed, in the past few years, an increasing number of reports concerning PFAAs have appeared in the literature, and over a dozen salient topical reviews have been published to highlight the biomonitoring, toxicological, and epidemiological findings for these compounds [3–15]. Hence, this chapter will provide a brief, overarching description of these perfluorinated chemicals, and readers are encouraged to consult the particular review papers for specific details.

Background

Naturally occurring fluorinated organic chemicals are rare. PFAAs are fairly contemporary chemicals, synthesized since the 1950s by electrochemical fluorination of an organic feedstock or by telomerization of tetrafluoroethylene units. Neither of these manufacturing processes is precise, thus yielding a family of target compounds as well as unintended by-products of various carbon-chain lengths and isomers [16]. The unique hydrophobic and oleophobic nature of PFAAs makes these chemicals ideal surfactants [17]. There are over 200 known industrial and consumer applications of PFAAs, including water, soil and oil repellents, lubricants, fire-fighting foams, and emulsifiers used in the production of fluoropolymers. PFAAs were initially considered metabolically inert. They are stable, nonreactive, and do not undergo metabolism. Structurally, they resemble fatty acids. In fact, in the early literature, they were often referred to as perfluorinated fatty acids. They bind to hepatic fatty acid-binding proteins, competing for

binding with the natural ligands [18–20]; however, PFAAs are not known to participate in biochemical reactions that use fatty acids as substrate. They also bind to other proteins in serum, liver, kidney, and testes [21–26]. PFAAs are known to serve as substrates and regulators of renal and hepatic organic anion transporters [27, 28] and as activators of nuclear receptors that regulate fatty acid and glucose metabolism and transport [29–38]. They have also been shown to alter cell membrane fluidity and membrane function via their surfactant effects [39–47], to interfere with intercellular communication through inhibition of gap junctions [48–50], and to disrupt mitochondrial bioenergetics and biogenesis [51–54, 203].

Historically, production of PFAAs is dominated by the C8 chemical, PFOS, and to a lesser extent, PFOA. In 2002, the major manufacturer of PFOS in the USA phased out production of this chemical, leading to a precipitous drop in global production. However, this market void has since been replenished to some extent by Asian (e.g., China) and European producers in recent years. In addition, increased production of PFOA has made it the most common PFAA in commerce. In 2006, the US Environmental Protection Agency initiated the PFOA Stewardship Program with industry, with the goal of eliminating emissions and product content of these chemicals by 2015. To accomplish this goal, shorter carbon-chain PFAAs such as perfluorobutane sulfonate (PFBS) and perfluorohexanoic acid (PFHxA), as well as different chemistries (such as ammonium 4,8-dioxa-3*H*-perfluorononanoate, ADONA [55]), are poised to replace the C8 compounds in commerce.

Environmental Fate and Transport of PFAAs

A summary of global production, emission, and environmental inventory for PFOS was provided by Paul *et al*. [56]. PFCAs are primarily derived from degradation of fluorotelomer alcohols and polyfluoroalkyl phosphates in the atmosphere, soil, and wastewater treatment plant (WWTP) sludge and from landfills [57–67]. The metabolic pathways for some fluorotelomer alcohols in *in vitro* and *in vivo* systems have been summarized [68–70]. These chemicals can be transferred from water to soil and taken up by plants [71, 72]. Armitage *et al*. [73] have recently described a model of global fate and transport of PFCAs. In general, two routes have been proposed to account for the global distribution of PFAAs, including remote regions such as the Arctic. The first hypothesis suggests an indirect atmospheric transport of PFAA precursors and subsequent degradation to PFSAs and PFCAs [74–77], whereas a second hypothesis favors a direct release of PFAAs and long-range ocean water transport [78–81] to the remote locations. While these two hypotheses remain a subject of debate, it is likely that both routes are involved in the distribution of these contaminants. At a local level, Davis *et al*. [82] have constructed a model of PFOA migration from a point source, where PFOA vapor and particulates

are emitted in the air, transported by wind, deposited on the surface soil, and leached to surface water and then to groundwater within the aquifer.

Environmental Exposure of PFAAs

Several reviews have previously summarized the biomonitoring studies on PFAAs in the environment, in wildlife, and in humans [3, 7, 9, 11, 12]. This chapter will only highlight the key features of these descriptions and provide an update of findings since the publication of these reviews. PFAAs are globally distributed and ubiquitously detected in all environmental media, including air, surface and drinking water, soil, sediment, and sludge recovered from wastewater treatment plants (WWTP). A number of Asian, European, and North American studies have documented PFAA particulates and telomer alcohol precursors in indoor air (~450 ng/m^3), house dust (~10–40 μg/g), and ambient air (~800 pg/m^3) [83–90]. Similarly, PFAAs in environmental and tap water have been detected worldwide, and a summary of these findings is available in recent reviews [91, 92]. Typically, PFAAs found in lakes and rivers may range from 0.3 to 2,600 ng/L and in drinking water from 0.1 to 70 ng/L [93–97] (although a PFOA level as high as 3,550 ng/L has been reported in West Virginia [98]). In that regard, health and safety guidelines for PFOS and PFOA in drinking water have been issued recently by various regulatory agencies [99–103]. Recent discoveries of PFAA-contaminated biosoils applied in farms and fields in Germany [104, 105] and in the USA [66, 67, 106] have raised significant research interests and public concerns [107]. These biosoils are derived from sewage sludge generated from municipal and industrial WWTP. Various studies have documented detection of PFAAs in both inflow and outflow of these treatment plants, suggesting that WWTP can be significant sources of these chemicals in the environment [108–112].

Since the seminal findings reported by Giesy and Kannan in 2001 [113–115], numerous studies have documented the widespread contamination of PFAAs in wildlife from the North Pole to the South Pacific. Several recent reviews have summarized these monitoring findings [7, 9] and described the various trends of bioaccumulation [116–120]. Human exposure to PFAAs was initially reported by occupational biomonitoring conducted by the manufacturers [121–123], followed by detection in selected samples from Red Cross blood donors [124]. Subsequently, reports from the National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention (CDC) revealed significant detection of PFOS, PFOA, perfluorohexane sulfonate (PFHxS), and perfluorononanoic acid (PFNA) in the US general population [125–128]. Serum levels of these chemicals from several NHANES reports are summarized in Table 1 and compared to levels in sera from occupational exposures and exposures of residents at PFAA-contaminated areas. Serum PFAA levels are understandably highest in the production workers who are routinely exposed to these chemicals. In the general population, levels of PFOS are higher than those of PFOA, and the

Table 1 Summary of major PFAAs reported in the National Health and Nutrition Examination Surveys (NHANES) and examples of exposure to residents at contaminated areas and occupational exposure [105, 121–123, 125–128, 158, 208]

	PFOS	PFOA	PFHxS	PFNA
NHANES 1999–2000	30.4	5.2	2.1	0.5
NHANES 2001–2002*	20.8	3.7	2.8	0.6
NHANES 2001–2002* (children)	30.5–42.5	6.1–7.6	4.5–18.7	0.6–1.2
NHANES 2003–2004	20.7	4.0	1.9	1.0
NHANES 2005–2006	17.1	3.9	1.7	1.1
NHANES 2007–2008	13.2	4.1	2.0	1.5
Arnsberg, Germany 2006	23.4–30.3	5.1–12.7	1.3–2.7	–
Little Hocking, WV 2007	23	368	–	–
Production workers	1,500–2,000	500–1,000	~500	Unknown

*denote values derived from pooled samples

levels of PFHxS and PFNA are substantially lower. By and large, profiles of PFAA exposure in humans comparable to those seen in the USA have been reported with other populations worldwide [129–136]. Among the four NHANES reports, there is a general trend for decline of serum PFAAs, with the exception of PFNA, the levels of which have doubled in the recent surveys. Such a declining trend is consistent with another report that follows the levels of PFAAs in sera from Red Cross blood donors [137]. Although the data are limited, levels of PFOS and PFOA appear to be higher in children than in adults, suggesting that children may be a vulnerable subpopulation for chemical exposure [126, 136]. Indeed, exposure to PFAAs appears to begin early in life, as PFOS and PFOA in particular have been detected in umbilical cord blood and in breast milk [138–147]. The routes of human exposure to PFAAs remain a subject of debate, although they likely involve migration of chemical from food packaging [148, 149], food intake [150–152], drinking water, and house dust. Exposure models from a recent review [153] suggested that food intake is the major exposure pathway for the general population, while drinking water exposure is dominant for populations near contaminated sites. Tolerable daily intake (TDI) of 100–300 ng/kg (body weight) for PFOS and 0.1–3 μg/kg for PFOA in food has been recommended by the European food regulatory authorities [154–156], and health advisories for PFOS (0.2 μg/L) and PFOA (0.04–0.4 μg/L) in drinking water have been issued by federal and state regulatory agencies in the USA [101–103] and in Europe [157] (Table 2). Considerably higher levels of PFAAs have also been detected among residents in areas, particularly in West Virginia, where contamination in drinking water was found [105, 158], although it is heartening to note that these levels began to decline once mitigation steps were taken.

Pharmacokinetic Disposition of PFAAs

Because of their physicochemical characteristics, most PFAAs possess unique pharmacokinetic properties based on their carbon-chain lengths and functional

Table 2 Recommended tolerable intake (TDI) levels of PFAAs by regulatory bodies

	PFOA	PFOS	References
US Environmental Protection Agency (drinking water)	0.4 μg/L	0.2 μg/L	[103]
Minnesota Department of Health (drinking water)	0.3 μg/L	0.3 μg/L	[102]
New Jersey Department of Environmental Protection (drinking water)	0.04 μg/L	–	[101]
Drinking Water Commission of German Ministry of Health (drinking water)	100 ng/kg BW	100 ng/kg BW	[100]
European Food Safety Authority (food)	1.5 μg/kg BW	150 ng/kg BW	[154]
UK Committee on Toxicity in Food, Consumer Products and the Environment (food)	3 μg/kg BW	300 ng/kg BW	[155, 156]
German Federal Institute for Risk Assessment (food)	100 ng/kg BW	100 ng/kg BW	[157]

BW body weight

groups, as well as the species, gender, and age of the subjects evaluated. Animal studies (typically with rodents) of various PFAAs have shown that they are well absorbed orally (within hours), are not metabolized, undergo extensive enterohepatic circulation, and readily cross the placenta. PFAAs are poorly eliminated (especially the long-chain PFAAs), and elimination is primarily via urinary excretion [159–161]. These chemicals are distributed mainly to the serum, kidney, and liver, with liver concentrations being several times higher than serum concentrations (with the exception of PFBA, perfluorobutanoate). The volume of distribution at steady state suggests that PFAA distribution is likely extracellular. These chemicals also have high binding affinity for a variety of proteins [17–25].

The elimination half-lives of several PFAAs in animal models and humans are summarized in Table 3 [158, 161–175, 179, 203]. In general, the rate of elimination is enhanced with decreasing carbon-chain length. Thus, the elimination half-lives of PFBS, PFBA, and PFHxA are shorter than those of PFOS, PFOA, and PFNA among most species examined. The lone exception is PFHxS, where limited data indicate that it does not follow this trend. Across the species evaluated, the rate of elimination is slowest in humans, with the half-life rank order being humans > monkey > mouse > rat. Few gender differences in PFAA clearance are observed in humans or monkeys. In contrast, marked sex differences are observed in the rat, particularly with PFCAs. Most notably, the half-lives of PFNA and PFOA in female rats are 20 and 50 times shorter than those in males, respectively. Interestingly, the gender difference in PFOA elimination is developmentally regulated in rats. The rapid elimination seen in female rats develops between 3 and 5 weeks of age [167]. Smaller sex differences are generally seen with PFSAs. On the other hand, the sex differences in PFAA elimination are consistently much smaller in the mouse than in the rat. In that regard, the mouse resembles humans more closely and thus provides a rodent model more amenable for extrapolation of results from toxicological studies, particularly those focusing on reproductive and developmental toxicity where pharmacokinetics in the pregnant females play a major role in determining the exposure of the conceptus.

Table 3 Summary of serum/plasma elimination half-lives of various PFAAs

Species	PFBS		PFHxS		PFOS		PFBA		PFHxA		PFOA		PFNA	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Rat	4.0 h	4.5 h	0.8 days		62–71 days	38–41 days	1.0–1.8 h	6–9 h	0.42 h	1.0 h	2–4 h	4–6 days	1.4 days	30.6 days
Mouse			25–27 days	28–30 days	31–38 days	36–43 days	3 h	12 h			17 days	19 days	25.8–68.4 days	34.3–68.9 days
Rabbit											7 h	5.5 h		
Dog											8–13 days	20–30 days		
Monkey	3.5 days	4.0 days	87 days	141 days	110 days	132 days	1.7 days		0.1–0.8 days	0.2–1.5 days	30 days	21 days		
Human	1 month		8.5 years		5.4 years		3 days				2.3–3.8 years			

PFBS perfluorobutane sulfonate; *PFHxS* perfluorohexane sulfonate; *PFOS* perfluorooctane sulfonate; *PFBA* perfluorobutanoate; *PFHxA* perfluorohexanoic acid; *PFOA* perfluorooctanoic acid; *PFNA* perfluorononanoic acid. References for rat [161, 163, 167, 170, 172–175, 179, 203], mouse [170, 171, 175, 179, 203], rabbit [167], dog [160], monkey [164, 170, 172, 173, 179, 203] and humans [158, 168, 169, 171]

The mechanisms underlying the sex difference for PFCA elimination in the rat are presently under active investigation and likely are related to renal clearance of the chemicals [176]. A number of studies have implicated the involvement of organic anion transporters (OATs) that are regulated by sex hormones [161, 177, 178]. A recent study by Weaver *et al*. [27] indicated that OAT1 and OAT3 are involved in renal secretion of perfluoroheptanoic acid (PFHpA), PFOA, and PFNA, while OATP1a1 contributes to the reabsorption of PFOA, PFNA, and perfluorodecanoic acid (PFDA). At present, it is not clear whether the diminished sex difference noted in the mouse for PFCA clearance is also related to these transporters. Ljubojevic *et al*. [178] reported that the renal expression pattern of OAT2 in the mouse resembles that in the rat, and both are under regulation by sex hormones. In contrast, Buist *et al*. [177] indicated that renal OAT2 mRNA levels are markedly higher in female than in male rat, but there is no sex difference in OAT2 expression in the mouse kidney [180]. Additional studies are needed to resolve this issue. On the other hand, it is encouraging that these transporters (such as OAT4 and urate transporter 1, URAT1) have been shown to play a key role in renal reabsorption of PFCA in humans [181], suggesting a potential common mechanism across species.

Toxicological Findings with PFAAs

The toxicology of PFOS and PFOA has been extensively reviewed in the past few years [5, 6, 9, 10]. Readers are encouraged to consult with these reviews for detailed descriptions. This chapter will highlight findings from primarily mammalian models, provide an update of information, and focus on recent discoveries with other PFAAs. Generally speaking, six major adverse effects have been identified with PFAA exposure in laboratory studies: tumor induction, hepatotoxicity, developmental toxicity, immunotoxicity, endocrine disruption, and neurotoxicity.

Tumor Induction

Neither PFOS, PFHxA, PFOA, nor PFDA is known to be mutagenic [5, 182–185]. A recent study suggested the genotoxic potential of PFOA in HepG2 cells but was likely associated with oxidative stress and ROS production [186]. However, DNA damage was observed only at high concentrations of PFOA (50–100 μM). In addition, while intracellular ROS production was increased by PFOA and PFOS in another study [187], no corresponding DNA damage was observed. PFBS and PFHxA did not generate ROS or DNA damage. PFNA caused DNA damage only at a cytotoxic concentration.

Significant positive trends were noted in the incidence of hepatocellular adenoma in rats exposed to high dietary doses of PFOS (20 ppm or 1.5 mg/kg/day) for 2 years [182], although this evidence was considered equivocal for carcinogenicity [156].

Significant increases in mammary fibroadenoma and adenoma were seen in the low-dose groups (0.5 and 2 ppm), but there was no dose–response relationship with this effect, as increases in the 5 ppm dose group were not statistically significant and a slight decrease of tumor incidence was seen at 20 ppm.

A significant increase in the incidence of mammary fibroadenoma in rats exposed to dietary doses of 30 or 300 ppm (16.1 mg/kg/day) PFOA for 2 years was also reported [188], but these findings were subsequently refuted by a review panel [189]. On the other hand, significant increases in the incidence of liver adenomas, pancreatic acinar cell tumors, and testicular (Leydig) cell adenomas were seen in rats exposed chronically to 300 ppm of PFOA in diet [5]. This liver–pancreas–testes triad of tumors is typical of many agonists of the peroxisome proliferator-activated receptor-alpha (PPARα). The hepatocellular tumors are most likely related to activation of the PPARα molecular pathway. Tumors observed in the testis have been associated with elevation of hepatic aromatase activity, leading to increases of serum estradiol, in concert with testicular growth factors [190, 191]. The mechanism(s) responsible for the PFOA-induced pancreatic tumors remain the subject of active investigation. In addition, using a unique tumor model of rainbow trout, Tilton *et al*. [192] showed that chronic PFOA exposure for 30 weeks resulted in enhanced liver tumor incidence, although the dose employed in this study (1,800 ppm or 50 mg/kg/day) was quite high.

Hepatotoxicity

Hepatomegaly primarily involving hepatocytic hypertrophy is perhaps a hallmark PFAA effect in laboratory animals, produced by PFOS, PFHxS, PFBS, PFDA, PFNA, PFOA, PFHpA, PFHxA, and PFBA [5, 9, 182, 185, 188, 193–198] and is likely associated with peroxisome proliferation. Chronic exposure to high doses of PFOA and PFOS led to hepatocellular vacuolation, degeneration and necrosis, accumulation of lipid droplets related to altered lipid metabolism and transport, and tumor induction [182, 188]. PFAAs, particularly the PFCAs (C6–C10), are known to induce hepatic peroxisomal fatty acid β-oxidation in rats and mice [194, 199, 200], leading to reduction of serum triglycerides and cholesterol [5, 201]. The hypolipidemic effect of PFOA is due, in part, to the reduced synthesis of cholesterol and an enhanced oxidation of fatty acids in the liver. However, despite an enhanced β-oxidation of fatty acids, Kudo *et al*. [202] have demonstrated an increase of glycerolipids and triglycerides in liver of rats treated with PFOA, which may be linked to increased *de novo* synthesis [202, 303]. The increase in triglyceride synthesis and accumulation in the liver, but a reduced level in circulation, prompted these investigators to suggest impaired hepatic secretion of triglycerides. In view of recent findings regarding the effects of PFAAs on various transporter proteins, this hypothesis is entirely conceivable, although future research on hepatic transporters that traffic lipids and other macromolecules are needed to clarify this issue. On the

other hand, the potential adverse effects of the apparent "fatty liver" produced by PFOA remain to be determined.

Recent toxicogenomic analyses of rodent livers after exposure to PFOA and PFOS revealed a strong PPARα signature [204–206] and supported previous findings from an *in vitro* system [207]. The involvement of PPARα signaling was further confirmed with studies using a transgenic mouse model where PPARα function was deleted [36, 209–210]. However, in contrast to the responses elicited by the potent PPARα agonist WY14, 643, where 99% of the observed changes in gene expression were eliminated in the PPARα-null mice, about 20% of the PFOA-induced genomic responses were still detected in the PPARα-knock out mice, suggesting a PPARα-independent mechanism for the perfluorinated chemical [211]. Further examination of the PPARα-independent genomic responses implicated another nuclear receptor, the constitutive androstane receptor (CAR) [35, 38], which is known to be involved in xenobiotic metabolism. Potential involvement of other nuclear receptors such as pregnane X receptor (PXR) and liver X receptor (LXR) in PFAA-induced hepatic responses is currently under active investigation [33, 213]. These nuclear receptors (PPAR, CAR, PXR, and LXR) are important regulators of fatty acid transport and metabolism, xenobiotic metabolism, and cholesterol and glucose homeostasis, which can readily account for some of the cellular responses elicited by PFAAs.

Developmental Toxicity

The adverse reproductive and developmental effects derived from exposure to PFAAs have been summarized in detail in previous reviews [6, 9]; thus, only salient features and updates of these effects are described here. Exposure to PFOS or PFOA during pregnancy in rats and mice produced overt anatomical defects in offspring (such as cleft palate) only at high doses, while other morphological abnormalities noted in fetuses chiefly reflected developmental delays [214–217]. Early pregnancy loss was noted with PFOA or PFBA exposure but only at very high doses, and the etiology of this effect is not clear. No frank terata or fetotoxicity was observed after gestational exposure to PFBA or PFDA [218, 219]. In contrast, when dams exposed to PFOS were allowed to give birth, dose-dependent deleterious effects were seen in the newborns [220, 221]. Although all pups were born alive and active, those exposed to high doses (5 or 10 mg/kg) became moribund within the ensuing hours and died soon afterward. Survival improved with lower PFOS exposure, but postnatal growth of surviving pups was somewhat stunted, and reductions of circulating thyroid hormones were observed. The PFOS-induced hypothyroxinemia was confirmed in a recent study that correlated PFOS accumulation with hormonal imbalance [222]. In addition, a critical prenatal window of PFOS exposure toward late gestation was noted for the adverse postnatal effects [223], potentially implicating immaturity of the newborn lung and pulmonary insufficiency as causes for neonatal death. However, no evidence of changes in lung phospholipids or markers

for alveolar differentiation was found to support underdevelopment of the neonatal lung [224]. Alternatively, because PFOS itself is a surfactant, one can speculate that the synthetic chemical may interact with endogenous pulmonary surfactant, thereby interrupting its function to facilitate the inflation of the neonatal lung after birth. The observation of a preferential accumulation of PFOS in the fetal lung adds support to this hypothesis [225]. Importantly, Xie *et al*. [41, 45] reported that PFOS (and to a lesser extent, PFOA) had a strong tendency to interact with dipalmitoylphosphatidylcholine (DPPC) and partition into lipid bilayers. Because DPPC is a major component of pulmonary surfactant, it is possible that such PFOS–DPPC physical interactions may interfere with the physiological function of pulmonary surfactant. However, the evidence available at present is still circumstantial, and definitive results from *in vivo* studies are needed to confirm respiratory distress related to impaired lung surfactant function as a pathophysiological mechanism for the PFOS-induced neonatal mortality.

In contrast to PFOS, the reproductive toxicological findings in rats exposed to PFOA were rather unremarkable [228], which might have been related to the unique ability of female rats to clear the chemical efficiently (half-life of 2–4 h, Table 3). Indeed, in mice, where elimination of PFOA is considerably less rapid (half-life of 17 days, Table 3) and chemical accumulation occurs in the females, a profile of neonatal mortality was noted when pregnant dams were exposed to high doses of PFOA (>10 mg/kg) [216]. The newborn mice appeared to survive slightly better and died less abruptly than those exposed to PFOS, perhaps partly due to a lesser effect of PFOA in interrupting lung surfactant function [41]. Among the surviving mice exposed to lower PFOA doses, neonatal growth deficits and developmental delays were seen. Evaluation of mammary differentiation of the nursing dams indicated significant reductions at postnatal day 10, suggesting that abnormal lactation function may play a role in the growth retardation of their offspring [229]. However, results from a cross-fostering study indicated that the developmental deficits seen in mouse pups were largely due to prenatal exposure to PFOA [230]. Interestingly, although growth impairment was noted in neonates exposed to relatively high doses (3–10 mg/kg) of PFOA during gestation, those exposed to low doses (0.01–0.3 mg/kg) displayed significant increases in body weight and serum insulin and leptin concentrations during mid-life [231]. In contrast, PFOA exposure of adult mice at comparable doses did not produce any weight effect, indicating a specificity of chemical perturbation during developmental periods. These paradoxical findings are intriguing and will require further elaboration but may reflect the subtle alterations of developmental programming of metabolic processes, where the adverse outcomes are manifested latently at adult ages, akin to a theory advanced by Barker [232]. In addition, mammary gland development in female mouse offspring exposed to PFOA was significantly delayed, leading to persistent abnormalities [233]. The functional sequelae of these morphological abnormalities are currently unknown, and future work should explore whether the lactational capability of these female mice (exposed to PFOA prenatally) is negatively impacted.

PFOA is known to be a PPARα agonist. In view of the important roles of this nuclear receptor in reproduction and development [234], Abbott and colleagues investigated the role of the PPARα molecular pathway in PFOA-induced developmental toxicity using a transgenic PPARα-null mouse model [235]. Wild-type (129 S1/SvlmJ) mice were slightly more sensitive to PFOA toxicity than CD-1 mice [216], but both strains displayed similar neonatal mortality, growth deficits, and developmental delays. However, these adverse outcomes were markedly attenuated in the PPARα-null mice, suggesting that PFOA developmental toxicity is dependent on expression of PPARα. In contrast, results from a follow-up study by the same investigators indicated that the developmental toxicity of PFOS was not dependent on this nuclear receptor function [236], thus possibly delineating distinct modes of action between PFCAs and PFSAs regarding their developmental effects. This contention is further supported by a recent developmental study with PFNA, where a near-identical profile of PPARα-dependent responses was detected [237].

Compared to long-chain PFAAs (>C8), the short-chain chemicals are much less toxic to the developing animal, in part due to their faster rate of clearance (Table 3). Thus, even at very high doses of PFBA (350 mg/kg, intended to match the body burden of PFOA), neither neonatal survival nor postnatal growth was compromised, although maternal hepatomegaly was detected (indicating the effectiveness of the PFBA dose regimen) and neonatal liver weight was transiently elevated [218]. A similar lack of overt reproductive and developmental toxicity has been reported for PFHxA [185], PFBS [196], PFHxS [239], and ADONA [55].

Immunotoxicity

DePierre and colleagues were the first to demonstrate the immunotoxic effects of PFOA in the C57BL/6 mouse, where thymic and splenic atrophy associated with an arrest of thymocyte and splenocyte proliferation and a marked reduction of cell populations were observed after subchronic dietary exposure to the chemical [240–242]. These effects appeared to be mediated by PPARα, as the PFOA-elicited alterations of lymphoid organ weight and cellularity were attenuated in the PPARα-null mice [243]. However, the precise role of this nuclear receptor and the extent of its involvement in the immunotoxicity of PFAAs have been challenged recently [244]. Fairley *et al*. [245] reported similar effects of thymic and splenic atrophy and decreased cellularities in BALB/c mice after dermal exposure to PFOA, along with an enhanced hypersensitive IgE response to ovalbumin. These results suggested that exposure to PFOA, although not allergenic itself, might enhance an individual's response to commonly encountered environmental allergens. Son *et al*. [246] administered PFOA in drinking water to ICR mice (50–250 ppm) and demonstrated an immunomodulatory effect of the chemical that altered T-lymphocyte phenotype in the spleen and thymus and elevated gene expression of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). Similar PFOA-induced immunomodulation was also observed

in C57BL/6J and C57BL/6N female mice, where IgM antibody synthesis was suppressed and IgG titer was elevated in response to a sheep red blood cell (SRBC) challenge [247]. While thymic and splenic atrophy and decreased IgM production were consistently seen in CD-1 (ICR) male mice given PFOA by oral gavage, no effect on production of anti-SRBC antibodies was noted in the rat. Moreover, an increase of serum corticosterone, increases in numbers of peripheral blood neutrophils and monocytes, and a decrease in absolute lymphocyte numbers were found in the PFOA-treated mice. This prompted the investigators to surmise that the immunotoxic responses were secondary to systemic toxicity of the perfluorinated chemical and the attendant stress evoked [248]. However, results from further investigation of this possibility with adrenalectomized mice (thereby removing the element of stress response) indicated that suppression of humoral immunity by PFOA was independent of the elevated serum corticosterone and not likely associated with stress [249].

Adverse immunological outcomes from exposure to PFOA are extended to other PFCAs such as PFNA [250–252]. Subchronic exposure to PFNA in mice led to reduction of lymphoid organ weight, cell cycle arrest, and apoptosis in spleen and thymus, accompanied by impaired production of IL-4 and interferon-γ by splenic lymphocytes and upregulation of IL-1β. Similar PFNA-induced histopathological changes were seen in the rat, along with alterations of serum cytokines, which in turn activated the mitogen-activated protein kinase (MAPK) signaling pathways that modulate the immune system. In addition, the splenic apoptosis caused by PFNA might be associated with oxidative stress, as the level of hydrogen peroxide was increased and superoxide dismutase activity and Bcl-2 protein levels were dramatically decreased in the spleen.

Immunotoxic responses are also detected in rodents treated with PFSA. Suppression of humoral immunity after exposure to a perfluorinated insecticide that can be metabolized to PFOS was reported [253]. Similar immunotoxic findings were extended to mice directly exposed to high doses of PFOS in a diet that produced a serum level of 340 μg/ml, although the effects were less pronounced than those seen with PFOA [254]. Significant immunomodulatory effects of PFOS were also seen in rats, although changes were generally less robust than those seen in mice [255]. Results from low-dose PFOS studies in mice were less definitive. Peden-Adams *et al.* [256] reported that exposure to PFOS by oral gavage in B6C3F1 mice, which produced serum concentrations of 0.09–0.67 μg/ml, suppressed T-cell-dependent (to SRBC challenge) or T-cell-independent (to trinitrophenyl conjugated lipopolysaccharide challenge, TNP-LPS) IgM antibody responses. Similarly, He and colleagues [257–259] showed that PFOS reduced subpopulations of lymphocytes in lymphoid organs and decreased natural killer cell activity in C57BL/6 mice, at exposure that yielded higher serum concentrations of 0.67–121 μg/ml. However, in a more recent study with B6C3F1 mice where PFOS was given in a diet that produced serum concentrations of 0.048 μg/ml, no adverse effects on adaptive immunity were evident [260]. The investigators speculated that routes of chemical administration might have played a role in these apparently disparate findings. Additional work is needed to clarify the low-dose effects of PFAAs on immune functions.

Interestingly, in a preliminary study where PFOS was given to mice at a dose that produced plasma levels of 0.19–0.67 μg/ml, thymus and spleen weights were not altered but the responses of these animals (emaciation and mortality) to influenza A virus challenge were increased significantly, suggesting that host resistance to pathogens was compromised by exposure to the perfluorinated chemical [261]. Detailed immunological mechanisms responsible for this observation remain to be explored. Gestational exposure to PFOS in mice has also been shown to suppress immune function later in life, indicating that the developing immune system is sensitive to PFAA insult and that these functional deficits might not be apparent until the animals reach adulthood [262].

In addition to their effects on adaptive immunity, influences of PFAAs on innate immunity have also been characterized [263, 264]. Short-term treatment with PFOS or PFOA led to significant reduction of white blood cells involving lymphopenia, reduction of macrophages in bone marrow, and augmented inflammatory responses to LPS. Dietary administration of the PFAAs also altered hepatic immune status by enhancing the number of intrahepatic immune cells, presumptive erythrocytes progenitors, and hepatic levels of erythropoietin.

Endocrine Disruption

The endocrine disruptive potentials of PFAAs have been summarized in a brief review [265]. In general, alterations of thyroid hormones and sex steroid hormones have been shown after exposure to primarily PFOS and PFOA, although PFDA-induced reductions of thyroid hormones have also been reported [266, 267]. Seacat *et al*. [268] first described alterations of circulating thyroid hormones in cynomolgus monkeys during chronic exposure to PFOS, which entailed significant reductions of triiodothyronine (T3) (by about 50%) that were greater and more consistent than those observed for total thyroxine (tT4, seen only in females) at serum levels of PFOS that reached 70–170 μg/ml. Values of thyroid-stimulating hormone (TSH) were quite variable and did not indicate compensatory elevation (by about twofold) until the end of the exposure period. This profile of primarily T3 reduction without an appreciative TSH response does not reflect classical hypothyroidism; rather, it resembles aspects of nonthyroidal illness syndrome, which is typically associated with a number of severe illnesses.

PFOS-induced alterations of thyroid hormones were confirmed in adult rat models [205, 214, 269]. However, in contrast to the monkeys, reductions of circulating tT4 were more pronounced and consistent than those of T3. These hormonal changes were abrupt. At an oral gavage dose that produced a serum PFOS level of 88 μg/ml, marked depressions of tT4 (by 50–75%) were seen within 1–3 days. In fact, it is interesting to note that thyroid hormones seem to be altered when serum PFOS level reaches the 70–90 μg/ml range, regardless of animal species (rat or monkey) or route of administration (diet, gavage, or drinking water), suggesting that PFOS effects on serum tT4 are directly related to

endogenous concentrations of the chemical. Furthermore, similar to the observation with monkeys, reductions of serum tT4 in rats failed to activate the hypothalamic–pituitary–thyroid (HPT) feedback mechanism to produce significant elevations of serum TSH.

A pronounced fall in serum tT4 with corresponding increases in TSH is typically noted during the course of pregnancy. Exposure of pregnant rats to PFOS exacerbated these hormonal shortfalls (both tT4 and T3) without further elevating the levels of TSH [214]. The effective dose of PFOS for tT4 reduction corresponded to maternal serum concentrations of 14–26 μg/ml (unpublished results). A similar effect of PFOS on serum tT4 was also seen in the pregnant mouse, although this rodent species appears to be less sensitive than the rat, with significant changes noted only at the doses that produced serum levels of 114–261 μg/ml [214, 270].

In utero exposure to PFOS led to postnatal mortality in the rat neonates, in a dose-dependent fashion [220]. Among the surviving pups, the ontogenetic increases of serum tT4 during the first 2 weeks of life were delayed or attenuated, with a lowest effective dose corresponding to serum PFOS levels of 60–72 μg/ml at 5 days of age and 30 μg/ml by 2 weeks. In contrast, only small changes were noted in the ontogenetic rises of T3 or TSH. Similar effects of PFOS on thyroid hormones in rats during development were also reported by Luebker *et al*. [221], where significant dose-related reductions of tT4 (46%) were noted on postnatal day 5 (serum PFOS level of 36 μg/ml). Consistent with the previous study, serum TSH remained unaltered. This lack of change in TSH was further corroborated by histological and morphometric evaluations of the fetal and neonatal thyroid glands, which indicated normal number and size distribution of follicles, as well as normal follicular epithelial cell height and colloid area, despite the PFOS-induced tT4 deficits [270]. In a cross-fostering study, Yu *et al*. [222] showed that pre- and postnatal exposure led to the most consistent effect of hypothyroxinemia and significant tT4 deficits were detected at rather low serum levels of PFOS (7–9 μg/ml). Although PFOS-related neonatal mortality was also observed in the mice, the ontogenetic increases of serum tT4 were not altered significantly in this species, a finding consistent with the relative insensitivity of mice to this chemical regarding thyroid hormone disruption [220].

In addition to the evaluation of PFOS effects on serum tT4, several studies have examined levels of circulating free T4 (fT4), the pool of hormone that is available for uptake by target cells and actions [214, 220, 221]. In these studies, fT4 was typically measured by analog radioimmunoassays (RIA) and reductions of free hormone produced by PFOS were similar to those observed in tT4. However, when the measurement of fT4 was carried out by including an equilibrium dialysis step prior to the standard RIA (ED-RIA), fT4 levels in the PFOS-treated rats were found to be comparable to those of controls [221]. Indeed, Chang *et al*. [271] further elaborated the merits of ED-RIA to eliminate the negative bias of fT4 determination produced by analog methods, primarily due to the high affinity for protein binding by PFOS. In light of these findings, the values of fT4 reported in previous PFOS studies may require reevaluation, and future investigations of these perfluorinated chemicals should employ this reference method.

Mechanisms underlying the PFOS-induced hypothyroxinemia are still under active investigation but do not likely involve altered *de novo* biosynthesis of the hormones or compromised integrity of the HPT axis. Yu *et al*. [269] reported no significant effects of PFOS on sodium iodide symporter gene expression (for iodide uptake) or thyroid peroxidase activity (for iodination of thyroglobulin and coupling into iodothyronine) in the thyroid gland. Chang *et al*. [272] showed that release of TSH from the pituitary in response to *ex vivo* TRH stimulation was not altered by PFOS exposure. In addition, when the hypothyroid drug propylthiouracil (PTU) was coadministered with PFOS, compensatory elevations of serum TSH that were equivalent to those elicited by PTU treatment alone were seen, indicating that the HPT axis in the PFOS-exposed rats was intact and fully functional. Importantly, in an acute exposure study, these investigators observed an abrupt fall of tT4, a transient increase in fT4 (determined by ED-RIA), and a corresponding transient decrease in TSH in circulation, accompanied by a brief increase in the expression of the gene for thyroid hormone-metabolizing enzyme UDP-glucuronosyltransferase 1A (UGT1A) in the liver, along with an increased 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*. [267] with PFDA and suggest that PFOS may act by displacing thyroid hormones from their transport proteins in circulation. Indeed, this hypothesis was confirmed by Weiss *et al*. [273] who demonstrated that perfluorinated chemicals (including PFOS) are capable of competing with T4 and displacing hormone binding to the human thyroid hormone transport protein transthyretin (TTR). Hence, a plausible scenario can be constructed to account for the hypothyroxinemic effects of PFOS in the rats. PFOS in circulation competes with T4 and displaces the hormone from binding to TTR (the primary thyroid hormone transport protein in the rat), initially leading to a transient elevation of fT4 (within 6 h) and a brief compensatory decrease of TSH. Concomitantly, hepatic metabolism of the hormone by UGT1A is enhanced (presumably in response to the transient elevation of free hormone), which results in an increase of hormonal clearance and urinary excretion of iodide. As the fT4 level returns subsequently to normal (within 24 h), a new equilibrium is reached between normal complements of fT4 and TSH, but a net reduction of total T4 (resulted from protein-binding displacement and metabolism) ensues. A lack of significant change in TSH receptor gene expression in the thyroid gland is also consistent with the transient nature of change in TSH [269, 270]. Moreover, maintenance of fT4 levels is indirectly supported by a general lack of thyroid hormone-specific responses in the rat [219, 272, 274], suggesting that the functional thyroid status has not been compromised significantly by short-term exposure to the chemical. However, the biochemical and physiological sequelae derived from long-term displacement of T4 as a result of chronic PFOS exposure have not been vigorously investigated. Significant elevation of TSH in monkeys after 6 months of daily treatment with PFOS does raise the possibility of compensatory responses of the HPT axis after prolonged chemical exposure [268].

Effects of PFOA on thyroid hormones are generally not as well characterized as those of PFOS. Butenhoff *et al*. [164] evaluated the toxicity of PFOA in male

cynomolgus monkeys and reported that T3 was reduced significantly within 5 weeks of treatment when a serum level of 158 μg/ml was attained. Recovery of T3 deficits was noted upon cessation of PFOA exposure. Serum tT4, fT4, or TSH was not altered throughout the study. The preferential effects of PFOA on serum T3 and a lack of TSH compensatory response are similar to those observed with PFOS. Martin *et al*. [205] showed that serum tT4 and fT4 (measured by analog RIA) were markedly (by about 80%) and abruptly (1 day after oral gavage treatment) depressed by PFOA in adult male rats, while serum T3 was also reduced, though to a lesser extent (by 25%). In contrast, none of these thyroid hormones were affected by PFOA in mature female rats, primarily because these animals were able to clear the chemical effectively (Table 3), confirming that the endocrine disrupting effects of PFOA are directly related to endogenous accumulation of the chemical. PFOA may also act by displacing T4 from its binding protein, as the chemical has been shown to compete for binding to human TTR at a potency equivalent to that of PFOS [273]. Alternatively, based on a toxicogenomic analysis of rat liver after an acute exposure to PFOA, Martin *et al*. [205] suggested a possible role of peroxisome proliferators in the thyroid hormone imbalance, although this hypothesis has yet to be explored in detail.

In addition to thyroid hormone disruption, changes in sex steroid hormone biosynthesis by PFAAs have also been reported. Some of this information has been summarized previously [9]. In brief, PFOA has been shown to decrease serum and testicular testosterone and to increase serum estradiol in male rats, presumably via induction of hepatic aromatase [190, 275]. PFOS, PFOA, and telomer alcohols have been shown to exhibit estrogenic activity in cultured tilapia hepatocytes, yeast cells, and medaka hepatocytes [276–278] and to inhibit testicular steroidogenic enzymes [279, 341]. In addition, the long-chain PFAA perfluorododecanoic acid (PFdoDA) has recently been shown to decrease testosterone synthesis in male rats and to decrease serum estradiol and gene expression of estrogen receptors in the female rats, possibly through oxidative stress pathways [281–285].

Neurotoxicity

Slotkin *et al*. [286] characterized the neurotoxic potential of perfluorooctane sulfonamide (PFOSA), PFOS, PFBS, and PFOA in a neuronotypic PC12 cell model. PFOSA was found to enhance differentiation of cells into cholinergic and dopaminergic phenotypes, PFOS promoted the cholinergic phenotype at the expense of dopaminergic cells, PFBS suppressed differentiation of both phenotypes, and PFOA had little to no effect. Changes in synaptic transmission and inhibition of neurite outgrowth brought forth by PFOS were reported in cultured rat hippocampal neurons; the effects were more pronounced with PFSAs than PFCAs, and C8 being the optimum chain length [280, 287]. Subtle behavioral changes were noted in adult mice exposed to PFOS [288]. Expression of transcription factors, c-fos and c-jun, and calcium-dependent signals were altered in the hippocampus and cerebral cortex of

rats given PFOS [289]. However, after a single oral treatment of PFOS at doses (125–250 mg/kg) where convulsion was noted in rats and mice, no morphological changes were seen in the brain and changes of CNS neurotransmitter levels were not detected [290].

Although Butenhoff *et al*. [291] reported no significant developmental neurotoxicity associated with gestational and lactational exposure to PFOS, using the current testing guidelines, subtle effects have been shown in the brain after developmental exposure to PFAAs. Liu and colleagues have shown aberrant expression of genes involved in calcium signaling pathways, neuroactive ligand-receptor interactions, and long-term potentiation/depression in neonatal and adult brains exposed to PFOS during perinatal periods [292, 293]. Johansson *et al*. [294, 295] also demonstrated changes in proteins involved in neurogenesis and synaptogenesis in the developing mouse brain after neonatal exposure to PFOS or PFOA, which were accompanied by neurobehavioral defects in adulthood. Similar perturbed cognitive performance was also reported in an avian model after exposure to PFOS or PFOA *in ovo* [296]. Overall, investigation of PFAA neurotoxicity is only emerging. Because the blood–brain barrier is not completely closed to chemical trafficking until late in gestation (human) or postnatally (rodent), PFAAs may readily reach the immature brain to produce long-lasting effects. Hence, future work should focus on the developing nervous system to better explore the neurotoxic potential of these perfluorinated chemicals.

Modes of Action for PFAAs

A clear understanding of the key events involved in the mode of action (MOA) of an adverse outcome will be instrumental to health risk assessment of chemical exposure. Although the toxicities of PFAA exposure have been better characterized with animal models in the past decade, little progress has been made to clarify the MOA for these chemicals. The lone exception is activation of nuclear receptors by PFAAs, particularly PPARα, for which there is a preponderance of evidence. Wolf *et al*. [297] have compared the relative potency of various PFAAs using mouse and human PPARα reporter cell constructs, and their results are summarized in Table 4. In general, PFCAs are more active than PFSAs, the long-chain PFCAs (>C6) are more potent than the short-chain homologues, and mouse PPARα appears to be more sensitive than that of human. As discussed above, PPARα activation has been shown to be associated with carcinogenicity, hepatotoxicity, developmental toxicity, immunotoxicity, and perhaps even endocrine disruption in laboratory rodents. In fact, key events of the PPARα pathway may play a critical role in the interpretation of PFAA-induced tumors observed in the rodent model, as expert panels have previously surmised that this mode of action is not likely to be relevant for humans [298, 299]. Recent studies using humanized PPARα mice also supported this species difference [198, 300]. However, this assertion has recently been challenged [301], and a final verdict for human relevance of the PFAA-related tumor induction

Table 4 Comparative potency of PFAAs for PPARα [297]

Compound	C_{20max} (μM)	
	Mouse	Human
PFNA (C9)	5	11
PFOA (C8)	6	16
PFDA (C10)	20	No activity
PFHxA (C6)	38	47
PFBA (C4)	51	75
PFHxS (C6)	76	81
PFOS (C8)	94	262
PFBS (C4)	317	206

must await further clarification. In the same vein, the relevance of other PFAA-evoked, PPARα-dependent effects (such as disruption of lipid metabolism, hepatotoxicity, developmental toxicity, and immunotoxicity) to human health risks will require additional scrutiny. In addition to PPARα and other nuclear receptor pathways, several possible mechanisms for PFAA toxicity have been suggested. These include oxidative stress [253, 286, 302–304], effects on other cell signaling pathways [252, 305, 306], and epigenetic changes [307]. Other putative mechanisms undoubtedly will emerge as investigation in this area intensifies in the future.

Epidemiology

Occupational biomonitoring studies have been conducted for PFAAs over the past several decades. Olsen and colleagues reported a lack of changes in serum hepatic enzymes, cholesterol, lipoproteins, or thyroid hormones associated with serum PFOS levels less than 6 μg/ml in the fluorochemical production plant workers (only few individuals had levels greater than 6 μg/ml) [122, 123]. Little change in mortality rate was seen in production workers, although the risk of death from bladder cancer was increased (with only three cases reported) [308]. Further analysis with larger cohorts of all living current and former employees did not support an association between bladder or other cancers and PFOS exposure [309, 310]. In fact, examination of health claim data (episodes of care) showed that illness and disorders reported among workers in the PFOS production plant were comparable to that of the non-PFOS-related work forces [311]. These investigators have also extended their epidemiological examination to PFOA occupational exposure and reported no significant associations between serum PFOA and reproductive hormones in men [121], serum cholesterol, or low-density lipoprotein; although high-density lipoprotein and free T4 were negatively associated with PFOA, triglycerides and T3 tended to be positively associated. Several explanations were offered by these authors to account for the inconsistent and marginal changes observed [313]. Results from a mortality study showed no association between PFOA exposure and liver, pancreatic, or testicular cancer (a tumor triad seen in

rodent models) in the production workers, but an inconsistent association was noted with prostate cancer, cardiovascular disease, and diabetes [314]. In reviewing 30 years of medical surveillance of PFOA production workers, Costa *et al*. [315] concluded that no specific clinical disease was associated with exposure to the fluorochemical, and biochemical parameters reflecting hepatic, renal, and hormonal functions appeared to be within reference ranges; however, a significant association of serum cholesterol and uric acid with PFOA was evident, indicating that further investigation of PFOA influences on intermediary metabolism is warranted. Based on the available information, Butenhoff *et al*. [316] provided a health risk characterization of PFOA exposure for the general population and suggested a wide "margin of exposure" that would represent a substantial protection of children, adult, and the elderly. Similarly, an epidemiological study of workers exposed to surfactant containing PFNA for more than a decade has been conducted, and no adverse clinical effects were detected from occupational exposure to this fluorochemical [317].

Prompted by the toxicity findings in animal models, a myriad of epidemiological investigations in general population have been launched over the past 5 years. The reproductive and developmental effects of PFOS and PFOA have by far attracted the most attention. Examining "time-to-pregnancy" among 1,240 pregnant women in the Danish National Birth Cohort from 1996 to 2002, Fei *et al*. [318] suggested that PFOA and PFOS exposure might be associated with a reduction of fecundity. Fetal growth, birth weight, and size have been negatively associated with maternal blood levels of both PFOS and PFOA in several cohort studies [139, 319, 320], although absence of such effects has also been reported in other studies [321–323]. Stein *et al*. [324] examined self-reported pregnancy outcomes in Mid-Ohio Valley residents between 2000 and 2006 (2,000–5,000 cases) and identified modest associations of PFOA with preeclampsia and birth defects and of PFOS with preeclampsia and low birth weight. Nolan *et al*. [325] evaluated a smaller Ohioan cohort exposed to PFOA-contaminated drinking water and found that PFOA was associated with maternal anemia and dysfunctional labor but not with congenital anomalies or delivery complication. The strengths and weaknesses of these studies and interpretations of their findings have been addressed in a thorough review [13]. Follow-up evaluations of infants and children in the Danish National Birth Cohort indicated no associations between prenatal exposure to PFAAs and risk of infectious diseases, developmental milestones, and behavioral and motor coordination problems [326–328]. A recent British cohort study also did not find an association between maternal PFAA exposure and altered age at menarche of their offspring [329].

The "C8 Health Project" was launched to investigate the potential health effects of exposure to PFOA from drinking water in the Mid-Ohio Valley areas. Associations of PFOS and PFOA with serum lipids and uric acid were reported among the local residents, although those with type II diabetes were not indicated [330–333]. A number of exploratory cross-sectional studies analyzing NHANES results have also been conducted. Lin *et al*. [334, 335] suggested that serum PFAAs were associated with altered glucose homeostasis, indicators of metabolic syndrome,

and elevated liver enzymes (particularly in obese subjects); Nelson *et al*. [336] indicated a positive association between serum PFAAs and cholesterol; Melzer *et al*. [337] showed a significant association of PFOS and PFOA with thyroid disease; and Hoffman *et al*. [338] reported an increased odds ratio of attention deficit hyperactivity disorder with higher serum PFAA levels. Typically, the odds ratios for these clinical disorders range from 1 to 2, although the trends are statistically significant. In light of the structural resemblance of PFAAs to fatty acids and their biochemical actions on PPAR pathways, iterative research with animal models to better elucidate the effects of these fluorochemicals on intermediary metabolism is a logical next step. On the other hand, in Danish cohorts, high PFAA levels were associated with fewer normal sperm [339], but no association was found with risk of prostate, bladder, or liver cancer in this population [340]. Steenland *et al*. [14] recently reviewed the epidemiological literature for PFOA and noted that available data were insufficient to draw firm conclusions regarding the role of fluorochemicals for any of the diseases of concern.

Summary

Since a smattering of papers on PFAAs first appeared in the literature before the turn of the century, there has been an explosion of studies on these chemicals just in the last 5 years. This chapter provides a summary of our current understanding of PFAA exposure in the environment and in human populations, their toxicological profiles in laboratory animals, and epidemiological findings in general and targeted populations. Improved sensitivity and reproducibility of analytical methods to readily detect multiple PFAAs at the parts per trillion level have afforded cross-study comparisons and the ability to track changes in trends. Continuous biomonitoring studies should provide updates regarding changes in PFAA exposure in the future. These changes in exposure are likely to occur as PFAAs in commerce (such as the C8 chemicals) are replaced by the short-chain homologues or entirely different chemistries. Descriptive characterization of the overt toxicity of PFAAs (particularly the long-chain homologues) in animal models should open the door for further investigation of the more subtle biochemical and physiological perturbations potentially elicited by these chemicals. These combined advances will facilitate an informed and reliable risk assessment of human and environmental health for these perfluorinated chemicals. However, two issues must be considered in extrapolating the data from animal studies to human health risks. As shown in Table 3, the accumulation of these chemicals varies tremendously between congeners of different chain length and functional group, and most importantly, the species differences between rodents and humans are profound. Simple correction factors will not be sufficient or appropriate to address these differences. Rather, a better understanding of the cellular and molecular mechanisms (such as the involvement of transporters, i.e. OATs) that control the clearance of these chemicals as well as possible homology between species is needed. Secondly, as indicated

by NHANES and numerous other monitoring studies, multiple PFAAs are detected in human and wildlife populations, and the profile of PFAA exposure is expected to change with time. Thus, the combined health risks of a mixture of these chemicals must be considered. In closing, many discoveries have been made with this intriguing family of chemicals in the past decade, but much more information will be needed to ascertain their adverse health effects.

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