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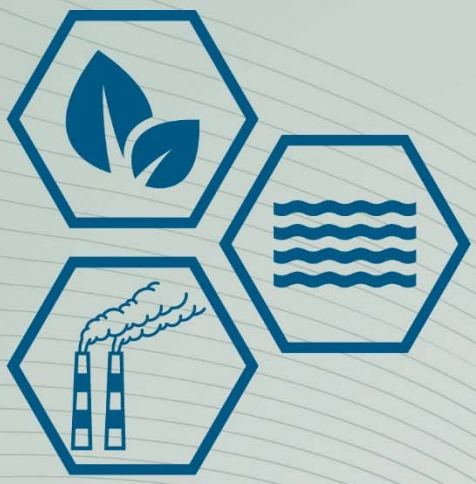

Toxicological Profile for Perfluoroalkyls

Released May 2021

Last Updated March 2020

U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

CS274127-A

DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance due to associated acute, intermediate, and chronic exposures;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, intermediate, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed through September 2018. New studies were added in 2019 following public comment, and NHANES data were updated. Staff from the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

VERSION HISTORY

Date	Description
May 2021	Final toxicological profile released
March 2020	Toxicological profile last updated
June 2018	Draft for public comment toxicological profile released
August 2015	Draft for public comment toxicological profile released
May 2009	Draft for public comment toxicological profile released

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ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

This toxicological profile on perfluoroalkyls discusses information on 10 perfluoroalkyls that have been measured in the serum collected from a representative U.S. population 12 years of age and older in the National Health and Nutrition Examination Survey (NHANES) 2003–2004 (Calafat et al. 2007b), as well as 2 compounds (PFBA and PFHxA) that have been identified in other monitoring studies. More recent NHANES monitoring studies have not evaluated additional perfluoroalkyl compounds (CDC 2019). The perfluoroalkyl compounds discussed in the profile include:

Compound	Acronym	CAS Registry Number
Perfluorobutanoic acid	PFBA	375-22-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroundecanoic acid	PFUnA	2058-94-8
Perfluorododecanoic acid	PFDoDA	307-55-1
Perfluorobutane sulfonic acid	PFBS	375-73-5
Perfluorohexane sulfonic acid	PFHxS	355-46-4
Perfluorooctane sulfonic acid	PFOS	1763-23-1
Perfluorooctane sulfonamide	FOSA	754-91-6

Perfluoroalkyls can exist in several ionic forms, most commonly as the anionic form or acidic form. In the environment, perfluoroalkyls are found in the anionic form (ITRC 2017). The names for the anionic and acidic forms (e.g., perfluorooctanoate and perfluorooctanoic acid) are often used interchangeably even though there are differences in physical and chemical properties and behavior in the environment, and the same acronym is used for both forms (e.g., PFOA). ATSDR has opted to the utilize the same terminology as NHANES (i.e., the acidic form names).

The term “perfluoroalkyls” used throughout the toxicological profile is referring to at least one of these 12 compounds and the information may not be applicable to other perfluoroalkyl compounds.

1.1 OVERVIEW AND U.S. EXPOSURES

The perfluoroalkyls discussed in this profile primarily consist of perfluorinated aliphatic carboxylic acids (PFCAs) and perfluorinated aliphatic sulfonic acids (PFSAs). These substances have been used extensively in surface coating and protectant formulations due to their unique surfactant properties (Kissa

2001; Schultz et al. 2003). Major applications have included protectants for paper and cardboard packaging products, carpets, leather products, and textiles that enhance water, grease, and soil repellency (3M 1999; Hekster et al. 2003; Kissa 2001; Schultz et al. 2003), and in firefighting foams (Schultz et al. 2003). Perfluoroalkyls such as PFOA have also been used as processing aids in the manufacture of fluoropolymers such as nonstick coatings on cookware (DuPont 2008; EPA 2008a).

Perfluoroalkyls are human-made substances that do not occur naturally in the environment. The perfluoroalkyl substances discussed in this profile, especially PFOS and PFOA, have been detected in air, water, and soil in and around fluorochemical facilities. However, these industrial releases have been declining since eight companies began voluntarily phasing out the production and use of several perfluoroalkyls in the early 2000s (3M 2007b, 2008a, 2008b; Barton et al. 2007; Davis et al. 2007; DuPont 2008; EPA 2007a, 2008a, 2016a). PFOA and PFOS may still be produced domestically, imported, and used by companies not participating in the PFOA Stewardship program. Under the Toxic Substances Control Act (TSCA), EPA has proposed a significant new use rule (SNUR) for long-chain perfluoroalkyl carboxylate (LCPFAC) chemical substances and sulfonates to ensure that the manufacture, import, or processing of LCPFAC chemical substances for any discontinued uses cannot begin without EPA review. EPA essentially excluded the use or import of all LCPFAC chemical substances by proposing a SNUR for LCPFACs and sulfonates (EPA 2015). Data are becoming more available regarding current releases of shorter-chain perfluoroalkyls (perfluorinated carboxylic acids with six or fewer carbons and perfluorosulfonic acids with five or fewer carbons) that are now being used in surface treatment products or perfluoropolyethers that are used as a replacement for PFOA in emulsion polymerization. Environmental fate and toxicity research of newer replacement substances is ongoing (De Silva et al. 2016; Gomis et al. 2018; Kabore et al. 2018).

In the environment, some of the perfluoroalkyls discussed in this profile can also be formed from environmental degradation of precursor compounds released during the manufacture and use of consumer products containing perfluoroalkyls (D'eon and Mabury 2007; D'eon et al. 2009; Martin et al. 2006; Prevedouros et al. 2006). Under the PFOA Stewardship Program with the U.S. Environmental Protection Agency (EPA), eight major fluoropolymer producers have phased out PFOA, precursor substances that can degrade to long-chain perfluoroalkyls such as PFOA, and higher homologues from emissions and products (EPA 2008a, 2016a).

1. RELEVANCE TO PUBLIC HEALTH

Due to the strength of the carbon-fluorine bonds, perfluoroalkyls are very stable in the environment and are resistant to biodegradation, photooxidation, direct photolysis, and hydrolysis (3M 2000; EPA 2008a; OECD 2002, 2007; Schultz et al. 2003). The perfluoroalkyl carboxylic acids and sulfonic acids have very low volatility due to their ionic nature (Kissa 2001; Prevedouros et al. 2006; SPARC 2008). As a group, perfluoroalkyls are persistent in soil and water (3M 2000; Prevedouros et al. 2006). Perfluoroalkyls are mobile in soil and leach into groundwater (Davis et al. 2007). Volatile fluorotelomer alcohols may be broken down into substances like PFOA, and atmospheric deposition can lead to contamination of soils and leaching into groundwater away from point sources. Perfluoroalkyls have been detected in many parts of the world, including oceans and the Arctic, indicating that long-range transport is possible (Armitage et al. 2006; Barber et al. 2007; Prevedouros et al. 2006; Wania 2007; Wei et al. 2007a; Yamashita et al. 2005, 2008).

Perfluoroalkyls have been detected in all environmental media including air, surface water, groundwater (including drinking water), soil, and food. Human exposure may occur from all of these media. Contaminated drinking water led to increased levels of exposure to PFOA, PFOS, and other perfluoroalkyls for some populations residing near fluoropolymer manufacturing facilities (ATSDR 2008; Emmett et al. 2006a; Steenland et al. 2009b). Median PFOA serum levels (measured in 2005–2006) of 45,276 non-occupationally exposed individuals residing in southeastern Ohio and West Virginia who were exposed to PFOA via contaminated drinking water (Shin et al. 2011b) were approximately 6 times greater than the median serum PFOA concentration in a representative sample of the U.S. general population (2005–2006 NHANES data; CDC 2018). Serum levels of PFOA and PFOS in the general population of the United States have sharply declined in recent years as U.S. production of these substances ceased (CDC 2019). For example, the geometric mean concentrations of PFOA and PFOS in the general population were 5.2 and 30.4 ng/mL (ppb), respectively, in 1999–2000; in 2015–2016, PFOA declined by 70% to 1.56 ng/mL and PFOS declined 84% to 4.72 ng/mL (CDC 2018, 2019).

Based on environmental measurements and theoretical models, one study has proposed that the major exposure pathways for PFOS for the general population in Europe and North America are food and water ingestion, dust ingestion, and hand-to-mouth transfer from mill-treated carpets (Trudel et al. 2008). For PFOA, major exposure pathways were proposed to be oral exposure resulting from migration from paper packaging and wrapping into food, general food and water ingestion, inhalation from impregnated clothes, and dust ingestion. This includes exposure to 8:2 fluorotelomer alcohol in food packaging and air, which can be broken down into PFOA. PFOS and PFOA exposure pathways are proposed to be similar for children except that exposure from hand-to-mouth transfer from treated carpets is expected to

be much greater in children. Based on these exposure pathways, adult uptake doses estimated for high-exposure scenarios were approximately 30 and 47 ng/kg/day for PFOS and PFOA, respectively (Trudel et al. 2008). PFOS and PFOA doses estimated for children under the age of 12 under high exposure scenarios were 101–219 and 65.2–128 ng/kg/day, respectively. Since PFOA and PFOS are no longer produced or used in the United States, current exposure levels may be lower than those predicted by Trudel et al. (2008). A study by Vestergren and Cousins (2009) evaluated potential exposure to perfluorocarboxylate homologues for different populations and also concluded that dietary intake was the primary background exposure pathway for the general population, while inhalation of indoor air was the main exposure pathway for occupationally exposed individuals with estimated intakes >150 ng/kg/day. Although not well studied, the available absorption data (Fasano et al. 2005; Franko et al. 2012) suggest that dermal contact may also contribute to the overall perfluoroalkyl body burden.

Perfluoroalkyls have been detected in human breast milk and umbilical cord blood. The reported maximum concentrations of PFOS and PFOA measured in human breast milk samples from women living in Massachusetts (samples were collected in 2004) were 0.617 and 0.161 ng/mL, respectively (Tao et al. 2008b). Maximum concentrations of other perfluoroalkyls were <0.06 ng/mL. In most umbilical cord samples collected in 2004–2005 in Maryland, the maximum concentrations of PFOS and PFOA were 34.8 and 7.1 ng/mL, respectively (Apelberg et al. 2007a, 2007b). Other perfluoroalkyls have been detected less frequently.

1.2 SUMMARY OF HEALTH EFFECTS

The toxicity of PFOA and PFOS has been evaluated in a large number of studies of humans and laboratory animals; less toxicity data are available for other perfluoroalkyls. However, comparison of the toxicity of perfluoroalkyls across species is problematic due to differences in elimination half-lives, lack of adequate mechanistic data, species differences in the mechanism of toxicity for some endpoints, and differences in measurement of exposure levels between epidemiological and experimental studies. Table 1-1 lists half-lives for PFOA, PFOS, PFHxS, PFNA, PFBS, and PFBA for human, nonhuman primates, rats, and mice to illustrate some of the species differences. For example, for PFOA, the estimated elimination half-life is measured in years in humans and in hours in female rats.

Table 1-1. Summary of Estimated Elimination Half-lives for Select Perfluoroalkyls[a]

	Humans	Nonhuman primates	Rats	Mice
PFOA	2.1–10.1 years	20.1–32.6 days	Males: 44–322 hours Females: 1.9–16.2 hours	
PFOS	3.3–27 years	110–170 days	179–1,968 hours	731–1,027 hours
PFHxS	4.7–35 years	87–141 days	Males: 382–688 hours Females: 1.03–41.28 hours	597–643 hours
PFNA	2.5–4.3 years		Males: 710–1,128 hours Females: 33.6–58.6 hours	619.2– 1,653 hours
PFBS	665 hours	8.0–95.2 hours	2.1–7.42 hours	
PFBA	72–81 hours	40.3–41.0 hours	1.03–9.22 hours	2.79–13.34 hours

[a]See Table 3-5 for additional information and citations.

The mechanisms of toxicity of perfluoroalkyls have not been fully elucidated. There is strong evidence that many of the adverse effects observed in laboratory animals involve the activation of peroxisome proliferator-activated receptor-α (PPARα), which can mediate a broad range of biological responses (Issemann and Green 1990). There are species differences in the activation of PPARα; rats and mice are the most sensitive species and guinea pigs, nonhuman primates, and humans are less responsive. Although humans are less responsive to PPARα agonists, they do have functional PPARα. This may explain some of the species differences in perfluoroalkyl toxicity. PPARα-dependent mechanisms have been associated with a variety of effects, including hepatocellular hypertrophy, alterations in lipid metabolism, decreased pup survival, and some immune effects. However, there is evidence that PPARα-independent mechanisms are also involved in PFOA and PFOS toxicity, including liver and immune toxicity; it is not known if species differences exist for these mechanisms. In general, epidemiological studies use serum perfluoroalkyl levels as a biomarker of exposure, which contrasts with experimental studies that utilize dose, expressed in mg/kg body weight/day units, or air concentrations as the dose metric. Although physiologically based pharmacokinetic (PBPK) models have been developed for rodents and humans, these models are not sufficient to allow for direct comparisons between administered doses in laboratory animals and serum concentrations in humans.

***Effects in Humans*.** Perfluoroalkyls have been detected in the serum of workers, residents living near perfluoroalkyl facilities, and the general population. A large number of epidemiological studies have evaluated possible associations between perfluoroalkyl exposure and a wide range of adverse health outcomes. However, most of the studies have focused on PFOA and/or PFOS; fewer studies have evaluated a smaller number of potential health outcomes for the remaining 10 perfluoroalkyls included in

this toxicological profile. Most of the epidemiological studies lack exposure monitoring data, and there is a potential for multiple routes of exposure (inhalation and oral); however, most of the studies used serum perfluoroalkyl level as a biomarker of exposure. The three primary sources of this information are occupational exposure studies, studies of communities living near a PFOA manufacturing facility with high levels of PFOA in the drinking water, and studies of populations exposed to background levels of perfluoroalkyls (referred to as general population studies). In the studies examined, workers have the highest potential exposure to a specific perfluoroalkyl, followed by the highly-exposed residents such as residents in the Mid-Ohio Valley who have elevated levels of PFOA and background levels of other perfluoroalkyls, and then the general population. In one study of workers at the Washington Works facility in West Virginia, the arithmetic mean serum PFOA level in 2001–2004 was 1,000 ng/mL (Sakr et al. 2007a); the arithmetic mean PFOA level in highly-exposed residents (without occupational exposure) near this facility was 423 ng/mL in 2004–2005 (Emmett et al. 2006a). By comparison, the arithmetic mean concentration of PFOA in the U.S. population was 4.91 ng/mL in 2005–2006 (calculated by ATSDR from NHANES data reported in CDC 2013). Although a large number of epidemiological studies have examined the potential of perfluoroalkyls to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causality. Based on a number of factors (described in Section 2.1), the available epidemiological studies suggest associations between perfluoroalkyl exposure and several health outcomes; however, cause-and-effect relationships have not been established for these outcomes:

- Pregnancy-induced hypertension/pre-eclampsia (PFOA, PFOS)
- Increases in serum hepatic enzymes, particularly alanine aminotransferase (ALT), and decreases in serum bilirubin levels (PFOA, PFOS, PFHxS)
- Increases in serum lipids, particularly total cholesterol and low-density lipoprotein (LDL) cholesterol (PFOA, PFOS, PFNA, PFDA)
- Decreased antibody response to vaccines (PFOA, PFOS, PFHxS, PFDA)
- Small (<20-g or 0.7-ounce decrease in birth weight per 1 ng/mL increase in either PFOA or PFOS blood level) decreases in birth weight (PFOA, PFOS)

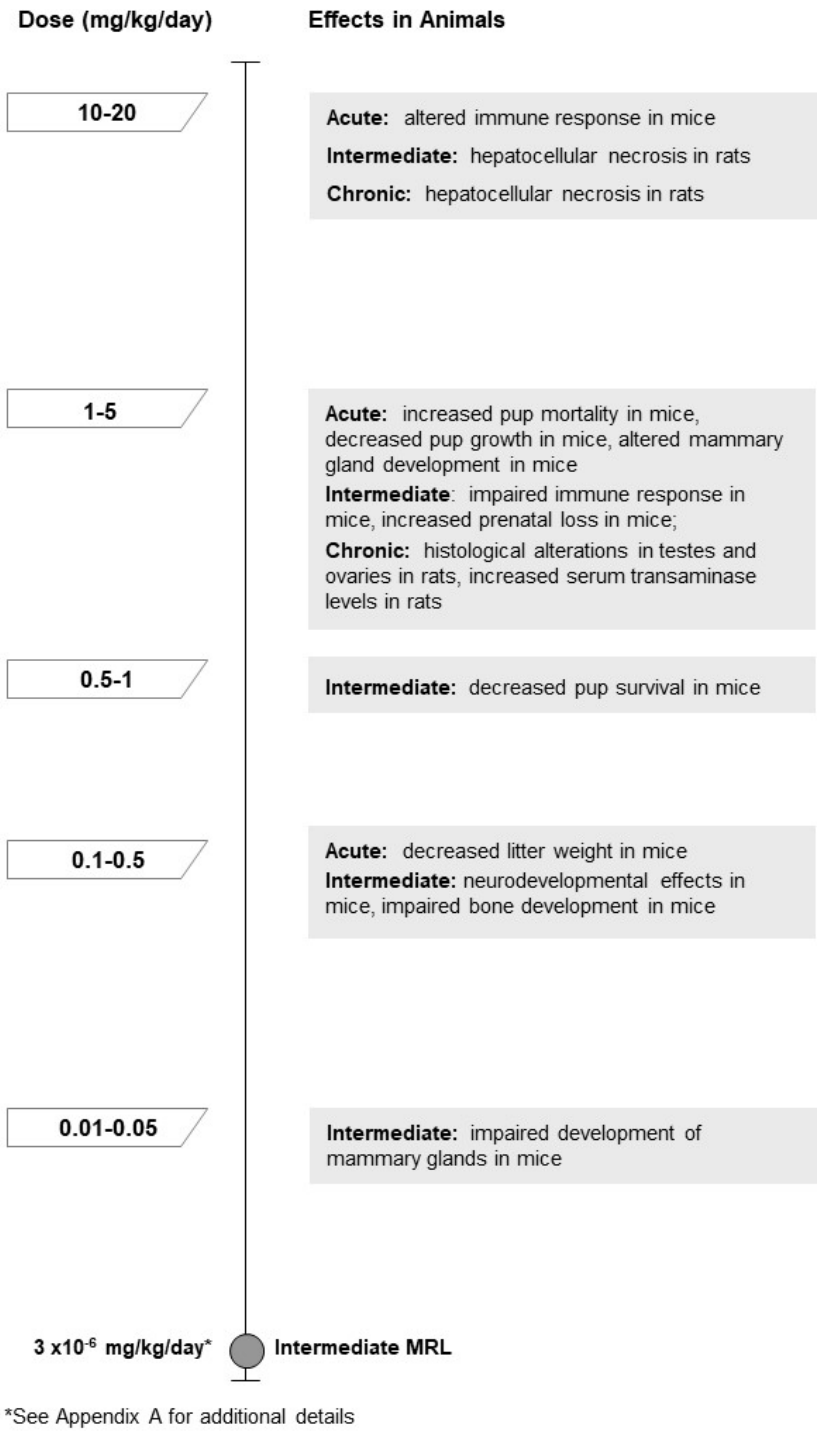
The International Agency for Research on Cancer (IARC 2017) concluded that PFOA is possibly carcinogenic to humans (Group 2B), and EPA (2016e, 2016f) concluded that there was suggestive evidence of the carcinogenic potential of PFOA and PFOS in humans. Increases in testicular and kidney cancer have been observed in highly exposed humans.

There is also some suggestive evidence for associations between perfluoroalkyls and additional health outcomes; there is less certainty in these associations due to inconsistencies across studies and/or a smaller number of studies examining a specific outcome. These health outcomes include osteoarthritis in women under 50 years of age (PFOA, PFOS) and decreased antibody response to vaccines (PFNA, PFUnA, PFDoDA). Additionally, associations between serum PFOA and PFOS and decreases in glomerular filtration rate and increases in serum uric acid levels and between serum PFOA, PFOS, PFHxS, and PFNA and increased risk of early menopause have been observed; these effects may be due to reverse causation, where the effect (disease) causes the change in serum perfluoroalkyl levels (exposure).

Effects in Laboratory Animals. Most of the information regarding the effects of perfluoroalkyls in animals is derived from oral studies; considerably less information is available from inhalation and dermal exposure studies. PFOA and PFOS are the most studied perfluoroalkyls, with considerably less data for the other compounds. Of the 233 animal studies reviewed in this toxicological profile, 42% examined PFOA, 31% examined PFOS, and 27% examined other perfluoroalkyls (8 studies on PFHxS, 17 studies on PFNA, 1 study on PFUnA, 5 studies on PFBS, 6 studies on PFBA, 9 studies on PFDA, 8 studies on PFDoDA, 1 study on FOSA, and 8 studies on PFHxA). The primary effects observed in rats and mice exposed to perfluoroalkyls are liver toxicity, developmental toxicity, and immune toxicity (see Figures 1-1, 1-2, and 1-3); not all of these effects have been observed or examined for all perfluoroalkyls. Based on limited data, the toxicity of perfluoroalkyls does not appear to be specific to the route of administration. It should be noted that, for the most part, adverse health effects in studies in animals have been associated with exposure concentrations or doses that resulted in blood levels of perfluoroalkyls that were significantly higher than those reported in perfluoroalkyl workers or in the general population. Furthermore, there are profound differences in the toxicokinetics of perfluoroalkyls between humans and experimental animals. The elimination $t_{1/2}$ of PFOA is approximately 4 years in humans compared with days or hours in rodents. These factors, plus issues related to the mode of action of perfluoroalkyls (see below), make it somewhat difficult at this time to determine the true relevance of some effects reported in animal studies to human health.

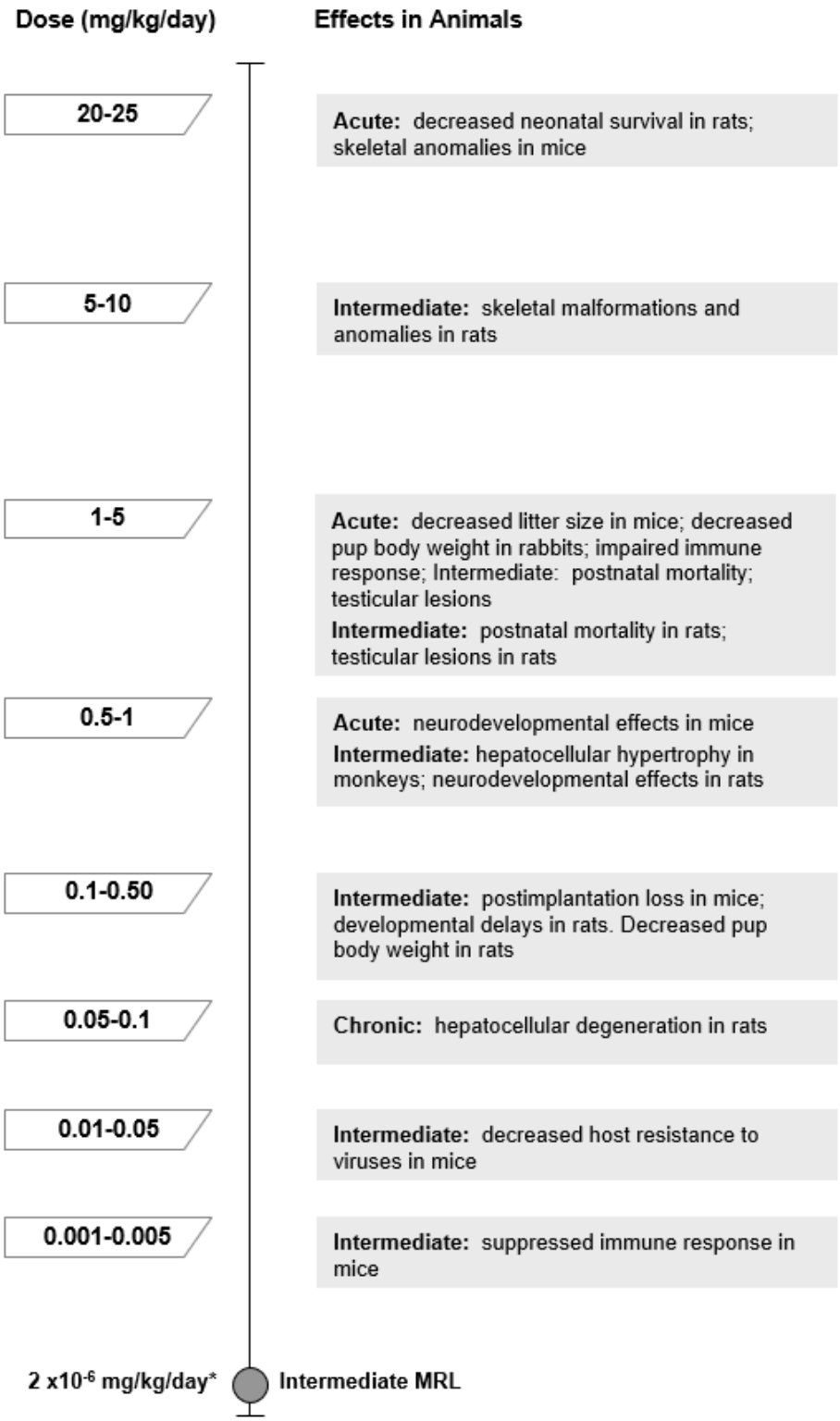
Liver Effects. Many studies have described morphological and biochemical alterations in the liver from rodents following acute and longer-term oral exposure to PFOA. Some of the effects observed in rats include increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol and triglyceride levels (e.g., Butenhoff et al. 2004b; Liu et al. 1996; Pastoor et al. 1987; Yang et al. 2001; see Section 2.9 for a complete list of citations). The observed hepatomegaly and hypertrophy are likely due

Figure 1-1. Health Effects Found in Animals Following Oral Exposure to PFOA



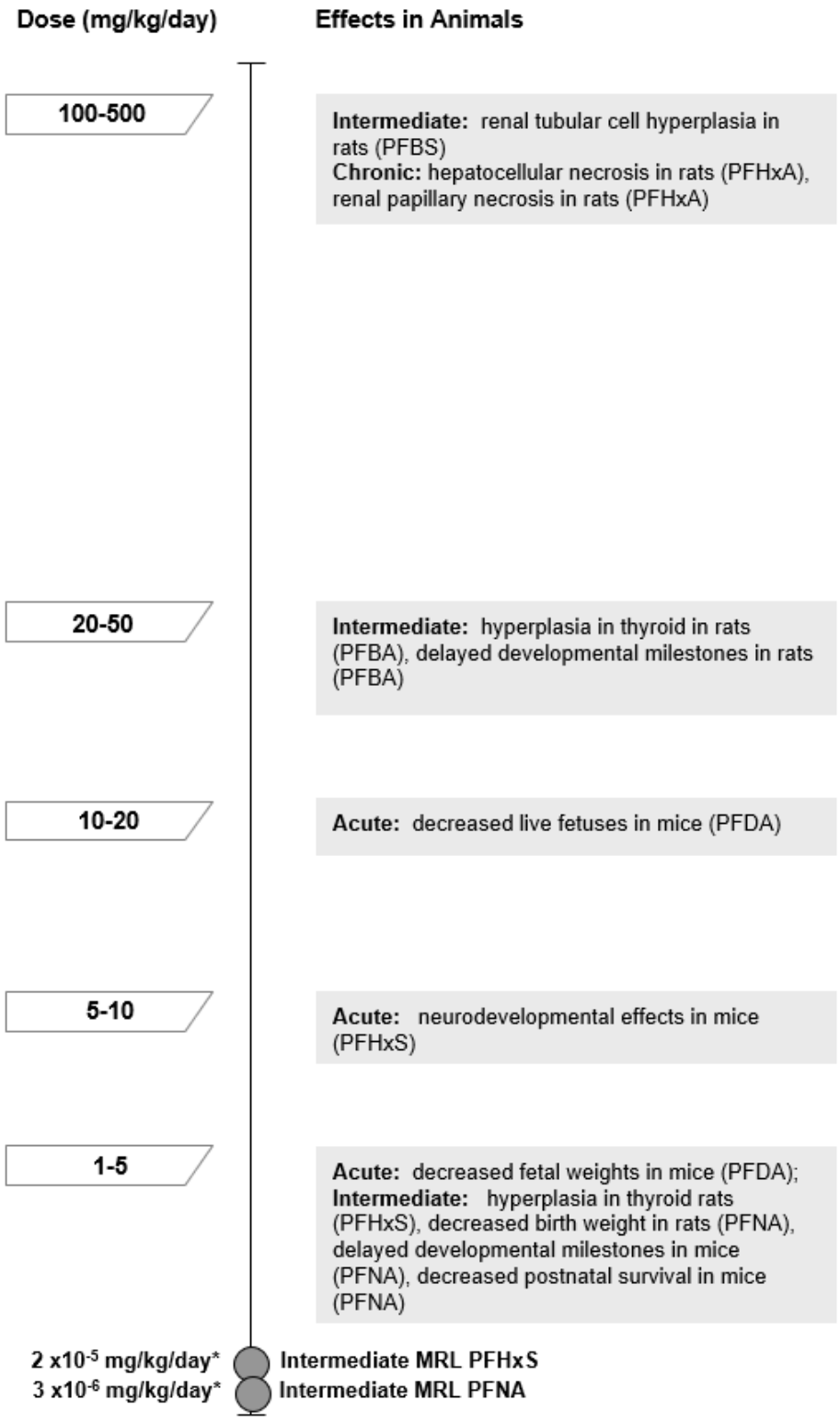
*See Appendix A for additional details

Figure 1-2. Health Effects Found in Animals Following Oral Exposure to PFOS



*See Appendix A for additional details

Figure 1-3. Health Effects Found in Animals Following Oral Exposure to Other Perfluoroalkyls



*See Appendix A for additional details

to expansion of the smooth endoplasmic reticulum and proliferation of peroxisomes, as confirmed by increased activity of biochemical markers and light and electron microscopy (Pastoor et al. 1987). It is important to note also that there appear to be different sensitivities for different endpoints. For example, in male rats dosed with PFOA for 14 days, absolute liver weight and fatty acid β-oxidation activity were significantly increased at 2 mg/kg/day, whereas hepatic microsomal concentration of total cytochrome P450 was significantly increased at 20 mg/kg/day (Liu et al. 1996). In general, longer-term studies with PFOA have shown that the hepatic effects are reversible once dosing ceases and that recovery tends to parallel the decline in blood levels of PFOA (Perkins et al. 2004). Studies in mice have provided similar results. However, studies in PPARα-null mice suggest that hepatomegaly may also be due to a PPARα-independent process in mice (Yang et al. 2002b), since PFOA induced hepatomegaly to the same extent in wild-type mice and PPARα-null mice, but failed to increase acyl-CoA oxidase activity in PPARα-null mice. PFOA exposure also resulted in increases in absolute liver weight in monkeys treated with ≥3 mg/kg/day for 26 weeks, an effect that was partly associated with significant mitochondrial proliferation, but not peroxisome proliferation (Butenhoff et al. 2002).

Similar to PFOA, PFOS exposure results in increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol and triglyceride levels in rodents (e.g., Elcombe et al. 2012a, 2012b; Era et al. 2009; Seacat et al. 2003; Thibodeaux et al. 2003). PFOS induced an increase in absolute liver weight, a decrease in serum cholesterol, and hepatocellular hypertrophy and lipid vacuolation in monkeys in a 26-week study (Seacat et al. 2002). Not unexpectedly, there was no evidence of peroxisome proliferation and no increase in hepatic palmitoyl-CoA oxidase, consistent with the fact that monkeys (and humans) seem to be refractory to peroxisome proliferative responses (Cattley et al. 1998; Klaunig et al. 2003).

Studies with other perfluoroalkyls have shown that, in general, liver weight and parameters of fatty acid β-oxidation are more severely affected as the carbon length increases up to about a 10-carbon chain length (Butenhoff et al. 2009a, 2012a; Goecke-Flora and Reo 1996; Goecke et al. 1992; Kudo et al. 2000, 2006; Permadi et al. 1992, 1993; van Otterdijk 2007a, 2007b). Significant peroxisome activity seems to require a carbon length >7 (Goecke-Flora and Reo 1996; Goecke et al. 1992), but increases over control levels have been reported with a four-carbon chain length (Permadi et al. 1993; Wolf et al. 2008a). In an *in vitro* study in COS-1 cells transfected with mouse PPARα, PFOA had the lowest effective concentration needed for PPARα activation followed by PFNA and PFDA, PFHxA, and PFBA (Wolf et al. 2008a). This pattern was not found for the sulfonates; the lowest effective concentration was for PFHxS followed by PFOS and PFBS. Wolf et al. (2008a) also found that carboxylate perfluoroalkyls activated PPARα at lower concentrations than the sulfonate perfluoroalkyls. In COS-1 cells transfected

with human PPARα, PFNA had the lowest effective concentration followed by PFOA PFHxA, PFHxS, PFOS, PFBS, PFBA, and PFDA (Wolf et al. 2008a). Studies have shown that the differential activity is also related to differential accumulation of the perfluoroalkyls in the liver (Kudo and Kawashima 2003; Kudo et al. 2000, 2006). Hydrophobicity, which increases as carbon length increases, seems to favor biliary enterohepatic recirculation, resulting in a more protracted toxicity (Goecke-Flora and Reo 1996). As discussed in greater detail in Section 2.9, the increases in liver weight and hepatocellular hypertrophy observed in the rat and mouse studies were considered rodent-specific adaptive responses and were not considered relevant to humans. However, other liver effects including biliary effects and hepatocellular necrosis were considered relevant to humans.

Developmental Effects. PFOA and PFOS have induced developmental effects in rodents. Most studies with PFOA have been conducted in mice. Specific effects reported include prenatal loss, reduced neonate weight and viability, neurodevelopment toxicity, altered bone development, and delays in mammary gland differentiation, eye opening, vaginal opening, and first estrus (Abbott et al. 2007; Albrecht et al. 2013; Cheng et al. 2013; Johansson et al. 2008; Koskela et al. 2016; Lau et al. 2006; Macon et al. 2011; Ngo et al. 2014; Onishchenko et al. 2011; Sobolewski et al. 2014; White et al. 2007, 2009, 2011; Wolf et al. 2007; Yahia et al. 2010). These effects occurred generally in the absence of overt maternal toxicity. Some of these effects, such as reduced pup survival from birth to weaning, have been observed in mice treated with as low as 0.6 mg/kg/day PFOA on gestation days (GDs) 1–17 (Abbott et al. 2007). This dose level resulted in mean serum PFOA concentrations of 5,200 and 3,800 ng/mL in dams and pups, respectively, on postnatal day (PND) 22. A cross-fostering study in mice showed that *in utero*, lactation only, and *in utero* and lactation exposure resulted in significant decreases in postnatal growth (Wolf et al. 2007). Alterations in spontaneous behavior were reported in 2- or 4-month-old male mice that were administered a single gavage dose of PFOA at the age of 10 days (Johansson et al. 2008). Increases in motor activity were also observed following *in utero* exposure to PFOA (Cheng et al. 2013; Onishchenko et al. 2011). Gestational exposure resulted in altered bone morphology and bone mineral density in the mature offspring (Koskela et al. 2016). Delays in ossification were found in another gestational exposure study in mice (Lau et al. 2006). A cross-fostering study showed that the delays in mammary gland development were observed following *in utero* exposure and following lactation-only exposure (White et al. 2009); however, the results of a 2-generation study showed that the delayed development did not appear to affect lactational support (White et al. 2011). No fetal toxicity or teratogenicity was reported in offspring of rabbits exposed to up to 50 mg/kg/day PFOA on GDs 6–18 (Gortner et al. 1982), suggesting that rabbits are less susceptible than mice to the developmental effects of PFOA, although comparing administered doses is probably not very informative due to toxicokinetic differences between species.

There were significant increases in body weight gain in mice aged 10–40 weeks that were exposed to low levels of PFOA (0.01–0.3 mg/kg/day) on GDs 1–17 (Hines et al. 2009). Increases in serum insulin and leptin levels were also observed, but there was no change in serum glucose or the response to a glucose challenge. A comparison of the effects of *in utero* exposure (GDs 1–17) to adult exposure (17 days at age 8 weeks) demonstrated that *in utero* exposure resulted in higher body weights, white fat weight, and brown fat weight at age 18 months (Hines et al. 2009).

Studies conducted with wild-type and PPARα knockout mice showed that PPARα was required for PFOA-induced postnatal lethality and that the expression of one copy of the gene was sufficient to mediate this effect (Abbott et al. 2007). Strain or PPARα expression did not affect serum PFOA levels. The mechanism of reduced postnatal viability has not been elucidated. Alterations in gene expression in both fetal liver and lung have been reported following exposure of mice to PFOA during pregnancy (Rosen et al. 2007). In the liver, PFOA altered the expression of genes linked to fatty acid catabolism, lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, retinol metabolism, proteosome activation, and inflammation. In the lung, transcriptional-related changes were predominantly associated with fatty acid catabolism. Although decreased pup survival appears to be linked to PPARα expression, there are insufficient data to determine whether other developmental effects observed in rats and mice are PPARα-independent.

PFOS significantly decreased birth weight and survival in neonatal rats exposed *in utero* (Chen et al. 2012b; Lau et al. 2003; Xia et al. 2011), and cross-fostering exposed pups with unexposed dams failed to improve survival rates (Lau et al. 2003). PFOS serum levels of pups at birth associated with significant decreased survival were approximately ≥70,000 ng/mL. In contrast to PFOA, the results of a study in wild-type and PPARα-null mice suggest that the decrease in pup survival was not dependent on PPARα activation (Abbott et al. 2009). Dosing rats late during gestation (GDs 17–20) caused significantly more lethality than dosing early (GDs 2–5) (Grasty et al. 2003). Since pups had difficulty breathing within minutes of birth and their lungs showed evidence of delayed lung maturation and other histological alterations (Grasty et al. 2003, 2005; Yahia et al. 2008), the possibility that this caused the early death has been suggested. Other effects included decreases in birth weight or pup body weight, delays in eye opening, cleft palate, and neurodevelopmental alterations (Butenhoff et al. 2009b; Case et al. 2001; Chen et al. 2012b; Era et al. 2009; Fuentes et al. 2006, 2007a, 2007b; Lau et al. 2003; Luebker et al. 2005a, 2005b; Onishchenko et al. 2011; Thibodeaux et al. 2003; Wang et al. 2015c; Yahia et al. 2008). Alterations in spontaneous motor activity were observed in mice. A decrease in activity was observed

when mice were placed in a novel environment (Fuentes et al. 2007a; Onishchenko et al. 2011); another study found a decrease in motor activity followed by increased activity (Johansson et al. 2009). Evaluation of immunological parameters in 8-week-old pups from mice exposed to PFOS during gestation showed reduced natural killer (NK) cell activity, suppressed IgM response to immunization, and alterations in splenic and thymic lymphocyte subpopulations (Keil et al. 2008).

Similar to PFOA and PFOS, increases in fetal mortality were observed in mice exposed to PFDA on GDs 6–15 (Harris and Birnbaum 1989) and decreases in litter size and pup survival were observed in mice exposed to PFNA (Wolf et al. 2010). In contrast, gestational exposure to PFBA, PFBS, or PFHxS did not result in alterations in pup survival or pup body weight (Das et al. 2008; Hoberman and York 2003; Lieder et al. 2009b). Decreases in spontaneous activity followed by an increase in activity were observed in mice exposed to PFHxS on PND 10 (Viberg et al. 2013); no alterations were observed in mice similarly exposed to PFDA (Johansson et al. 2008).

Immunological Effects. A number of studies have examined the immunotoxicity of perfluoroalkyls in rats and mice; these data suggest that mice are considerably more sensitive than rats. PFOA- and PFOS-induced immunological alterations in adult mice are characterized by thymus and spleen atrophy, alterations in thymic and splenic lymphocyte phenotypes, and impaired response to T-dependent antigens (DeWitt et al. 2008, 2009; Dong et al. 2009; Guruge et al. 2009; Lefebvre et al. 2008; Loveless et al. 2008; Qazi et al. 2012; Yang et al. 2000, 2002a; Zheng et al. 2009). The lowest lowest-observed-adverse-effect level (LOAEL) for immune effects in mice exposed to PFOA was 3.75 mg/kg/day administered for 15 days; this dosing level resulted in a mean PFOA serum level of 75,000 ng/mL (DeWitt et al. 2008). For PFOS, several studies identified LOAELs of 0.02–0.8 mg/kg/day (Dong et al. 2009, 2011; Zheng et al. 2009) and one study identified a LOAEL of 0.00166 mg/kg/day for suppressed response to a T-dependent antigen (Peden-Adams et al. 2008). PFOA applied to the skin of mice increased serum IgE levels following a challenge with ovalbumin relative to mice treated with ovalbumin alone, which led the investigators to suggest that PFOA may increase the IgE response to environmental allergens (Fairley et al. 2007). More limited data are available for other perfluoroalkyls. Thymic and/or splenic alterations were observed in rats and mice administered ≥1 mg/kg/day PFNA (Fang et al. 2008, 2009, 2010). No histological alterations were observed in rodents exposed to PFHxS (Butenhoff et al. 2009a), PFDA (Harris et al. 1989), PFBS (3M 2001), or PFBA (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

Cancer Effects. PFOA, as many other PPARα agonists, induced hepatocellular adenomas, Leydig cell adenomas, and pancreatic acinar cell adenomas in rats (Biegel et al. 2001). An increase in hepatocellular adenomas was also observed in rats chronically exposed to PFOS (Butenhoff et al. 2012b). Liver tumors induced by PFOA are believed to be mediated largely through PPARα activation, and considered to be of limited or no relevance to humans (EPA 2016h), based on species differences in response to PPARα activation. Although Leydig cell tumors are also commonly induced by peroxisome proliferating agents, the mode of action by which these tumors are induced by PFOA, and thus their relevance to humans, is much less clear (Corton et al. 2014; EPA 2016h; Klaunig et al. 2003). One mode of action proposed for the induction of Leydig cell tumors involves PFOA-induced decreases in circulating testosterone levels, leading to increased production of gonadotropin releasing hormone and circulating luteinizing hormone (LH), which promotes Leydig cell proliferation. Reduced testosterone levels may occur through decreased biosynthesis, or via the conversion of testosterone to estradiol via the enzyme aromatase, both of which may be related to PPARα activation (EPA 2016h). However, the data supporting a PPARα-dependent mode of action for Leydig cell tumors is not sufficiently established to rule out human relevance (EPA 2016h). Likewise, the mechanism of PFOA-induced pancreatic acinar cell tumors may include a PPARα-dependent component, but the mechanism has not been fully elucidated, and relevant data are limited. A proposed mode of action involves stimulation of PPARα leading to reduced bile flow and/or changes in bile acid composition with subsequent increase in cholecystokinin (CCK), which stimulates pancreatic cell proliferation and tumor formation (EPA 2016h). Support for this mode of action is limited to information demonstrating increased biliary excretion of PFOA in wild-type and PPARα null mice (Minata et al. 2010) and data showing altered expression of bile acid transporters (OATPs and MRPs) in exposed laboratory animals (Cheng and Klassen 2008a; Maher et al. 2008). The limitations in available data on the mode of action for pancreatic tumor development preclude a conclusion regarding the human relevance of PFOA-induced pancreatic tumors (EPA 2016h).

1.3 MINIMAL RISK LEVELS (MRLs)

ATSDR develops MRLs as screening tools to help identify chemicals that may be of concern. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to determine areas and populations potentially at risk for health effects from exposure to a particular substance. Exposure above the MRLs does not mean that health problems will occur. Instead, it may act

as a signal to health assessors to look more closely at a particular site where exposures may be identified. MRLs do not define regulatory or action levels for ATSDR.

ATSDR uses the point of departure (POD)/uncertainty factor approach to derive MRLs. Potential PODs are no-observed-adverse-effect levels (NOAELs), LOAELs, or the lower limit of the benchmark dose (BMDL). MRLs are set below levels that, based on current information, might cause adverse health effects in the people most sensitive to such substance-induced effects. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys) are not used as a basis for establishing MRLs. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. ATSDR does not extrapolate across exposure durations to derive MRLs for durations with limited databases.

Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals. ATSDR utilizes uncertainty factors to account for uncertainties associated with: (1) extrapolating from a LOAEL to a NOAEL; (2) extrapolating from animals to humans; and (3) to account for human variability. Default values of 10 are used for each of these categories of uncertainty factors; a value of 1 can be used if complete certainty exists for a particular uncertainty factor category. A partial uncertainty factor of 3 can be used when chemical-specific data decreases the uncertainty. On a case-by-case basis, ATSDR also utilizes modifying factors to account for MRL-specific database deficiencies.

Oral MRLs have been derived for several perfluoroalkyls. A summary of the MRLs derived for perfluoroalkyls is presented in Table 1-2 and detailed discussions of MRLs are provided in Appendix A. The database was not considered adequate for derivation of inhalation MRLs. Though inhalation data are available for PFOA and PFNA, these studies examined a limited number of endpoints and the data are not adequate for identifying the most sensitive targets of toxicity or establishing dose-response relationships. No inhalation data are available for other perfluoroalkyls.

Table 1-2. Overview of Minimal Risk Levels Derived for Perfluoroalkyls

	Inhalation MRLs			Oral MRLs		
Compound	Acute	Intermediate	Chronic	Acute	Intermediate	Chronic
PFOA	X[a]	X	X	X	$3x10^{-6}$ mg/kg/day (Table 1-3)	X
PFOS	X	X	X	X	$2x10^{-6}$ mg/kg/day (Table 1-4)	X
PFHxS	X	X	X	X	$2x10^{-5}$ mg/kg/day (Table 1-5)	X
PFNA	X	X	X	X	$3x10^{-6}$ mg/kg/day (Table 1-6)	X
PFDA	X	X	X	X	X	X
PFUnA	X	X	X	X	X	X
PFHpA	X	X	X	X	X	X
PFBS	X	X	X	X	X	X
PFBA	X	X	X	X	X	X
PFDoDA	X	X	X	X	X	X
PFHxA	X	X	X	X	X	X
FOSA	X	X	X	X	X	X

[a]X indicates that no MRL was derived.

FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

The oral databases were considered adequate for derivation of intermediate-duration oral MRLs for PFOA, PFOS, PFHxS, and PFNA based on laboratory animal data. The databases were not considered adequate for derivation of MRLs for the other perfluoroalkyls. Hepatic, immune, and developmental endpoints were the most sensitive targets in laboratory animals exposed to PFOA (see Figure 1-4) and PFOS (see Figure 1-5), respectively. The most sensitive targets were hepatic and thyroid endpoints for PFHxS and body weight and developmental endpoints for PFNA. As discussed in Section 1.2, toxicokinetic and mechanistic differences exist between humans and laboratory animals, in particular differences in elimination rates and the relevance of effects associated with activation of PPARα. The uncertainties in the relevance of animal data for developing screening levels are decreased by focusing on health outcomes also reported in epidemiological studies or involving PPARα-independent mechanisms of action and estimating a POD using serum perfluoroalkyl concentrations. The MRL values for PFOA, PFOS, PFHxS, and PFNA are summarized in Tables 1-3, 1-4, 1-5, and 1-6 and discussed in greater detail in Appendix A.

Figure 1-4. Summary of Sensitive Targets of PFOA – Oral

Developmental endpoints are the most sensitive target of PFOA.
Numbers in circles are the lowest LOAELs for all health effects in animals.

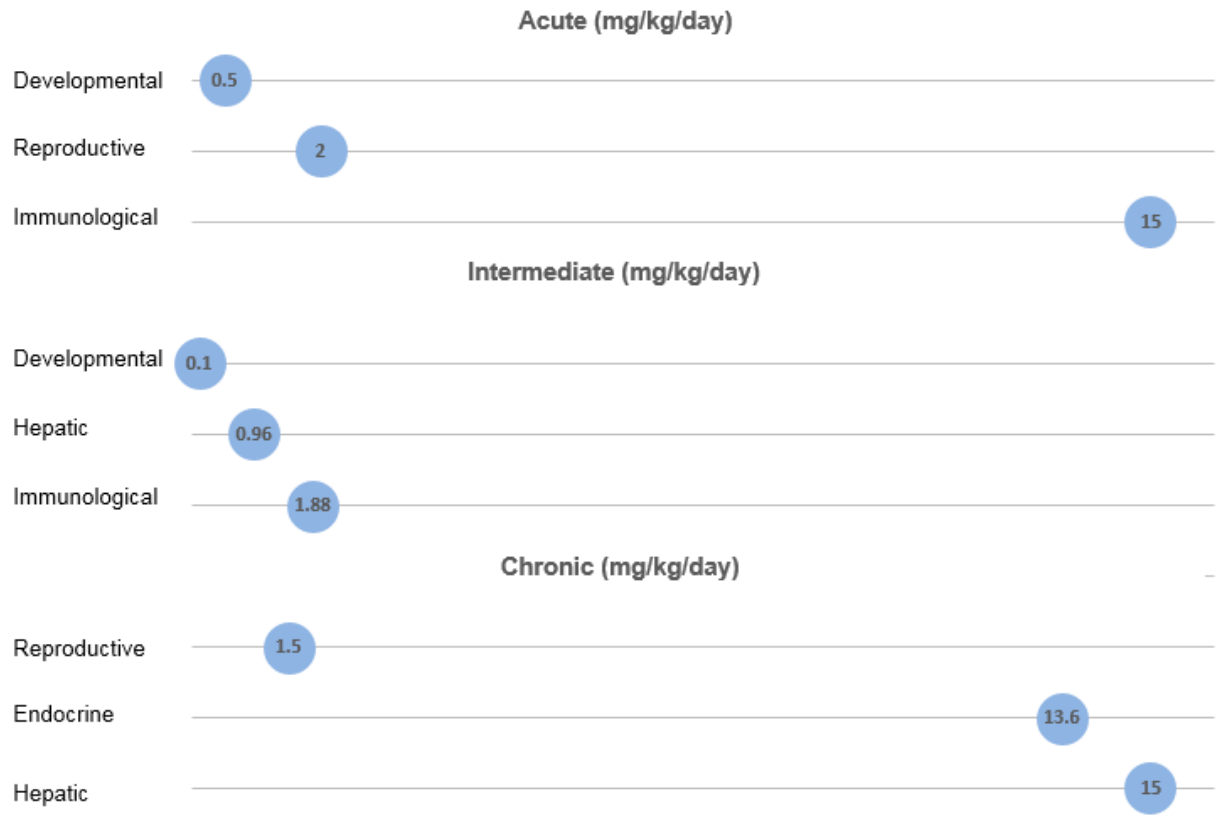


Figure 1-5. Summary of Sensitive Targets of PFOS – Oral

The immune system and developing organism are the most sensitive targets of PFOS.
Numbers in circles are the lowest LOAELs for all health effects in animals.

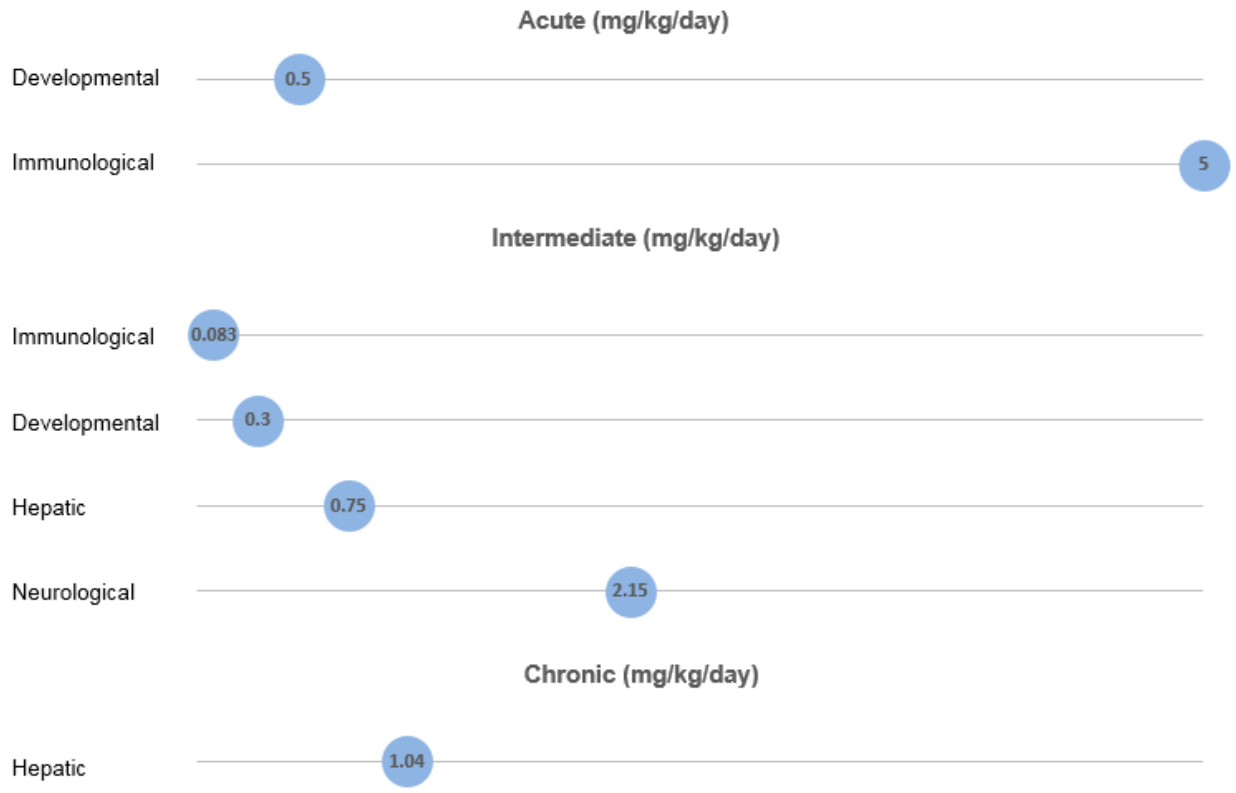


Table 1-3. Minimal Risk Levels (MRLs) for PFOA[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty factor	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$3x10^{-6}$	Skeletal effects in mice	0.000821 ($LOAEL_{HED}$)	300	Koskela et al. 2016
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; PFOA = perfluorooctanoic acid

Table 1-4. Minimal Risk Levels (MRLs) for PFOS[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$2x10^{-6}$	Delayed eye opening and decreased pup weight in rats	0.000515 ($NOAEL_{HED}$)[b]	30 10	Luebker et al. 2005a
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PFOS = perfluorooctane sulfonic acid

Table 1-5. Minimal Risk Levels (MRLs) for PFHxS[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$2x10^{-5}$	Thyroid follicular epithelial hypertrophy/ hyperplasia in rats	0.0047 ($NOAEL_{HED}$)	30 10	Butenhoff et al. 2009a
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PFHxS = perfluorohexane sulfonic acid

Table 1-6. Minimal Risk Levels (MRLs) for PFNA[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$3x10^{-6}$	Decreased body weight and developmental delays in mice	0.001 ($NOAEL_{HED}$)	30 10	Das et al. 2015
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PFNOA = perfluorononanoic acid

CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of perfluoroalkyls. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

This document discusses information on perfluoroalkyls that have been measured in the serum collected from a representative U.S. population ≥12 years of age in the 2003–2004 NHANES (Calafat et al. 2007b), as well as two compounds (PFBA and PFHxA) that have been identified in other monitoring studies. More recent NHANES monitoring studies have not evaluated additional perfluoroalkyl compounds (CDC 2019). The perfluoroalkyl compounds discussed in the profile are listed below. They are discussed in the profile in following order, based on the abundance of epidemiological data:

Perfluorooctanoic acid (PFOA, CAS Registry Number 335-67-1)
Perfluorooctane sulfonic acid (PFOS, CAS Registry Number 1763-23-1)
Perfluorohexane sulfonic acid (PFHxS, CAS Registry Number 355-46-4)
Perfluorononanoic acid (PFNA, CAS Registry Number 375-95-1)
Perfluorodecanoic acid (PFDA, CAS Registry Number 335-76-2)
Perfluoroundecanoic acid (PFUnA, CAS Registry Number 2058-94-8)
Perfluoroheptanoic acid (PFHpA, CAS Registry Number 375-85-9)
Perfluorobutane sulfonic acid (PFBS, CAS Registry Number 375-73-5)
Perfluorobutanoic acid (PFBA, CAS Registry Number 375-22-4)
Perfluorododecanoic acid (PFDoDA, CAS Registry Number 307-55-1)
Perfluorohexanoic acid (PFHxA, CAS Registry Number 307-24-4)
Perfluorooctane sulfonamide (FOSA, CAS Registry Number 754-91-6)

The term perfluoroalkyls used throughout the profile is referring to at least one of these 12 compounds and may not be applicable to other perfluoroalkyl compounds.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figures 2-1, 2-2, 2-3, 2-4, and 2-5 provide an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to perfluoroalkyls, but may not be inclusive of the entire body of literature. ATSDR's approach for assessing study quality and weight-of-evidence evaluation is described in the Agency's Guidance for the Preparation of Toxicological Profile document (https://www.atsdr.cdc.gov/toxprofiles/guidance/profile_development_guidance.pdf).

Summaries of the epidemiological studies, including details on the study design and results, are presented in tables in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*; briefer summaries of the studies are presented in summary tables for each endpoint. For studies in which the population was divided into perfluoroalkyl exposure categories, such as quartiles, the risk ratio reported in the summary table is for the lowest exposure category with a statistically significant association; risk ratios for higher exposure categories are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls* tables. In general, associations were also found for higher exposure categories.

Summaries of experimental studies are separated by exposure route and are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6. The inhalation data for PFOA and other perfluoroalkyls are presented in Tables 2-1 and 2-2, respectively. A large number of experimental studies have evaluated the oral toxicity of PFOA and PFOS, the results of these studies are presented in Tables 2-3 and 2-4, respectively. Lesser amounts of data are available for the remaining 10 perfluoroalkyl compounds; the study results for these compounds are presented in Table 2-5. Table 2-5 is divided by exposure duration and by compound. The dermal data for PFOA is presented in Table 2-6. In addition, the NOAEL and LOAEL values from inhalation and oral studies are graphically presented in Figures 2-6, 2-7, 2-8, 2-9, and 2-10.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing NOAELs or LOAELs reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant

dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints (ATSDR 2003). ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

A User's Guide has been provided at the end of this profile (see Appendix C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

The discussion of the available data for each health effect is divided into several subsections. Each health effect section begins with an overview, which contains a brief discussion of the available data and conclusions that can be drawn from the data. Compound-specific discussions follow the overview; the perfluoroalkyls are discussed in the following order: PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, and FOSA. It is noted that for most health effects, there are no data for a number of the perfluoroalkyls. The health effect endpoints examined in epidemiological and experimental data for each perfluoroalkyl is summarized in Figures 2-1 and 2-2, respectively. The compound-specific discussions are further divided into Epidemiological Studies and Laboratory Animal Studies; for data-rich endpoints, a compound-specific summary is also included. Each perfluoroalkyl is treated separately in this chapter. Although there is some evidence of similar health outcomes for some compounds, there is evidence of qualitative and mechanistic differences (Peters and Gonzalez 2011).

The health effects of perfluoroalkyls have been evaluated in a large number of epidemiological and animal studies; the literature search framework for identifying these studies is discussed in Appendix B. As illustrated in Figures 2-3, 2-4, and 2-5, most of the health effects data come from epidemiological studies. For PFOA, PFOS, and other perfluoroalkyls, 74, 76, and 70%, respectively, of the health effect studies were in humans; it is noted that most epidemiological studies examined more than one perfluoroalkyl. More than half (52%) of the epidemiological studies were cross-sectional studies, 29% were prospective studies, and the remainder were retrospective, case-control, cohort, or longitudinal studies. Three population categories were examined in epidemiological studies: workers at facilities involved in the production or use of perfluoroalkyls (most of the studies involved workers at two U.S. facilities and typically involved higher than background exposure to PFOA and PFOS), communities living near a PFOA manufacturing facility with high levels of PFOA in the drinking water (almost all of

the studies involved residents living near a PFOA production facility in West Virginia; elevated PFOA exposure and background exposure to other perfluoroalkyls), and populations exposed to background levels of perfluoroalkyls (referred to as general population studies). Most of the studies of communities living near perfluoroalkyl manufacturing facilities are part of the C8 Health Project and C8 Health Study (C8 is a synonym for PFOA). The C8 Health Project was a population study of Ohio and West Virginia residents living near the DuPont Washington Works facility in West Virginia and was funded by DuPont as part of a class action settlement agreement. The Washington Works facility began using PFOA in 1951 and peak use was in the late 1990s. At the time of enrollment (2005–2006), blood samples were collected from over 69,000 participants who lived, worked, or attended school in six contaminated water districts surrounding the facility for at least 12 months between 1950 and December 2004 (Frisbee et al. 2009); the six water districts were Little Hocking Water Association, Tuppers Plains Chester Water District, Village of Pomeroy, Lubeck Public Service District, Mason County Public Service District, or private water sources within these areas. The participants ranged in age from 1.5 to >100 years, with an average age of 39.1 years.

Serum perfluoroalkyl levels were used as the biomarker of exposure in almost all of the epidemiological studies since most of the studies did not provide external exposure levels. The highest levels of serum PFOA were found among workers, followed by the community members, and then the general population. One study of PFOA workers in 2004–2005 reported an average serum PFOA level of 1,000 ng/mL (Sakr et al. 2007a). A study of community members living near this same facility reported a mean serum PFOA level of 423 ng/mL in 2004–2005. In the United States, the mean geometric mean serum PFOA level in 2005–2006 was 3.92 ng/mL (CDC 2018). In a study of two PFOS facilities, mean serum PFOS levels in workers were 960–1,400 ng/mL in 2000 (Olsen et al. 2003a); the geometric mean serum PFOS levels in the U.S. general population in 1999–2000 was 30.4 ng/mL (CDC 2018). Bach et al. (2015b) investigated whether transport of blood samples under ambient temperature conditions and processing delays impact serum perfluoroalkyl concentrations. Using the conditions of the Danish National Birth Cohort study, Bach et al. (2015a) found relative differences between serum samples that were transported with processing delays and those processed immediately of 1% (winter sampling) to 3% (summer sampling) for PFOA, -29–2% for PFOS, 12–11% for PFHxS, -5–3% for PFNA, -39–0% for PFDA, -77 to -7% for PFUnA, and 38–17% for PFHpA. This discrepancy has not been verified for other Danish National Birth Cohort studies or for other studies.

Most of the epidemiological studies provided a single serum perfluoroalkyl concentration, which has been shown to be a reliable biomarker of recent exposure; however, it does not provide information on

historical exposure. The lack of historical exposure data is a particular limitation of the occupational and community population studies where past exposures were typically higher than current exposures.

Another limitation of the epidemiological studies involves co-exposure to multiple perfluoroalkyls. A number of the epidemiological studies have found strong correlations between serum levels of different perfluoroalkyls. *In vitro* studies (Carr et al. 2013; Wolf et al. 2014) have shown that at lower concentrations, binary pairs of perfluoroalkyls demonstrate concentration and response additivity, but deviate from additivity at higher concentrations (Wolf et al. 2014). These possible interactions (or dose additivity) complicate the interpretation of the epidemiological data.

Although a large number of epidemiological studies have examined the potential of perfluoroalkyls to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causality. ATSDR evaluated the available epidemiological data to assess whether the preponderance of the evidence suggested a possible association between perfluoroalkyl exposure and a particular health effect. This approach took into consideration the consistency of the findings across studies, the quality of the studies, dose-response, and plausibility. It should be noted that although the data may provide evidence for an association, it does not always imply that the observed effect is biologically relevant because the magnitude of the change may be within the normal limits or not indicative of an adverse health outcome. Plausibility depends primarily on experimental toxicology studies that establish a plausible biological mechanism for the observed effects. ATSDR's toxicological profile development guidance (https://www.atsdr.cdc.gov/toxprofiledocs/additional_resources.html/#Profile_Development) describes in detail the weight-of-evidence approach that includes quality assessment of every study included in the profile.

The available epidemiological studies suggest associations between perfluoroalkyl exposure and several health outcomes; however, cause-and-effect relationships have not been established for these outcomes:

- **Hepatic effects.** Increases in serum enzymes and decreases in serum bilirubin, observed in studies of PFOA, PFOS, and PFHxS, are suggestive of liver alterations. In addition, the results of epidemiological studies of PFOA, PFOS, PFNA, and PFDA suggest an association between perfluoroalkyl exposure and increases in serum lipid levels, particularly total cholesterol and LDL cholesterol; see Section 2.9 for detailed discussion and citations.

- **Cardiovascular effects.** There is suggestive epidemiological evidence for an association between serum PFOA and PFOS and pregnancy-induced hypertension and/or pre-eclampsia; see Section 2.5 for detailed discussion and citations.

- **Immune effects.** Evidence is suggestive of an association between serum PFOA, PFOS, PFHxS, and PFDA levels and decreased antibody responses to vaccines; there is also limited evidence for PFNA, PFUnA, and PFDoDA; see Section 2.14 for detailed discussion and citations.

- **Developmental effects.** Evidence is suggestive of an association between serum PFOA and PFOS and small decreases in birth weight; the decrease in birth weight is <20 g (0.7 ounces) per 1 ng/mL increase in blood PFOA or PFOS level; see Section 2.17 for detailed discussion and citations.

As presented in Figures 2-3, 2-4, and 2-5, most of the available literature on the health effects of perfluoroalkyls in laboratory animals was conducted in oral studies, with a few inhalation and dermal exposure studies identified. The most commonly examined endpoints were liver, body weight, developmental, reproductive, and immunological.

The results of the animal studies suggest the following:

- **Hepatic effects.** Evidence from acute, intermediate, and/or chronic oral studies in rats, mice, and monkeys indicates that the liver is a sensitive target of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFBA, PFBS, PFDoDA, and PFHpA toxicity. The effects include increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipid levels. These effects were considered specific to rodents and were not considered relevant to humans. Some degenerative and necrotic effects that are likely relevant to humans have also been observed for PFOA, PFOS, and PFHpA. See Section 2.9 for detailed discussion and citations.

- **Immune effects.** Evidence from acute and intermediate oral studies in mice indicates that immune endpoints are sensitive targets of PFOA and PFOS toxicity. The most commonly reported effect was an impaired response to antigens. No alteration in antigen response was observed in the one study of PFNA. Immune function has not been tested for the other perfluoroalkyls examined in this profile. See Section 2.14 for detailed discussion and citations.

- **Reproductive effects.** Impaired mammary gland development has been observed in mice orally exposed to PFOA. In general, studies of PFOA and PFOS have not found alterations in fertility. See Section 2.16 for detailed discussion and citations.

- **Developmental effects.** Evidence from acute and intermediate oral studies in rats and/or mice indicates that developmental endpoints are targets of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, and PFBA toxicity. The developmental effects include decreases in pup body weight, decreases in pup survival, and alterations in locomotor activity. See Section 2.17 for detailed discussion and citations.

Figure 2-1. Health Effect Endpoints Examined in Epidemiological Studies

Health Effect Endpoint	Perfluoroalkyl											
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFBA	PFDoDA	PFHxA	FOSA
Body weight	•	•	•	•	•	•				•		•
Respiratory	•											
Cardiovascular	•	•	•	•	•	•	•	•	•	•	•	•
Gastrointestinal		•										
Hematological	•	•										
Musculoskeletal	•	•	•	•								
Hepatic	•	•	•	•	•	•	•	•	•	•		
Renal	•	•	•	•	•			•		•	•	
Dermal												
Ocular												
Endocrine	•	•	•	•	•	•				•		
Immunological	•	•	•	•	•	•	•	•		•	•	•
Neurological	•	•	•	•								
Reproductive	•	•	•	•	•	•		•		•	•	•
Developmental	•	•	•	•	•	•	•		•	•		•
Other noncancer	•	•	•	•	•	•	•					•
Cancer	•	•	•	•	•	•	•			•		•

FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

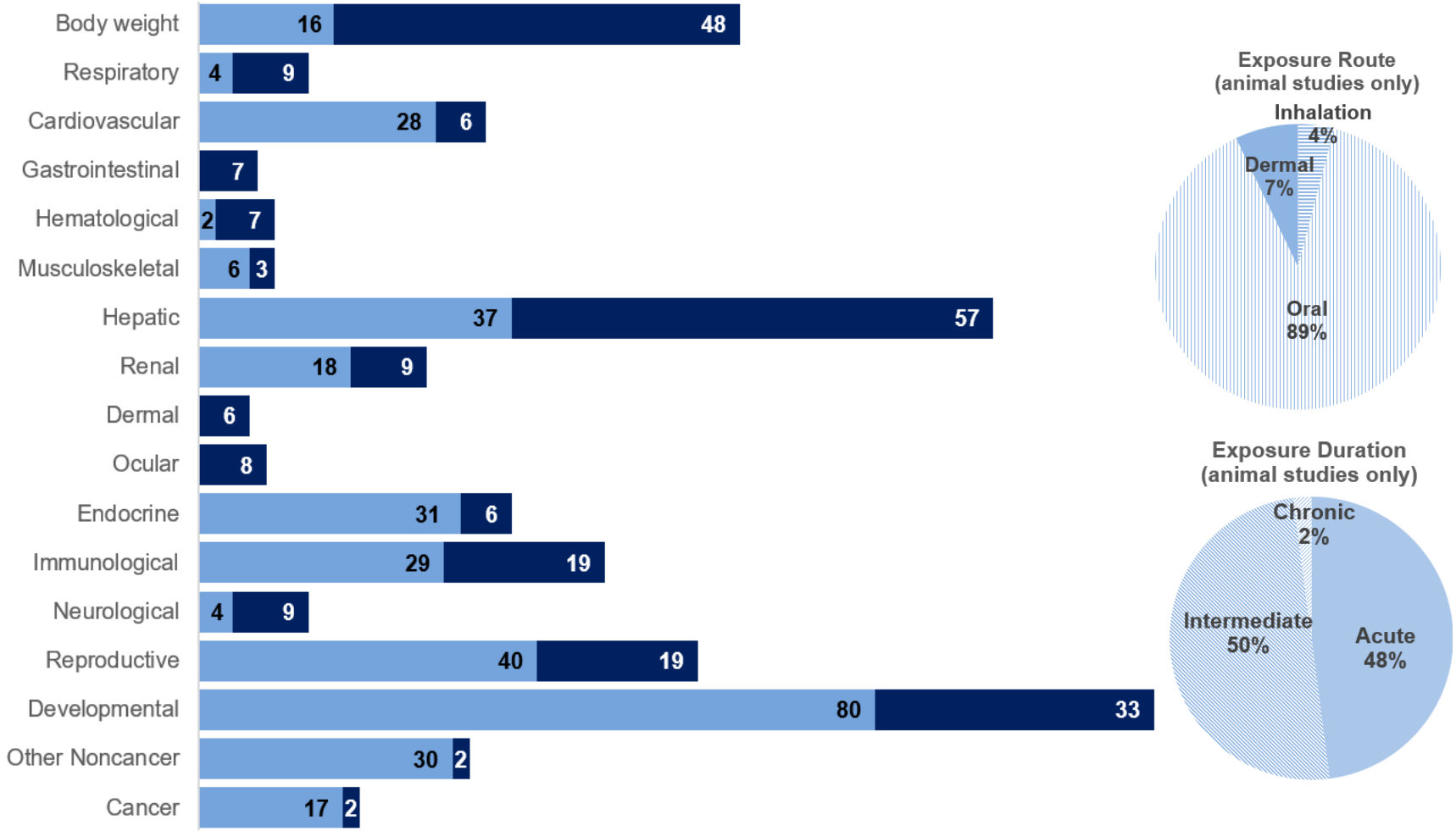
Figure 2-2. Health Effect Endpoints Examined in Laboratory Animal Studies

Health Effect Endpoint	Perfluoroalkyl											
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFBA	PFDoDA	PFHxA	FOSA
Body weight	•	•	•	•	•	•		•	•	•	•	•
Respiratory	•	•	•	•	•			•	•		•	
Cardiovascular	•	•	•		•			•	•	•	•	
Gastrointestinal	•	•	•		•			•	•	•	•	
Hematological	•	•	•		•	•		•	•	•	•	
Musculoskeletal	•	•	•					•	•		•	
Hepatic	•	•	•	•	•	•		•	•	•	•	•
Renal	•	•	•		•	•		•	•	•	•	
Dermal	•	•							•			
Ocular	•	•						•	•		•	
Endocrine	•	•	•		•			•	•	•	•	
Immunological	•	•	•	•	•			•	•		•	
Neurological	•	•	•		•			•	•	•	•	
Reproductive	•	•	•	•				•	•	•	•	
Developmental	•	•	•	•	•	•		•	•	•	•	
Other noncancer	•			•								
Cancer	•	•										

FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Figure 2-3. Overview of the Number of Studies Examining PFOA Health Effects*

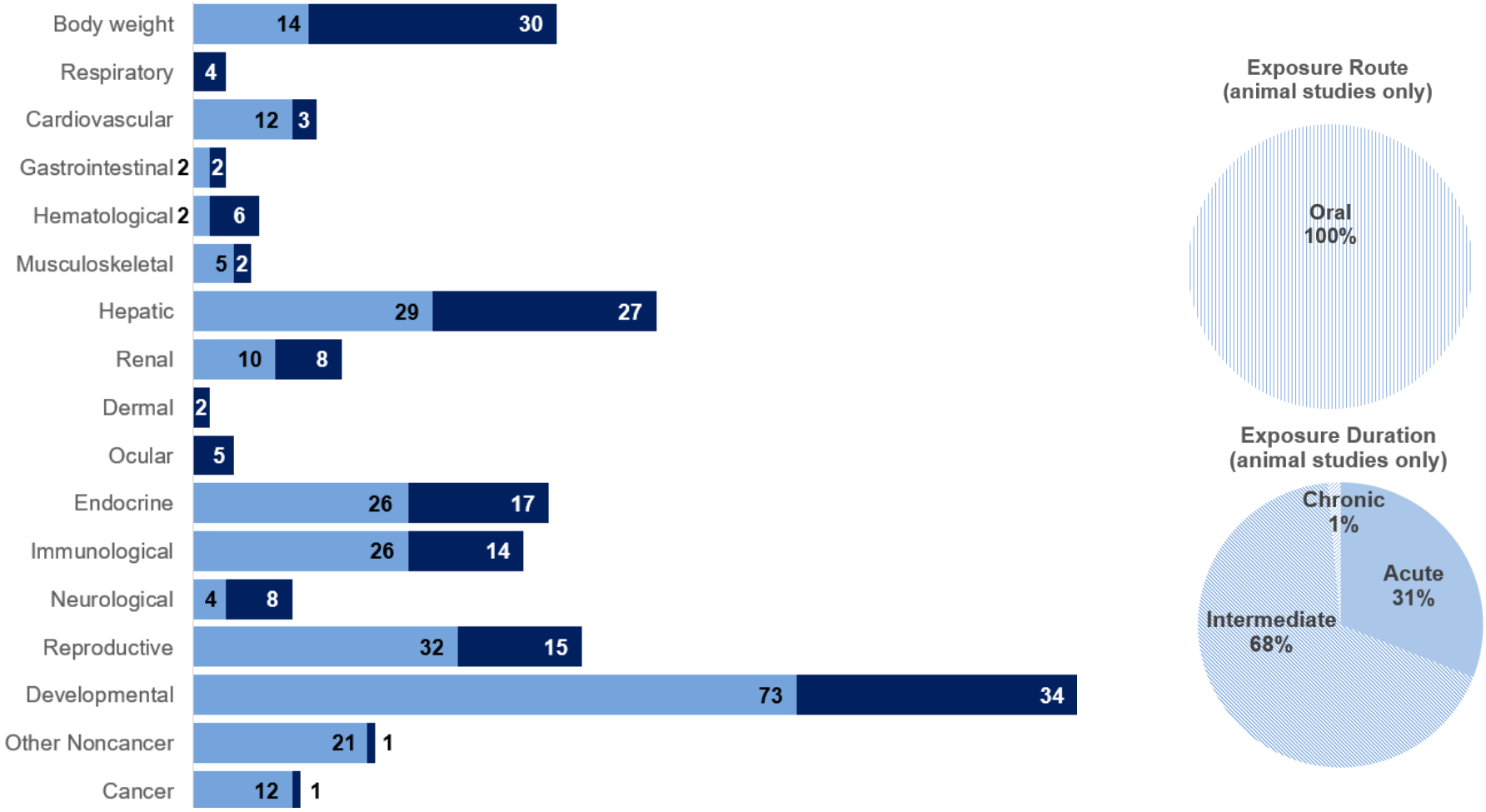
Developmental, hepatic, and body weight effects of PFOA were the most widely examined potential toxicity outcomes
More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 363 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. In this figure, the number of human studies is referring to the number of publications; most human studies examined multiple endpoints.

Figure 2-4. Overview of the Number of Studies Examining PFOS Health Effects*

Developmental, hepatic, and reproductive effects of PFOS were the most widely examined potential toxicity outcomes
More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)

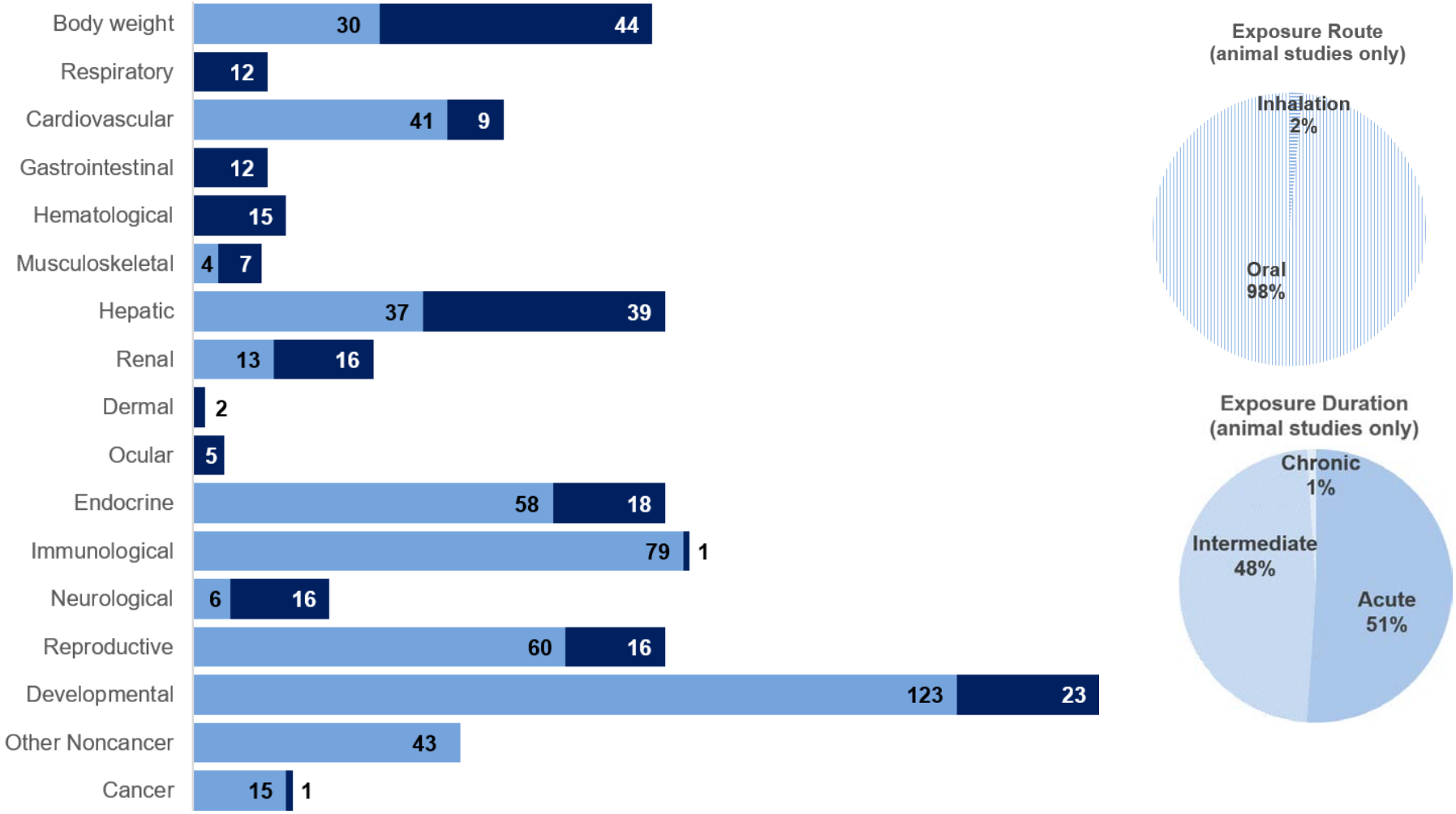


*Includes studies discussed in Chapter 2. A total of 301 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. In this figure, the number of human studies is referring to the number of publications; most human studies examined multiple perfluoroalkyls.

Figure 2-5. Overview of the Number of Studies Examining Other Perfluoroalkyls Health Effects*

Developmental, hepatic, and body weight effects of other perfluoroalkyls were the most widely examined potential toxicity outcomes

More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 213 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. Most human studies examined multiple perfluoroalkyls; within each publication, the results for each perfluoroalkyl is counted as a study.

Table 2-1. Levels of Significant Exposure to PFOA – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
ACUTE EXPOSURE									
1	Rat (albino) 5 M,F	1 hour (NS)	18,600	CS, BW, GN, HP	Resp		18,600		Red nasal discharge; dry rales
					Ocular		18,600		Red material around the eyes; lacrimation
					Neuro		18,600		Excessive salivation
Griffith and Long 1980									
APFO									
2	Rat (CD) 36 M	4 hours	380, 810, 830, 2,200, 4,800, 5,700	CS, HE, BI, GN, HP	Death			980 M	LC_{50}
					Bd wt			380 M	Weight loss for 1–2 days after exposure (data not shown)
					Resp			380 M	Pulmonary edema
					Gastro		380 M		Stomach irritation
					Hepatic	810			Liver enlargement at 810 mg/m^3; no histological alterations
					Ocular	380 M		810 M	Corneal opacity and corrosion
Kennedy et al. 1986									
APFO									
3	Rat (CD) 24 M	2 weeks 6 hours/day 5 days/week	0, 1, 7.6, 84	CS, HE, BI, GN, HP	Bd wt	7.6	84		7% lower body weight on exposure day 5
					Resp	84			
					Cardio	84			
					Gastro	84			
					Hemato	84			
					Musc/skel	84			
					Hepatic	84			Increased absolute and relative liver weight; hepatocellular hypertrophy at 7.6 mg/m^3
					Renal	84			
					Dermal	84			
					Ocular	84			
					Endocr	84			
					Neuro	84			No histological alterations
					Repro	84			No histological alterations
Kennedy et al. 1986									
APFO									

Table 2-1. Levels of Significant Exposure to PFOA – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
4	Rat (Sprague-Dawley) 12 F	GDs 6–15 6 hours/day	0, 0.1, 1, 10, 25	MX, DX, OW, CS, HP	Death			25	3/12 deaths on GDs 12, 13, and 17
					Bd wt	1	10		12% decrease weight gain on GDs 6–15
					Hepatic	25			18% increase absolute liver weight at 25 mg/m^3
					Develop	10	25		10% decreased neonatal body weight on PND 1
Staples et al. 1984 APFO									

[a]The number corresponds to entries in Figure 2-6.

APFO = ammonium perfluorooctanoate (ammonia salt of PFOA); BI = biochemical changes; BW or Bd wt = body weight; F = female(s); Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE or Hemato = hematological; HP = histopathology; LC_{50} = lethal concentration, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; PFOA = perfluorooctanoic acid; PND = postnatal day; Repro = reproductive; Resp = respiratory

Figure 2-6. Levels of Significant Exposure to PFOA – Inhalation
Acute (≤14 days)

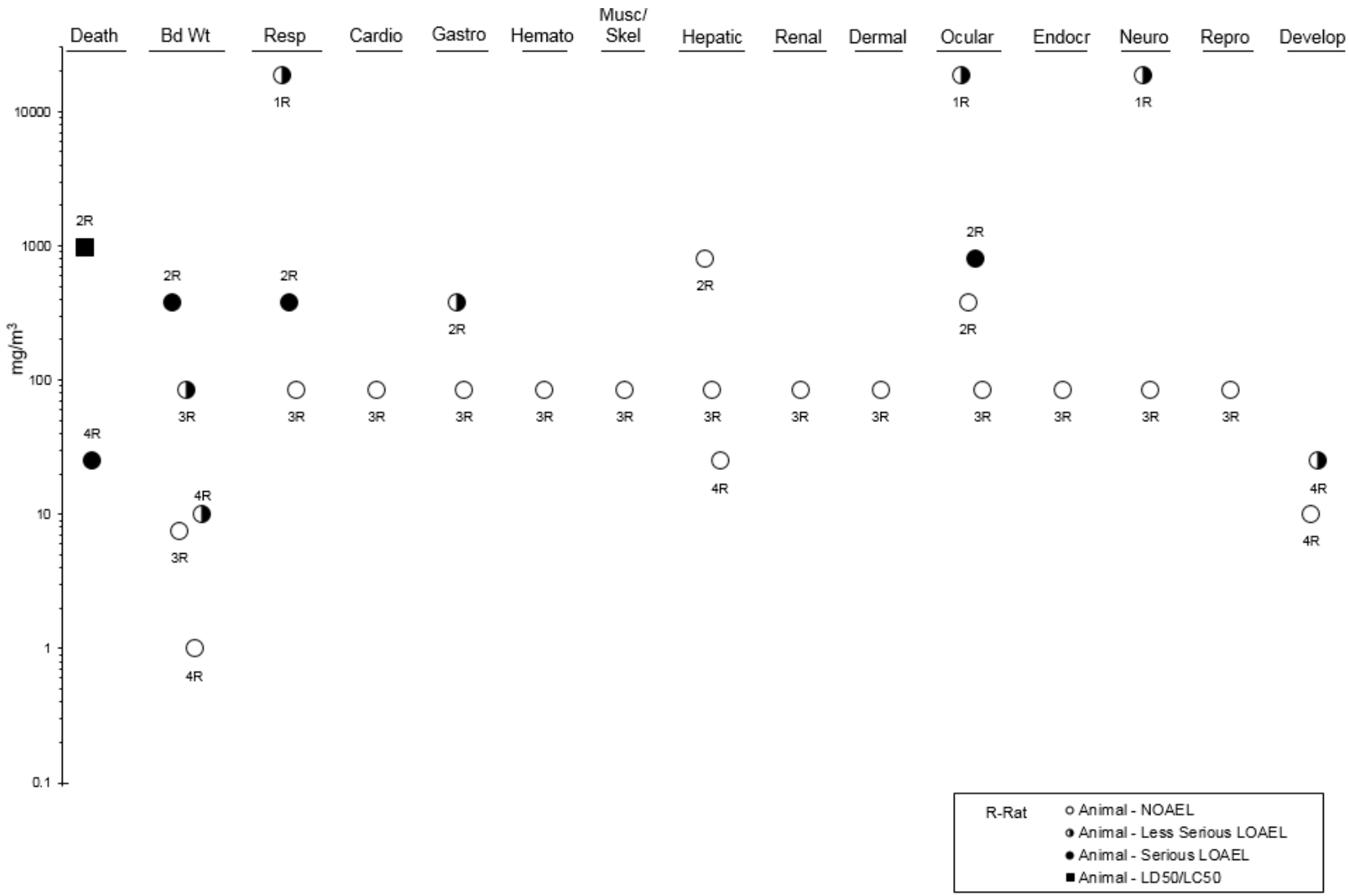


Table 2-2. Levels of Significant Exposure to Other Perfluoroalkyls – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
ACUTE EXPOSURE									
PFNA									
1	Rat (CD) 6 M	4 hours	67–4,600	LE	Death			820	14-day LC_{50}
Kinney et al. 1989									
Exposure was nose-only.									
2	Rat (CD) 10 M	4 hours	0, 67, 590	BW, OW	Bd wt	67	590		Reduced 18% 5 days after exposure
					Resp	67	590		Lung noise; labored breathing during and after exposure
					Hepatic	67			28% increase in absolute liver weight 5 days after exposure to ≥67 mg/m^3
Kinney et al. 1989									
Exposure was nose-only.									

[a]The number corresponds to entries in Figure 2-7.

BW or Bd wt = body weight; LC_{50} = lethal concentration, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; OW = organ weight; PFNA = perfluorononanoic acid; Resp = respiratory

Figure 2-7. Levels of Significant Exposure to Other Perfluoroalkyls – Inhalation
Acute (≤14 days)

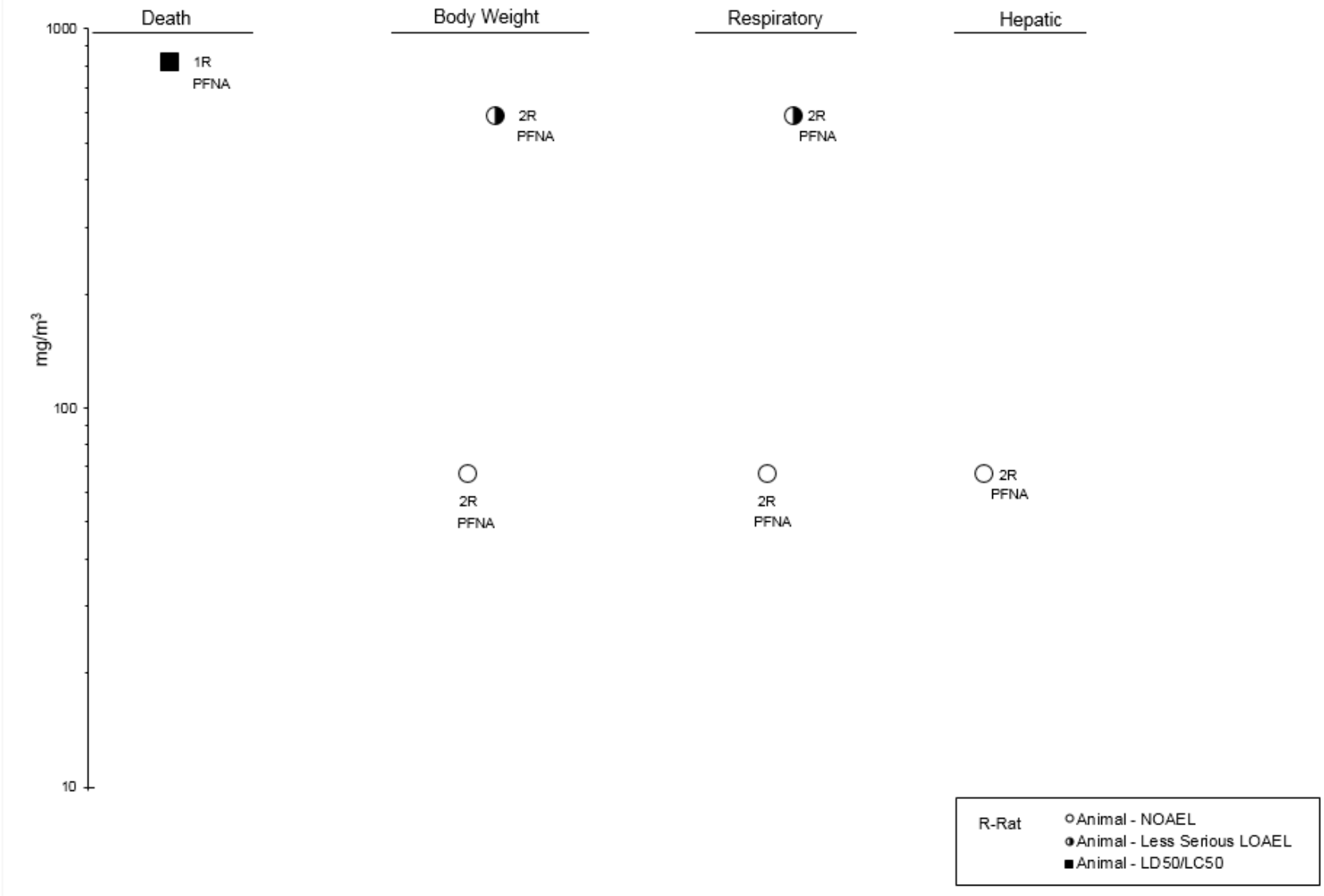


Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Monkey (Rhesus) 10 M,F	2 weeks (G)	0, 3, 10, 30, 100	LE, CS, HE, BI, GN, HP	Death			100	Unspecified number out of four died on week 2
Griffith and Long 1980									
APFO									
2	Rat (CD) 10 M	14 days (GW)	0, 25	BI, OW	Repro		25		184% increase in serum estradiol
Biegel et al. 1995									
APFO									
3	Rat (CD) 75 M	14 days (GW)	0, 1, 10, 25, 50	BW, OW, BI, HP	Bd wt	10	25		14% reduction in final body weight
					Hepatic	50			46% increase in relative liver weight at ≥10 mg/kg/day
					Repro	1	10		63% increase in serum estradiol
Cook et al. 1992									
APFO									
4	Rat (Sprague-Dawley) 18 M	1 or 7 days (F)	0, 18, 23		Hepatic	23			Increased liver weight, decreased serum cholesterol, triglyceride, hepatocellular hypertrophy at ≥18 mg/kg/day
Elcombe et al. 2010									
APFO									
5	Rat (albino) 25 M,F	Once (GO)	100, 215, 464, 1,000, 2,150		Death			680 M 430 F	LD_{50} LD_{50}
Griffith and Long 1980									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
6	Rat (albino) 40 M,F	28 days (F)	M: 0, 3, 10, 30, 10, 300, 1,000, 3,000; F: 0, 3.4, 11.3, 34, 113, 340, 1,130, 3,400		Death			1,000 M 1,130 F	5/5 males and 5/5 females died before end of 1st week of study
Griffith and Long 1980									
APFO									
7	Rat (Wistar) 8 M	7 days ad lib (F)	0, 16	BW, OW, BI, EA	Bd wt	16			
					Hepatic	16			66% increase in absolute liver weight
Haughom and Spydevold 1992									
APFO									
8	Rat (Sprague-Dawley) 3 M	14 days (F)	0, 20	OW, EA	Hepatic	20			45% increase in relative liver weight
Ikeda et al. 1985									
PFOA									
9	Rat (Sprague-Dawley) 16 M	14 days (GW)	0, 0.5, 5, 50	BW, OW, CS, HE, BI	Bd wt	50			
					Hepatic	50			2-fold increased mean relative liver weight at 50 mg/kg/day
					Immuno	50			No alterations in spleen weight or splenocyte phenotype
Iwai and Yamashita 2006									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
10	Rat (Wistar) 5–12 M	Once (GO)	0, 50	BW, FX	BW	50			
					Neuro	50			No alteration in performance on novel object recognition test
Kawabata et al. 2017									
PFOA									
11	Rat (Wistar) 30 M	1 week (F)	0, 1.2, 2.4, 4.7, 9.5	BW, OW, EA, HP	Bd wt	38			
					Hepatic	9.5			Significant increase in absolute and relative liver weight at ≥4.7 mg/kg/day
Kawashima et al. 1995									
PFOA									
12	Rat (SD-IGS BR) 10 M	14 days (GW)	0, 0.3, 1, 3, 10, 30,	BW, OW, BC	Bd wt	1	3		24% decrease in overall body weight gain
					Hepatic	30			Decreased serum cholesterol levels at ≥0.3 mg/kg/day
Loveless et al. 2006									
APFO									
13	Rat (CD) 15 M	14 days (G)	0, 0.2, 2, 20, 40	BW, OW, EA	Bd wt	2	20		14% lower final body weight
					Hepatic	40			34% increase in absolute and relative liver weight at ≥2 mg/kg/day
					Repro	0.2	2		2-fold increase in serum estradiol
Liu et al. 1996									
APFO									
14	Rat (Sprague-Dawley) 24 M	1, 3, 7 days (GW)	0, 50	BW, BI, EA, HP	Bd wt		50		17% weight loss
					Hepatic	50			2-fold increase in relative and absolute liver weight
Pastoor et al. 1987									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
15	Rat (Sprague-Dawley) 12 or 25 F	GDs 6–15 (GO)	0, 100	DX, MX, BW	Bd wt			100	33% reduced maternal body weight gain
					Develop	100			No alterations in fetal body weight or teratology
Staples et al. 1984									
APFO									
16	Mouse (Kunming) 12 F	GDs 1–7 or 13 (GW)	0, 2.5, 5, 10	BC, OW, RX	Repro		2.5		Decreases in progesterone levels at ≥5 mg/kg in GD 13 group; increased serum estradiol level at 10 mg/kg/day on GD 7; decreases in number of corpora lutea at ≥2.5 mg/kg/day in GD 1–7 groups and at ≥5 mg/kg/day in GD 13 groups
					Develop	5	10		Increased resorbed embryos at 10 mg/kg/day in GD 13 group
Chen et al. 2017b									
PFOA									
17	Mouse (SV129 wild type) 4 M	7 days (G)	0, 10	BW, HP	Bd wt	10			
					Hepatic	10			Hepatocellular hypertrophy, steatosis, and increased hepatic triglyceride levels
Das et al. 2017									
PFOA									
18	Mouse (C57BL/6N) 6 F	10 days (W)	0, 3.75, 7.5, 15	FX	Immuno	7.5	15		Altered response to sRBC
DeWitt et al. 2009									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
19	Mouse (CD) 40 M,F	28 days (F)	M: 0, 5.4, 18.0, 54, 180, 540, 1,800, 5,400; F: 0, 5.8, 19.5, 58, 195, 580, 1,950, 5,800		Death			180 M 195 F	5/5 died before 2nd week of study 5/5 died before 2nd week of study
Griffith and Long 1980									
APFO									
20	Mouse (C57BL/6N) 16 F	GDs 6–17 (DW)	0, 0.5, 1		Bd wt	1			
					Develop		0.5		7–10% decrease in litter weight on PND 2
Hu et al. 2010									
APFO									
21	Mouse (CD-1) 10 M	Once (G)	0, 0.58, 8.70	CS, OF, DX	Develop		0.58		Decreased spontaneous activity and altered response to cholinergic stimulant
Johansson et al. 2008									
APFO									
10-day-old mice were administered a single dose of PFOA; neurodevelopmental testing was conducted when the pups were 2 or 4 months of age									
22	Mouse (CD-1) 5 M,F	14 days (F)	0, 5.3, 54, 537	OW	Hepatic	537			123–155% increase in absolute liver weight in 14 days at ≥5.3 mg/kg/day
Kennedy 1987									
APFO									
23	Mouse (CD-1) 10 M	14 days (GW)	0, 0.3, 1, 3, 10, 30,	BW, OW, BC	Bd wt	3		10	6–12% decreased in body weight gain
					Hepatic	30			Decreased serum cholesterol levels at ≥0.3 mg/kg/day
Loveless et al. 2006									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
24	Mouse (C57BL/6N) 3 M	2–10 days (F)	0, 78, 390	BW, OW, EA	Bd wt			78	25% body weight loss after 10 days of treatment
					Hepatic	390			74% increase in absolute liver weight at ≥78 mg/kg/day
Permadi et al. 1992									
PFOA									
25	Mouse (C57BL/6N) 4 M	2–10 days (F)	0, 78, 390	BW, OW, EA	Bd wt			78	25% body weight loss after 5 days of treatment
					Hepatic	390			74% increase in absolute liver weight in 5 days at ≥78 mg/kg/day
Permadi et al. 1993									
PFOA									
26	Mouse (C57BL/6) 4 M	10 days (F)	0, 2	BW, FI, BC, HP	Bd wt	2			
					Hemato	2			
					Hepatic	2			68% increase in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol levels
					Immuno	2			
Qazi et al. 2010a									
PFOA									
27	Mouse (BALB/c) 5 F	7 days (GW)	20	BW, OW, BC	Bd wt		20		36% decrease in body weight gain
					Hepatic	20			32% increase in relative liver weight
					Immuno		20		Inhibition of T-and B-lymphocyte proliferation in response to sRBC; decreased phagocytosis by peripheral blood cells and NK cell activity; decreased IgM antibody formation in response to OVA
Vetvicka and Vetvickova 2013									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
28	Mouse (CD-1) 14 F	GDs 8–17 GDs 1217 (GW)	0, 5	DX, GN	Develop		5		Altered mammary gland development in female pups; reduced pup weight on PND 20
White et al. 2007									
APFO									
29	Mouse (CD-1) 56 F	GDs 8–17 (GW)	0, 5		Bd wt	5			
					Hepatic	5			40–120% increased relative liver weight in lactating dams on PNDs 1–10
					Repro		5		Immature mammary gland morphology in lactating dams on PNDs 1–10
					Develop		5		Delayed mammary gland development (30–60%) in female pups on PNDs 1–10
White et al. 2009									
APFO									
30	Mouse (CD-1) 12–14 F	GDs 7–17 GDs 10–17 GDs 13–17 GDs 15–17	0, 5		Develop		5		Delayed mammary gland development (31–47%) in female pups on PNDs 22–32 and at 18 months
White et al. 2009									
APFO									
31	Mouse (CD-1) 6–14 F	GDs 7–17, GDs 10–17 GDs 13–17 GDs 15–17 (GW)	0, 5, 20	DX, MX, BW, OW	Bd wt	20			No alterations in dams dosed on GDs 15–17
					Hepatic	20			Increase in relative liver weight in dams dosed on GDs 13–17, 10–17, or 7–17 at ≥5 mg/kg/day
					Develop		5		Reduced pup body weight at weaning, 43% in males and 35% in females
Wolf et al. 2007									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
32	Mouse (C57BL/6N) 8 M	7 days (F)	0, 24	CS, BW, OW, BI	Bd wt		24		>10% reduced final body weight
					Hepatic	24			2-fold increase in absolute liver weight
Xie et al. 2003									
PFOA									
33	Mouse (C57BL/6N) 8 M	10 days (F)	0, 30	BW, OW, BI, CS	Bd wt		30		17% decrease in final body weight
					Hepatic	30			>90% increase in absolute and relative liver weight
					Immuno			30	86% reduction in absolute thymus weight; 30% reduction in absolute spleen weight
Yang et al. 2000									
PFOA									
34	Mouse (C57BL/6N) 8 M	10 days (F)	0, 1, 3.5, 11.5, 23, 58	CS, BW, OW, OF, BI	Hepatic	1			35% increase in absolute liver weight at ≥1 mg/kg/day
					Immuno		11.5		40–50% decrease in spleen and thymus weights
Yang et al. 2001									
PFOA									
35	Mouse (C57BL/6N) 8–12 M	7 days (F)	0, 24	CS, BW, BI, OF	Immuno		24		Decreased humoral response to immunization with horse red blood cells
Yang et al. 2002a									
PFOA									
36	Mouse (C57BL/6N) 16 M	7 days ad lib (F)	0, 33	BW, OW, BI, OF	Bd wt		33		14% decreased mean body weight
					Hepatic	33			86% increase in absolute liver weight
					Immuno		33		40% reduction in spleen weight and 79% reduction in thymus weight
Yang et al. 2002b									
PFOA									
Experiments with PPARα-null mice suggested PPARα-dependent and -independent immune effects									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
37	Rabbit (New Zealand) 18 F	GDs 6–18 1 time/day (GW)	0, 1.5, 5, 50	CS, MX, DX, BW	Develop	50			
Gortner et al. 1982									
PFOA									
INTERMEDIATE EXPOSURE									
38	Monkey (Cynomolgus) 4–6 M	26 weeks 1 time/day (C)	0, 3, 10, 30/20	BC, BW, CS, EA, FI, GN, HE, HP, LE, OP, OW, UR	Bd wt	10	20		12% decrease by week 10
					Resp	20			
					Cardio	20			
					Gastro	20			
					Hemato	20			
					Musc/skel	20			
					Hepatic		3		36% increase in absolute liver weight at ≥3 mg/kg/day; increased serum triglyceride levels at 30/20 mg/kg/day
					Renal	20			
					Dermal	20			
					Ocular	20			
					Endocr	3	10		Significant decrease in serum TT4 (27–35%) and FT4 (30–38%)
					Immuno	20			No histological alterations
					Neuro	20			No histological alterations
					Repro	20			No histological alterations
Butenhoff et al. 2002									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
39	Monkey (Rhesus) 10 M,F	90 days 1 time/day (G)	0, 3, 10, 30, 100	LE, CS, HE, BI, GN, HP	Death			30	One male and two females died during weeks 7–12
					Bd wt	10		30	33% body weight loss by week 6
					Cardio	10			
					Gastro	10	30		Emesis
					Hemato	30			
					Hepatic	10			
					Renal	10			
					Immuno	10	30		Atrophy of lymphoid follicles in spleen and lymph nodes
					Neuro	10	30		Hypoactivity and prostration
					Repro	100			No histological alterations in testes or ovaries
Griffith and Long 1980									
APFO									
40	Monkey (Cynomolgus) 8 M	30 day 1 time/day (C)	0, 2, 20	CS, BW, FI, BI, HE, EA, GN, HP	Bd wt	20			
					Hemato	20			
					Hepatic	20			
					Endocr	20			No alterations in serum levels of thyroid hormones and TSH and histopathology of adrenals
					Repro	20			No alterations in serum estradiol, estriol, or histopathology of the testes
Thomford 2001									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
41	Rat (CD) 156 M	1 year ad lib (F)	0, 13.6	CS, BW, FI, OW, GN, HP	Bd wt		13.6		>10% reduced weight gain
					Hepatic	13.6			Increased relative liver weight
					Repro		13.6		Significant increase in serum estradiol at 1, 3, 6, 9, and 12 months (~100–180%); prolactin was decreased at all time points, but not always significantly
Biegel et al. 2001									
APFO									
42	Rat (Sprague-Dawley) 30 M,F	70–90 days 1 time/day (GW)	0, 1, 3, 10, 30	MX, DX, OF, BW, GN, HP	Bd wt	3	10		>11% reduced body weight
					Hepatic	1	3		Increased absolute and relative liver weight in P-generation (36%) and F1-generation (30%) males; hepatocellular hypertrophy and less commonly necrosis in F1 males (incidence not reported)
					Renal		3		Increased absolute and relative kidney weight in P-generation (14%) and F1-generation (11%) males
					Endocr	10	30		Vacuolation of zona glomerulosa of adrenal gland
					Repro	30			No alteration in reproductive performance in P0 or F1 generation
					Develop	10		30	Increased number of dead pups on PNDs 6–8
Butenhoff et al. 2004b									
APFO									
43	Rat (Wistar) 5 F	GD 1 to PND 21 ad lib (W)	0, 1.6		Develop		1.6		17–18% reduced motor coordination and increased locomotor activity in pups on PNDs 34–35
Cheng et al. 2013									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
44	Rat (Sprague-Dawley) 10 M	Daily 28 days (G)	0, 5, 20	OW, HP	Resp		5		
					Hepatic		5		Cytoplasmic vacuolization, necrosis, hypertrophy, increased liver weight at ≥5 mg/kg/day; fatty degeneration, angiectasis and congestion in the hepatic sinusoid or central vein at 20 mg/kg/day
					Neuro		5		Cachexia and lethargy
Cui et al. 2009									
PFOA									
45	Rat (Sprague-Dawley) 10 M	28 days (F)	0, 18		Hepatic		18		Increased liver weight (43% on day 29), decreased serum cholesterol (39% on day 29) and triglyceride (73% on day 29), hepatocellular hypertrophy and hyperplasia
Elcombe et al. 2010									
APFO									
46	Rat (CD) 40 M,F	28 days ad lib (F)	M: 0, 3, 10, 30, 100, 300, 1,000, 3,000; F: 0, 3.4, 11.3, 34, 113, 340, 1,130, 3,400	BW, FI, HP	Bd wt	10 M	30 M	100 M	30 mg/kg/day: 11% reduction in final body weight; 100 mg/kg/day: 33% reduction in final body weight
					Hepatic	3 M			Hepatocyte hypertrophy
Griffith and Long 1980									
APFO									
47	Rat (CD) 30 M,F	90 days ad lib (F)	M: 0, 1, 3, 10, 30, 100; F: 0, 1.1, 3.4, 11, 34, 110	CS, BW, FI, HE BI, GN, HP, OW	Bd wt	30 M 110 F	100 M		33% reduction in final mean body weight
					Resp	100 F			
					Cardio	110 F			
					Gastro	110 F			
					Musc/skel	110 F			
					Hepatic	100 M			Hepatocyte hypertrophy; 50% increase in absolute liver weight
					Renal	110 F			

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Dermal	110 F			
					Ocular	110 F			
					Endocr	110 F			
					Immuno	110 F			
					Neuro	110 F			No histological alterations
					Repro	100 M 110 F			No histological alterations
Griffith and Long 1980									
APFO									
48	Rat (CD) 10 M	28 days (G)	0, 0.29, 0.96, 9.6, 29		Bd wt	0.96	9.6		10% decrease in final body weight
					Hemato	29			
					Hepatic	0.29	29		34% decrease in serum triglyceride levels, minimal hepatocellular hypertrophy at ≥0.29 mg/kg/day; minimal focal necrosis at 29 mg/kg/day
					Immuno	29			
Loveless et al. 2008									
APFO									
49	Rat (CD) 55 M	13 weeks ad lib (F)	0, 0.06, 0.64, 1.94, 6.5	CS, BW, FI, OW, GN, HP	Bd wt	6.5			
					Resp	6.5			
					Hepatic	6.5			Minimal to moderate hepatocellular hypertrophy at ≥0.64 mg/kg/day
					Neuro	6.5			No histological alterations
					Repro	6.5			No histological alterations
Perkins et al. 2004									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
50	Mouse (129S1/SvlmJ WT) 17 F	GDs 1–17 (GW)	0, 0.1, 0.3, 0.6, 1, 3, 5, 10, 20	MX, DX, BW	Bd wt	10 F			
					Hepatic	20			Increased absolute and relative liver weight of dams on PND 22 at ≥1 mg/kg/day
					Develop	0.3		0.6	Significantly reduced pup survival (46%) from birth to weaning
Abbott et al. 2007									
APFO									
Body weight NOAEL is for changes during pregnancy.									
51	Mouse (wild-type Sv/129) 5–6 F	GDs 1–17 1 time/day (GW)	0, 3		Hepatic	3			28% increased liver weight, hepatocellular hypertrophy with increased peroxisomes
					Develop			3	31.5% reduced pups per litter on PND 20
Albrecht et al. 2013									
PFOA									
52	Mouse (C57BL/6N) 8 F	15 days ad lib (W)	0, 0.94, 1.88, 3.75, 7.5, 15, 30	BW, OW, OF	Bd wt	7.5		15	Weight loss (~5%)
					Immuno	1.88	3.75		Reduced sRBC-specific response to IgM antibody titers
DeWitt et al. 2008									
APFO									
53	Mouse (wild-type C57BL/6-Tac and PPARα knockout) 8 F	15 days (W)	0, 7.5, 30	BW, OW, BC	Bd wt		30		14–20% decrease in body weight in wild-type mice
					Immuno	7.5	30		16 and 14% reduction in sRBC-specific antibody response in wild-type and PPARα knockout mice, respectively; 29.8% decrease in relative spleen weight in wild-type mice
DeWitt et al. 2016									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
54	Mouse (C57BL/6N) 8 F	15 days (W)	0, 0.94, 1.88, 3.75, 7.5	BW, OW, BC	Bd wt	7.5			
					Immuno	0.94	1.88		Decrease in DNP-specific IgM antibody responses; decreases in relative spleen (17%) and thymus weights (14%) at 7.5 mg/kg/day
DeWitt et al. 2016									
PFOA									
55	Mouse (C57BL/6N) 8 F	10, 13, 15 days (W)	0, 3.75, 7.5	BC	Bd wt	7.5			
DeWitt et al. 2016									
PFOA									
56	Mouse (CD-1) 6–14 dams; 21–37 F offspring	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1, 5	HP	Hepatic	0.3	1		Increase in severity of chronic inflammation at ≥1 mg/kg/day; Ito cell hypertrophy and centrilobular hepatocellular hypertrophy at 5 mg/kg/day
Filgo et al. 2015a, 2015b									
PFOA									
Animals exposed *in utero* on GDs 1–17 and examined at 18 months of age; the offspring were also examined by Hines et al. 2009									
57	Mouse (129/Sv WT) 3–7 dams; 6–10 F offspring	GDs 1–17 (GW)	0, 0.1, 0.3, 0.6, 1	HP	Hepatic	1			Increased severity of centrilobular hepatocyte hypertrophy at ≥0.3 mg/kg/day
Filgo et al. 2015a, 2015b									
PFOA									
Animals exposed *in utero* on GDs 1–17 and examined at 18 months of age; the offspring were also examined by Abbott et al. 2007									
58	Mouse (129/Sv PPARα-knockout) 5–9 dams; 6–10 F offspring	GDs 1–17 (GW)	0, 0.1, 0.3, 1, 3	HP	Hepatic	1	3		Centrilobular hepatocyte hypertrophy and bile duct hyperplasia at 3 mg/kg/day
Filgo et al. 2015a, 2015b									
PFOA									
Animals exposed *in utero* on GDs 1–17 and examined at 18 months of age; the offspring were also examined by Abbott et al. 2007									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
59	Mouse (CD-1) NS F	GDs 1–17 (GW)	0, 0.1, 0.3, 1	NX	Develop	0.3	1		Increased ambulatory activity and decreased methamphetamine-induced activity
Goulding et al. 2017									
PFOA									
60	Mouse (CD) 40 M,F	28 days ad lib (F)	M: 0, 5.4, 18.0, 54, 180, 540, 1,800, 5,400; F: 0, 5.8, 19.5, 58, 195, 580, 1,950, 5,800	BW, FI, HP	Death			54 M 58 F	4/5 died before end of 4th week 5/5 died before 4th week of study
					Bd wt		5.4 M	5.8 F	Males: final body weight 20% lower than controls; females: final body weight 25% lower than controls
					Hepatic	18			3-fold or greater increased absolute and relative liver weight and hepatocellular hypertrophy at ≥5.4 mg/kg/day
Griffith and Long 1980									
APFO									
61	Mouse (CD-1) 5–14F	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1, 3, 5	BW FX	Develop		5 F		Decreased birth weight (approximately 8%) and body weight at weaning (24%)
Hines et al. 2009									
APFO									
62	Mouse (CD-1) 5 M,F	21 days ad lib (F)	0, 0.0018, 0.0054, 0.018, 0.054, 0.18, 0.54, 1.8, 5.4	OW	Hepatic	5.4			39–41% increase in absolute liver weight at ≥5.4 mg/kg/day
Kennedy 1987									
APFO									
63	Mouse (C57BL/6) 10 F	GDs 1–21 (F)	0.3	BW, DX	Develop		0.3[b]		Altered femur and tibial bone morphology, decreased tibial mineral density
Koskela et al. 2016									
PFOA									
Offspring were examined at 13 and 17 months of age									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
64	Mouse (CD-1) 9–45 M	GDs 1–17 1 time/day (GW)	0, 1, 3, 5, 10, 20, 40	MX, DX, BW, OW	Bd wt	5		10	32% reduced weight gain during pregnancy
					Hepatic	1			38% increase in absolute liver weight at ≥1 mg/kg/day
					Develop		1	5	Reduced ossification of proximal phalanges and advanced preputial separation at ≥1 mg/kg/day; 20% decrease in pup body weight on PND 23 at ≥3 mg/kg/day; increased number of dams with full litter resorptions, decrease in neonatal survival, tail and limb defects, delay in eye opening, and delay in first estrus at ≥5 mg/kg/day
Lau et al. 2006									
APFO									
65	Mouse (CD) 20 M	28 days (G)	0, 0.29, 0.96, 9.6, 29		Bd wt	0.96		9.6	Weight loss (86% of controls)
					Hepatic	0.29	0.96		Mild hepatocellular hypertrophy at ≥0.29, moderate to severe hypertrophy and single cell necrosis at ≥0.96 mg/kg/day; decreased serum cholesterol (31%) and triglyceride (53%) at ≥9.6 mg/kg/day
					Immuno	0.96 M	9.6 M		Decreased response to sRBC, decreased number of splenic and thymic lymphocytes
Loveless et al. 2008									
APFO									
66	Mouse (CD-1) 13 F	GDs 1–17 (G)	0, 0.3, 1.0, 3.0	DX	Develop		0.3		Impaired development of mammary glands in offspring
Macon et al. 2011									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
67	Mouse (CD-1) 13 F	GDs 10–17 (G)	0, 0.01, 0.1, 1.0	DX	Develop		0.01		Developmental delays in mammary gland development
Macon et al. 2011									
PFOA									
68	Mouse (C57BL/6J-Apc+/+) 20–21 F	GDs 1–17 (GW)	0, 0.01, 0.1, 3.0	BC, BW, FI, HE, OW	Develop	0.1	3		Decrease in number of successful births
Ngo et al. 2014									
PFOA									
69	Mouse (C57BL/6/Bk1) 6 M, 10 F	GDs 1–21 ad lib (F)	0, 0.3		Develop		0.3		Altered exploratory behavior in adult offspring (increased in males and decreased in females); increased global activity in males
Onishchenko et al. 2011									
PFOA									
70	Mouse (CD-1) 17–21 F dams; 4–6 M,F pups	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1	BI, BW, HP, OF, OW	Hepatic		0.01		Increased severity of hepatocellular hypertrophy at PND 91 and periportal inflammation on PND 21 at ≥0.01 mg/kg/day (incidence was not reported); decreased serum total cholesterol, LDL, and HDL levels in high-fat fasted animals on PND 91 at ≥0.3 mg/kg/day
Quist et al. 2015a, 2015b									
PFOA									
Subgroup of female offspring were fed a high-fat diet (50% calories from diet) for 6 weeks									
71	Mouse (C57BL/6) 6 M,F	6 weeks (F)	0.55	BC, BW, FI, OW	Hepatic	0.55			66–67% increase in relative liver weight in males; increased plasma cholesterol levels in males (35%) and females (70%)
Rebholz et al. 2016									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
72	Mouse (BALB/c) 6 M,F	6 weeks (F)	0.55	BC, BW, FI, OW	Hepatic	0.55			54–65% increase in relative liver weight; 20% increase in plasma cholesterol in males
Rebholz et al. 2016									
PFOA									
73	Mouse (C57BL/6) 6 F	GD 7 to PND 21 (F)	0, 0.1	BH, BW	Develop		0.1		Increased horizontal and ambulatory locomotor activity and decreased resting time in males; decrease in novel object recognition in males and females
Sobolewski et al. 2014									
PFOA									
74	Mouse (ICR) 10 M	21 days ad lib (W)	0, 0.5, 2.6, 18, 47	BW, OW, GN, HP	Bd wt	2.6		18	17% decrease in weight gain
					Hepatic	18	47		27% increase in relative liver weight at ≥0.5 mg/kg/day; increases in ALT at ≥2.6 mg/kg/day; hepatocytomegaly at 18 mg/kg/day; necrosis at 47 mg/kg/day
					Renal	47			
Son et al. 2008									
APFO									
75	Mouse (ICR) 10 M	21 days (W)	0, 0.49, 2.64, 17.63, 47.21	FX HP	Immuno		47.21		Marked hyperplasia in spleen white pulp and thymic atrophy
Son et al. 2009									
PFOA									
76	Mouse (C57BL/6N) 7–8 M	3 weeks (F)	0, 5	HP	Hepatic		5		Hepatocellular hypertrophy and degeneration
Tan et al. 2013									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
77	Mouse (CD-1) 4–12 F	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1.0	BW, DX, OW	Develop		0.01		Developmental delays in the mammary glands on PNDs 35 (26%) and 56 (30%)
Tucker et al. 2015 PFOA									
78	Mouse (C57BL/6) 2–6 F	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1.0	BW, DX, OW	Develop	0.1	0.3		Developmental delays in the mammary glands on PNDs 21 (38%) and 61 (25%)
Tucker et al. 2015 PFOA									
79	Mouse (CD-1) 14–16 F	GDs 1–17 GDs 8–17 1 time/day GDs 12–17 (GW)	0, 5	MX, DX, GN, HP	Repro		5		Delayed mammary gland differentiation
White et al. 2007 APFO									
80	Mouse (CD-1) 14 F	GDs 1–17 1 time/day (GW)	0, 5	DX, GN	Develop			5	Increased prenatal loss; 40% reduced neonatal body weight on PNDs 5 and 10
White et al. 2007 APFO									
81	Mouse (CD-1) 28–48 F	GDs 1–17 1 time/day (GW)	0, 3, 5		Develop		3		Delayed mammary gland development in female pups on PNDs 22–63 and at 18 months
White et al. 2009 APFO									
82	Mouse (CD-1) 10–12 F	GDs 1–17 1 time/day (GW)	0, 1, 5		Repro		1		Delayed mammary gland lactational differentiation in dams on PND 22
					Develop	1		5	323% increased prenatal loss, 16.7% decreased live fetuses, 24.3% decreased neonatal survival
White et al. 2011 APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
83	Mouse (CD-1) 10–12 F	GDs 1–17 1 time/day (GW)	0, 0.0024, 1.0024		Repro		0.0024		Delayed mammary gland lactational differentiation in dams on PND 22
		GD 7 to PND 22 (W); 3-generation study			Develop		0.0024		Delayed mammary gland development in female pups on PNDs 22–63
White et al. 2011									
APFO									
84	Mouse (CD-1) 28–48 F	GDs 1–17 1 time/day (GW)	0, 3, 5	CS, BW, MX, DX, OW	Bd wt	5			
					Hepatic	5			Significant increase in relative and absolute maternal liver weight on PND 22 at ≥3 mg/kg/day
					Develop		3	5	3 mg/kg/day: reduced weight gain through lactation (14.8% in males and 20.6% in females); delayed eye opening and hair growth at ≥3 mg/kg/day; decreased pup survival from birth to weaning at 5 mg/kg/day
Wolf et al. 2007									
APFO									
85	Mouse (ICR) 5–10 F	GDs 0–17 or GDs 0–18 (GW)	0, 1, 5, 10	BW, FI, WI, OW, HP, DX	Hepatic	1	10		35% increased maternal relative liver weight with hepatocellular hypertrophy at ≥1 mg/kg/day; single cell necrosis and mild calcification at 10 mg/kg/day
					Renal	1			35% increased maternal relative kidney weight with renal hypertrophy
					Develop	1		5	14% increased neonatal mortality, 9.5% reduced fetal body weight
Yahia et al. 2010									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
86	Mouse (BALB/c) 5 F	5 days/week 4 weeks starting at PND 21 (GW)	0, 1, 5, 10	BW, OW, HP	Bd wt	5	10		13% decrease in body weight gain
					Hepatic	10			Increases in liver weight and hepatocellular hypertrophy at ≥1 mg/kg
					Develop		1		Delay in vaginal opening at 1 mg/kg and mammary gland growth inhibition at 5 and 10 mg/kg
Yang et al. 2009									
PFOA									
87	Mouse (C57BL/6)) 5 F	5 days/week 4 weeks starting at PND 21 (GW)	0, 1, 5, 10	BW, OW, HP	Bd wt	5	10		10% decrease in body weight gain
					Hepatic	10			Increases in liver weight and hepatocellular hypertrophy at ≥1 mg/kg
					Develop	1	5		Delay in vaginal opening at 5 mg/kg and mammary gland growth stimulation a 5 mg/kg and inhibition at 10 mg/kg
Yang et al. 2009									
PFOA									
88	Mouse (C57BL/6)) 2–5 F	5 days/week 4 weeks (GW)	0, 5	BC	Repro		5		Increased progesterone levels
Zhao et al. 2010									
PFOA									
89	Mouse (PPARα knockout) NR F	5 days/week 4 weeks (GW)	0, 5	HP	Develop		5		Mammary gland growth stimulation
Zhao et al. 2010									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
CHRONIC EXPOSURE									
90	Rat (CD) 156 M	2 years ad lib (F)	0, 13.6	CS, BW, FI, OW, GN, HP	Bd wt		13.6		>10% reduction in weight gain most of the study
					Hepatic	13.6			Increased relative liver weight
					Repro		13.6		Increased incidence of Leydig cell hyperplasia; elevated serum LH at 18 months
					Other noncancer		13.6		Increased incidence of acinar cell hyperplasia in pancreas
					Cancer			13.6	CEL: testicular Leydig cell adenomas and pancreatic acinar cell adenomas
Biegel et al. 2001									
APFO									
91	Rat (Sprague-Dawley) 50–65 M, 50–65 F	2 years ad lib (F)	0, 1.5, 15	CS, FI, BW, OW, HE, BI, GN, HP	Bd wt	1.5 F	15 F		10.3% lower terminal body weight
					Resp	15			
					Cardio	15			
					Gastro	15			
					Hemato	15			
					Hepatic	1.5	15		Increased serum ALT and AST at ≥1.5 mg/kg/day; hepatocellular hypertrophy at 15 mg/kg/day; hepatocellular necrosis at 15 mg/kg/day only at 1 year
					Renal	15			
					Ocular	15			
					Endocr	15			
					Immuno	15			
					Neuro	15			

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Repro	1.5 M 15 F	15 M		Vascular mineralization in the testes
					Other noncancer		1.5 M		Inflammation of the salivary gland
					Cancer			15	CEL: testicular Leydig cell adenomas

3M 1983; Butenhoff et al. 2012c

APFO

[a]The number corresponds to entries in Figure 2-8.
[b]Used to derive an intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day based on the predicted TWA serum PFOA level of 8.29 µg/mL at the LOAEL dose and an empirical clearance model to estimate a HED. The $LOAEL_{HED}$ of 0.000821 mg/kg/day was divided by an uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, and 10 for human variability).

ad lib = *ad libitum*; ALT = alanine aminotransferase; APFO = ammonium perfluorooctanoate (ammonium salt of PFOA); AST = aspartate aminotransferase; BC = biochemistry; BI = biochemical changes; BW or Bd wt = body weight; C = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DW = drinking water; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FT4 = free thyroxine; FX = fetal toxicity; G = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; GO = gavage in oil vehicle; GW = gavage in water vehicle; HDL = high-density lipoprotein; HE or Hemato = hematological; HED = human equivalent dose; HP = histopathology; Immuno = immunotoxicological; LD_{50} = lethal dose, 50% kill; LE = lethality; LDL = low-density lipoprotein; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NK = natural killer; NOAEL = no observed-adverse-effect level; NX = neurotoxicity; OF = organ function; OP = ophthalmology; OW = organ weight; PFOA = perfluorooctanoic acid; PND = postnatal day; PPARα = peroxisome proliferator-activated receptor-α; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; sRBC = sheep red blood cell; TSH = thyroid-stimulating hormone; TT4 = total thyroxine; TWA = time-weighted average; UR = urinalysis; W = water; WI = water intake

Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Acute (≤14 days)

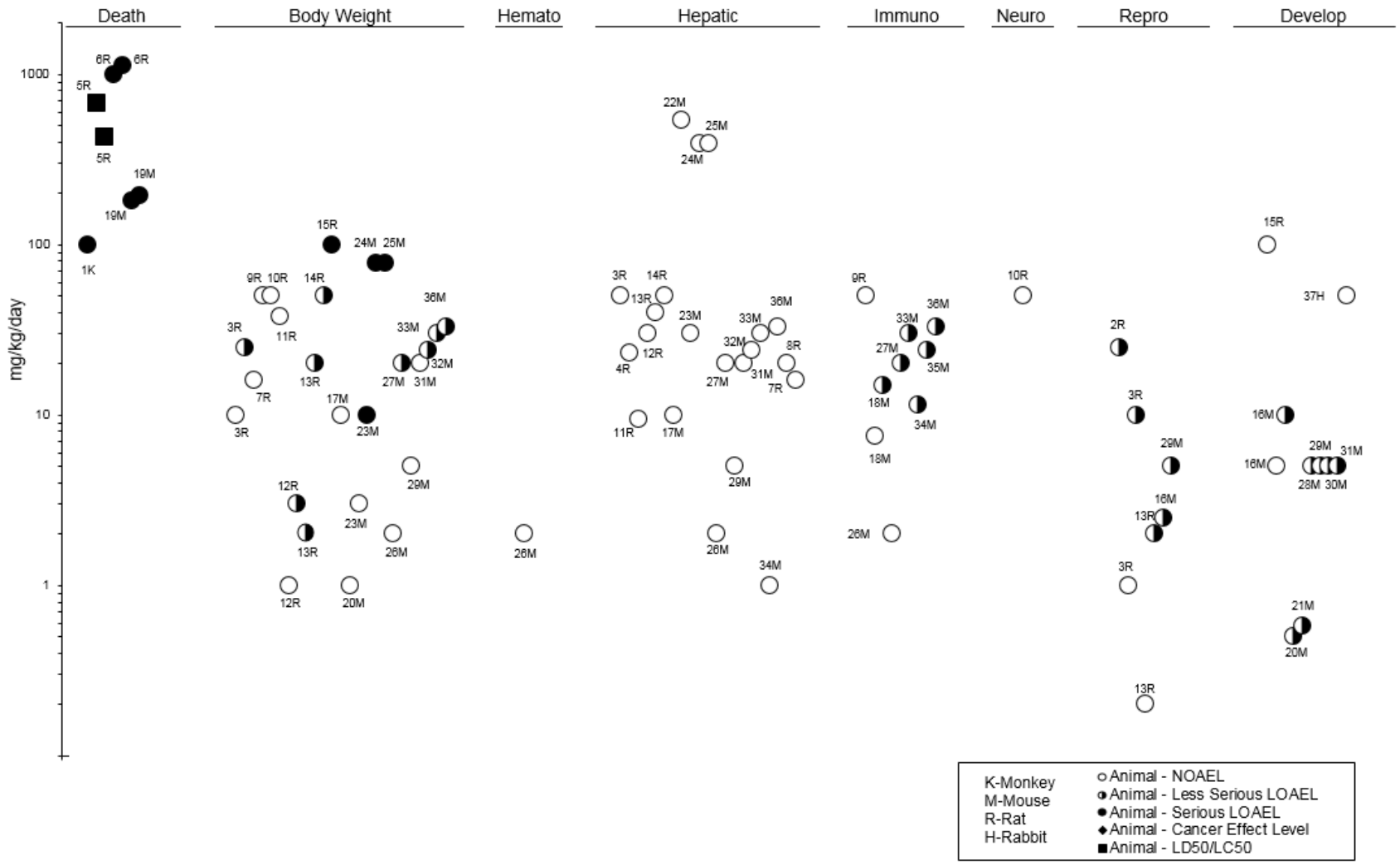


Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Intermediate (15–364 days)

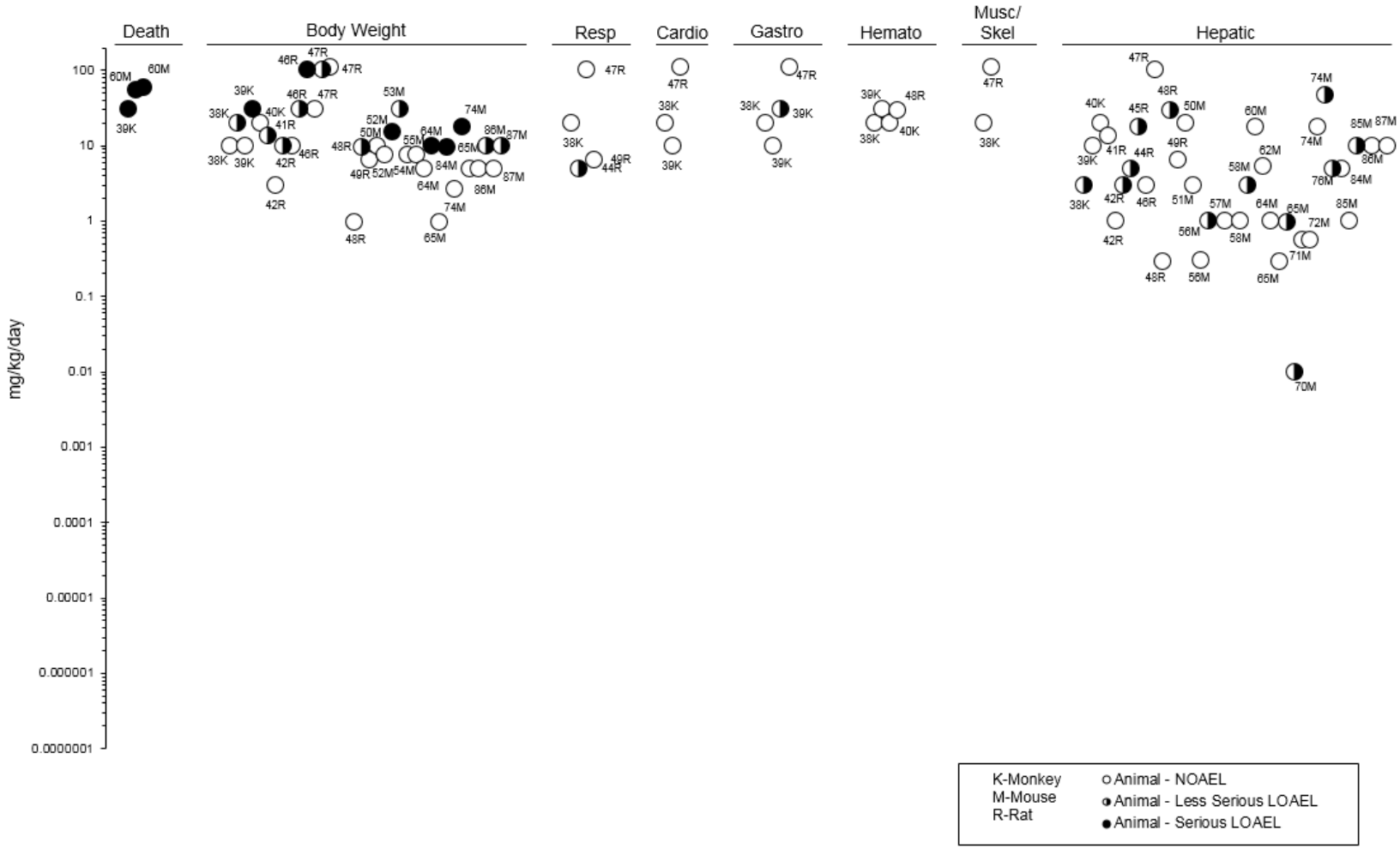


Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Intermediate (15–364 days)

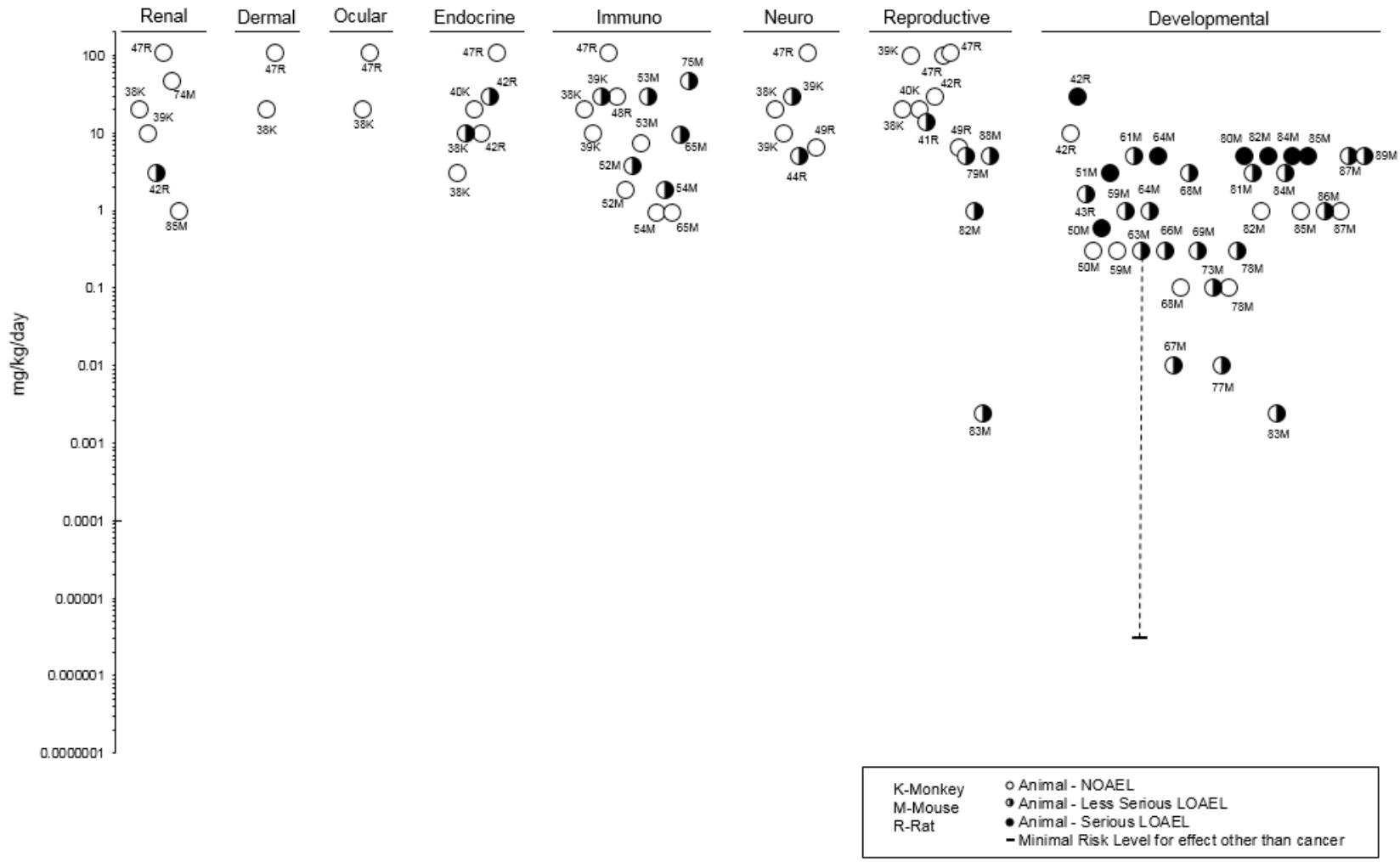


Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Chronic (≥365 days)

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Monkey (cynomolgus) 4–6M, 4–6F	Once or 3 times in 315 days (G)	0, 9, 13.3 (M), 14 (F)	BW, BC	Bd wt	13.3 M 14 F			
					Hepatic		13.3 M 14 F		Decrease in HDL cholesterol; values still within normal range
					Renal	13.3 M 14 F			
					Endocr		13.3 M 14 F		Decreased serum T4; values still within normal range
Chang et al. 2017									
PFOS potassium salt									
2	Rat (Sprague-Dawley) 5–15 F	Once (GW)	0, 15	BI, OF	Endocr		15		Transient decrease in serum TT4 (24, 38, and 53% after 2, 6, and 24 hours, respectively)
Chang et al. 2008b									
PFOS potassium salt									
3	Rat (Sprague-Dawley) 30 M	1 day (F)	0, 1.97, 10.3	HP	Hepatic	10.3			
					Endocr	10.3			
Elcombe et al. 2012a									
PFOS potassium salt									
4	Rat (Sprague-Dawley) 30 M	7 day (F)	0, 0, 1.72, 8.17	HP	Hepatic	8.17			Decreased serum cholesterol (38%) and triglyceride (55%) levels at 8.17 mg/kg/day
					Endocr	8.17			
Elcombe et al. 2012a									
PFOS potassium salt									
5	Rat (Sprague-Dawley) 10 M	7 day (F)	0, 1.79, 8.96	HP	Hepatic	8.96			Hepatocellular hypertrophy, increased liver weight, decreased serum cholesterol at ≥1.79 mg/kg/day
					Endocr	8.96			
Elcombe et al. 2012b									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
6	Rat (Sprague-Dawley) NS F	2 day GDs 19–20 1 time/day (GW)	0, 25, 50	BW, MX, DX	Develop			25	Decreased neonatal survival (82% of controls on PND 1)
Grasty et al. 2003									
PFOS potassium salt									
7	Rat (Sprague-Dawley) NS F	4 days GDs 2–5, 6–9, 10–13, 14–17, or 17–20 (GW)	0, 25	MX, DX	Bd wt			25	Weight loss during treatment when treated on GDs 2–5 (22%) or 6–9 (17%)
					Develop			25	Decreased neonatal survival (90% survival on GDs 2–5; 30% survival on GDs 17–20)
Grasty et al. 2003									
PFOS potassium salt									
8	Rat (Sprague-Dawley) NS F	2 days GDs 19–20 1 time/day (G)	0, 25, 50		Develop			25	Increased neonatal mortality
Grasty et al. 2005									
PFOS potassium salt									
9	Rat (Wistar) 8 M	7 days ad lib (F)	0, 15	BW, OW, BI, EA	Hepatic	15			40% increase in absolute liver weight
Haughom and Spydevold 1992									
PFOS potassium salt									
10	Mouse (wild-type 129S1/Svlm) 8–20 F	GDs 15–18 1 time/day (GW)	0, 4.5, 6.5, 8.5, 10.5	DX	Develop			4.5	31% reduced percentage of live pups per litter on PND 15
Abbott et al. 2009									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
11	Mouse (ICR) 5–7 F	GDs 11–15 1 time/day (GW)	0, 50	BW, OW, DX	Hepatic	50			103% increased maternal relative liver weight
					Develop		50		6.1% increased cleft palate and 12.7% reduced body weight in fetuses
Era et al. 2009									
PFOS potassium salt									
12	Mouse (CD-1) 10–11 F	GDs 6–18 1 time/day (GW)	0, 1.5, 3, 6	MX, DX, BW, CS, OW	Bd wt	6			
					Hepatic	6			21% increase in absolute liver weight at ≥3 mg/kg/day
					Endocr	6			No alterations in serum T3 or T4 levels
					Develop	6			
Fuentes et al. 2006									
PFOS potassium salt									
13	Mouse (CD-1) 8–10 F	GDs 12–18 (GW)	0, 6	CS, BW, BH, MX, DX	Bd wt	6			
					Develop		6		Reduced body weight of pups on PNDs 4 and 8
Fuentes et al. 2007b									
PFOS potassium salt									
14	Mouse (CD-1) 8–10 F	GDs 12–18 (GW)	0, 6	DX	Develop		6		Decreased distance traveled in open field test at 3 months of age
Fuentes et al. 2007a									
PFOS potassium salt									
15	Mouse (NMRI) 12 M pups	Single dose (GO)	0, 11.3	BH, BW, OF	Develop		11.3		Altered spontaneous behavior (≤60, 87.5, or 60% changes in total activity, rearing, and locomotion)
Hallgren et al. 2015									
PFOS									
16	Mouse (CD-1) 10 M pups	Once (G)	0, 0.75, 11.3	CS, OF, DX	Develop		0.75 M		24% decreased total spontaneous activity at 2 months of age; no significant alteration at 4 months
Johansson et al. 2008									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
17	Mouse (CD-1) 10 F	GDs 11–16 (G)	0, 0.5, 2.0, 8.0	BC, BW, DX, HP	Bd wt		8.0		21% reduction in maternal body weight gain on GDs 14–17
					Repro		0.5		Decreases in mean fetal placental weight and placental capacity
					Develop		0.5	2.0	Post-implantation losses at ≥0.5 mg/kg/day; 24 and 35% reduction fetal body weight and 31 and 52% reduction in the number of live fetuses at 2.0 and 8.0 mg/kg/day
Lee et al. 2015a									
PFOS									
18	Mouse (C57BL/6) 4M	10 days (F)	0, 6	BW FI BC HP	Bd wt	6			
					Hemato	6			
					Hepatic	6			41% increase in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol levels
					Immuno	6			
Qazi et al. 2010a									
PFOS									
19	Mouse (BALB/c) 5 F	7 days (GW)	20	BW, OW, BC	Bd wt		20		41% decreased body weight gain
					Hepatic	20			59% increase in relative liver weight
					Immuno		20		Inhibition of T lymphocyte proliferation in response to sRBC; decreased phagocytosis by peripheral blood cells and NK cell activity; decreased IgM antibody formation in response to OVA
Vetvicka and Vetvickova 2013									
PFOS									
20	Mouse (CD-1) 4 M	14 days 1 time/day (GO)	0, 1, 5, 10		Bd wt	10			
					Hepatic	10			~70% increased absolute liver weight at ≥5 mg/kg/day
					Repro	10			
Wan et al. 2011									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
21	Mouse (C57BL/6) 10 M	1 day (GO)	0, 300, 400, 500, 600, 700	BW, CS, HP, LE	Death			579	LD_{50}
Xing et al. 2016									
PFOS									
22	Mouse (C57BL/6N) 12 M	7 days (G)	0, 5, 20, 40	FX	Immuno		5		Impaired response to T-cell mitogens; suppressed response to sRBC
Zheng et al. 2009									
PFOS									
23	Rabbit (New Zealand) 22 F	GDs 6–20 1 time/day (GW)	0, 0.1, 1.0, 2.5, 3.75	MX, DX, BW, CS	Bd wt	0.1		1 F	21% decreased mean maternal body weight gain on GDs 7–21; no effect on food consumption
					Develop	1	2.5	3.75	Decreased fetal body weight; 10% at 2.5 mg/kg/day and 24% at 3.75 mg/kg/day; 10/22 does aborted between GD 22 and 28 at 3.75 mg/kg/day
Case et al. 2001									
PFOS potassium salt									
INTERMEDIATE									
24	Monkey (Cynomolgus) 4–6 M, 4–6 F	26 weeks 1 time/day (C)	0, 0.03, 0.15, 0.75	CS, BW, OW, HE, BI, GN, HP	Bd wt	0.15 M 0.75 F	0.75 M		13.5% reduction in final body weight
					Resp	0.75			
					Cardio	0.75			
					Gastro	0.75			
					Hemato	0.75			
					Musc/skel	0.75			
					Hepatic	0.15	0.75		47–55% increased absolute liver weight; 50–60% decreased serum cholesterol; hepatocellular hypertrophy, mild bile stasis, and lipid vacuolation at 0.75 mg/kg/day
					Renal	0.75			
					Dermal	0.75			

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Ocular	0.75			
					Endocr	0.15	0.75		Increased TSH and decreased total T3
					Immuno	0.75			
					Neuro	0.75			No histological alterations
					Repro	0.15	0.75 M		Significant decrease in serum estradiol on days 62 (48%), 91 (42%), and 182 (96%); no histological alterations
Seacat et al. 2002									
PFOS potassium salt									
25	Monkey (Cynomolgus) 6 M,F	4 weeks 1 time/day (C)	0, 0.02, 2	CS, BW, FC, HE, BI, GN, HP, EA	Bd wt	2			
					Resp	2			
					Hemato	2			
					Hepatic	2			
					Renal	2			
					Ocular	2			
					Endocr	2			
					Immuno	2			No histological alteration
					Repro	2			No histological alterations
Thomford 2002a									
PFOS potassium salt									
26	Rat (Sprague-Dawley) 25 F	GD 0 to PND 20 1 time/day (GW)	0, 0.1, 0.3, 1		Repro	1			
					Develop	0.3	1		~30% increased locomotor activity and concurrent failure to habituate to test environment in male pups on PND 17
Butenhoff et al. 2009b									
PFOS potassium salt									
27	Rat (Sprague-Dawley) 25 F	GD 0 to PND 20 1 time/day (GW)	0, 0.1, 0.3, 1		Develop		1		2.1-fold increased fetal thyroid cell proliferation on GD 20
Chang et al. 2009									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
28	Rat (Sprague-Dawley) 10 F	GDs 1–21 1 time/day (GW)	0, 0.1, 2		Develop	0.1		2	~5-fold increased postnatal mortality and severe lung histopathology in pups
Chen et al. 2012b PFOS									
29	Rat (Sprague-Dawley) 10 M	Daily 28 days (G)	0, 5, 20	HP	Death			20	100% by day 26
					Resp		5		Pulmonary congestion
					Hepatic		5		Hepatocellular hypertrophy and focal necrosis at ≥5 mg/kg/day; fatty degeneration at 20 mg/kg/day
					Neuro		5		Cachexia and lethargy
Cui et al. 2009 PFOS									
30	Rat (Sprague-Dawley) 15M, 15F	Daily 28 days (F)	M: 0, 0.13, 1.23, 2.98, 5.89 F: 0, 0.14, 1.33, 3.47, 7.01		Cardio	5.89 M			
					Hemato	3.47 F	7.01 F		Decreased red blood cells (8.9%), hemoglobin (10%), hematocrit (8.8%)
					Hepatic	5.89 M			Increased relative liver weight; hepatocellular hypertrophy in males at 5.89 mg/kg/day
					Renal	5.89 M			
					Endocr	0.14	1.23		Decreased T4 level (82% in males; 48% in females)
Curran et al. 2008 PFOS potassium salt									
31	Rat (Sprague-Dawley) 30 M	28 days (F)	0, 1.54, 7.34	HP	Hepatic	7.34			Hepatocellular hypertrophy and decreased serum cholesterol levels at ≥1.54 mg/kg/day
					Endocr	7.34			
Elcombe et al. 2012a PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
32	Rat (Wistar) 5–6 M	Daily 28 days (G)	0, 0.12, 0.5, 2.0, 8.5	FX	Neuro	2	8.5		Tonic convulsions in response to stimuli
Kawamoto et al. 2011									
PFOS									
33	Rat (Sprague-Dawley) NS	GDs 2–21 (GW)	0, 1, 2, 3, 5, 10		Develop	1		2	Reduced serum T4 in pups at 1 mg/kg/day; approximately 60% survival at weaning versus 80% in controls at 2 mg/kg/day
Lau et al. 2003									
PFOS potassium salt									
34	Rat (Sprague-Dawley) 15M, 15F	28 days (F)	M: 0, 0.14, 1.33, 3.21, 6.34; F: 0, 0.15, 1.43, 3.73, 7.58	OW, BW	Bd wt Hepatic Immuno	1.33 M 6.34 M 6.34 M	3.21 M		12% decrease in terminal body weight Increased relative liver weight at ≥0.14 mg/kg/day
Lefebvre et al. 2008									
PFOS									
35	Rat (Sprague-Dawley) 10 dams; 12–13 pups	GDs 12–18 (GO)	0, 5, and 20	BC, BW, DX, OW	Bd wt Develop	5 5	20 20		30% reduction in body weight of dams 13% reduction in body weight of male pups
Li et al. 2016									
PFOS									
36	Rat (Sprague-Dawley) 5M	21 days (G)	0, 5, 10	BW, BC, OW, HP	Bd wt Repro	10	5		Delayed maturation of testicular Leydig cells, decreased seminal vesicle weight, decreased epididymal sperm count, decreased serum testosterone levels
Li et al. 2018									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
37	Rat (Sprague-Dawley) 19 M	28 days (GO)	0, 0.5, 1.0, 3.0, 6.0	BC, BW, HP, OF, OW	Neuro	0.5	1		Degeneration of gonadotropic cells of the pituitary gland at ≥1.0 mg/kg/day; dense chromatin, condensed ribosomes, loss of morphology in the hypothalamus at ≥3.0 mg/kg/day
					Repro	0.5	1		Loss/degeneration of spermatozoids, marked edema in the testes
Lopez-Doval et al. 2014									
PFOS									
38	Rat (Sprague-Dawley) 35 M,F	84 days (6 weeks prior to mating GD 0 to PND 21) 1 time/day (GW)	0, 0.1, 0.4, 1.6, 3.2	MX, DX, BW, OW, OF, GN, HP, FC	Bd wt	1.6	3.2		>10% reduction in body weight
					Repro	3.2			No alterations in mating and fertility parameters
					Develop	0.1[b]	0.4	1.6	Delayed eye opening and transient decrease in F2 pup body weight (13%) on LDs 7–14 at ≥0.4 mg/kg/day; decreased pup survival to postpartum day 21 at ≥1.6 mg/kg/day
Luebker et al. 2005a									
PFOS potassium salt									
39	Rat (Sprague-Dawley) 50 F	90 day 1 time/day	0, 1.6	MX, DX, BW, OW, OF, GN		Develop		1.6	Increased pup mortality during PNDs 1–4
Luebker et al. 2005a									
PFOS potassium salt									
Cross-foster study									
40	Rat (Sprague-Dawley) 20 F	62–67 days 42 days prior to mating through GD 20 or PND 4 (G)	0, 0.4, 0.8, 1, 1.2, 1.6, 2	MX, DX, BW, CS, BI	Bd wt	1.6	2		22% reduction in body weight gain during premating; food consumption reduced 5.8%
					Hepatic	2			16% reduction in serum total cholesterol on PND 5 at ≥0.4 mg/kg/day; increased liver weight in dams at ≥0.8 mg/kg/day
					Endocr		0.4		46% reduction in total T4 on PND 5
					Repro	2			No alteration in fertility

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Develop		0.4	1.6	>10% decrease in mean pup weight per litter on PND 5 at ≥0.4 mg/kg/day; ~50% decrease mean pup survival per litter on PND 5 at ≥1.6 mg/kg/day
Luebker et al. 2005b									
PFOS potassium salt									
41	Rat (Sprague-Dawley) 10 M	25 days (GW)	0, 0.5, 1.0, 3.0 and 6.0	OF, OW	Endocr		0.5		Decreases in serum corticosterone (~58%) and ACTH levels (~11%), decrease in corticotrophin releasing hormone levels in hypothalamus (~8%); decrease in relative adrenal weight (~43%),
Pereiro et al. 2014									
PFOS									
42	Mouse (C57) 12 M	5 weeks (GO)	0, 0.5, 10	BW, OW, HP, RX	Bd wt	0.5	10		17% decrease in body weight
					Repro	0.5	10		Decreases in sperm concentration, serum testosterone levels; vacuolation in testicular spermatogonia, spermatocyte, and Leydig cells
Qu et al. 2016									
PFOS									
43	Rat (Sprague-Dawley) 21 dams; 10–12 M,F pups/litter	GDs 2–6 (G)	0, 18.75	BW, OF, OW	Bd wt		18.75		Reduced body weight in dams; approximately 98% on GD 8 and 33% on GD 20
					Develop		18.75		Decreased birth weight in females only (approximately 11%); increased systolic blood pressure in male offspring at 7 and 52 weeks and in female offspring at 37 and 65 weeks; reduced nephron endowment
Rogers et al. 2014									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
44	Rat (Sprague-Dawley) 7 M	28 days (GW)	0, 3.0, and 6.0	BI, BW, OW	Bd wt	6			
					Endocr		3		Decreased serum prolactin (~78%) and estradiol concentrations (~18%)
Salgado et al. 2015 PFOS									
45	Rat (Sprague-Dawley) 25 M,F	4 weeks ad lib (F)	M: 0, 0.05, 0.18, 0.37, 1.51; F: 0, 0.05, 0.22, 0.47, 1.77	CS, CO, OW, HE, BI, GN, HP	Bd wt	1.77 F			
					Hemato	1.77 F			
					Hepatic	1.77 F			
					Renal	1.77 F			
					Ocular	1.77 F			
					Immuno	1.77 F			No histological alterations
					Neuro	1.77 F			No histological alterations
					Repro	1.51 M 1.77 F			No histological alterations
Seacat et al. 2003 PFOS potassium salt									
46	Rat (Sprague-Dawley) 25 M,F	14 weeks ad lib (F)	M:0, 0.03, 0.13, 0.34, 1.33; F:0, 0.04, 0.15, 0.4, 1.56	CS, CO, OW, HE, BI, GN, HP	Bd wt	1.56 F			
					Hemato	0.34 M	1.33 M		45% increase in non-segmented neutrophils
					Hepatic	1.33 M			Increased absolute and relative liver weight; increased serum ALT; hepatocyte hypertrophy and vacuolation at 1.33/1.55 mg/kg/day
					Renal	1.56 F			
					Ocular	1.56 F			
					Endocr	1.56 F			
					Immuno	1.56 F			No histological alterations
					Neuro	1.56 F			No histological alterations
					Repro	1.33 M 1.56 F			No histological alterations
Seacat et al. 2003 PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
47	Rat (Sprague-Dawley) 25–50 F	GDs 2–20 (GW)	0, 1, 2, 3, 5, 10	MX, DX, BW, FI, WI, OW, BI	Bd wt	1	2		Decreased mean body weight gain, 10% at 2 mg/kg/day and 33% at 5 mg/kg/day
					Hepatic	10			
					Endocr		1		Reduced total and free T4 and T3
					Develop		10		Increased incidences of cleft palate
Thibodeaux et al. 2003									
PFOS potassium salt									
48	Rat (Wistar) 10 or 15 dams; 6–10 M,F pups	GD 1 to PND 1, PNDs 1–7 or 35, or GD 1 to PND 7 or 35	0, 0.8, 2.4	DX	Develop		0.8		Decreased spatial learning ability in prenatally or postnatally exposed offspring at ≥0.8 mg/kg/day and in offspring exposure pre- and postnatally at 2.4 mg/kg/day; decreased memory ability in offspring exposure pre- and postnatally at 2.4 mg/kg/day
Wang et al. 2015c									
PFOS									
49	Rat (Sprague-Dawley) 10 F	GDs 2–21 1 time/day (GW)	0, 0.1, 0.6, 2		Develop	0.6		2	5-fold increased neonatal mortality on PNDs 1–3
Xia et al. 2011									
PFOS									
50	Rat (Sprague-Dawley) 8–10 M	91 days (W)	0, 0.27, 0.79, 2.37	BC	Endocr		0.27 M		42% decrease in total T4 levels
					Develop		3.2 F		19–36% reduced serum T4 levels in pups on PNDs 21–35 after gestation- and/or postnatal-only exposure
Yu et al. 2009a									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
51	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 3	BW, FI, BC, OW	Bd wt	3			
					Hepatic	3			Decreased plasma triglyceride, total cholesterol, non-HDL cholesterol, and HDL cholesterol levels; increased hepatic triglyceride levels, increased liver weight
Bijland et al. 2011									
PFOS									
52	Mouse (C57BL/6N) 10 M	60 days (G)	0, 0.00833, 0.08333, 0.41667, 0.83333, 2.0833	FX	Immuno	0.0083	0.0833		Impaired response to sRBC
Dong et al. 2009									
PFOS potassium salt									
53	Mouse (C57BL/6N) 12 M	60 days (G)	0, 0.00833, 0.0167, 0.0833, 0.4167, 0.8333	FX	Immuno	0.0167	0.0833		Impaired response to sRBC
Dong et al. 2011									
PFOS potassium salt									
54	Mouse (ICR) 6–8 F	GDs 1–17 1 time/day (GW)	0, 20		Bd wt		20		35% reduced maternal body weight
					Develop		20		89% increased cleft palate, 25% reduced body weight in fetuses
Era et al. 2009									
PFOS potassium salt									
55	Mouse (CD-1) 10 M	4 weeks (GW)	0, 3, 6	CS, BW, BH, MX, DX	Develop		3		Impaired retention of the task in the water maze test
Fuentes et al. 2007c									
PFOS potassium salt									
56	Mouse (B6C3F1) 30 F	21 days (G)	0, 0.005, 0.025		Immuno	0.005	0.025		Decreased host resistance to influenza virus
Guruge et al. 2009									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
57	Mouse (B6C3F1) 10–12 F	GDs 1–17 1 time/day (GW)	0, 0.1, 1, 5	MX, DX, BW, CS, OF	Develop	0.1	1		42.5% reduced NK cell activity in male pups at 8 weeks of age
Keil et al. 2008									
PFOS potassium salt									
58	Mouse (CD-1) 21–22 F	GDs 1–17 1 time/day (GW)	0, 1, 5, 10, 15, 20	DX, OW, BI	Develop		1	10	1 mg/kg/day: delayed eye opening at ≥1 mg/kg/day, ~ 0% postnatal survival at weaning versus 90% in controls at 10 mg/kg/day
Lau et al. 2003									
PFOS potassium salt									
59	Mouse (CD-1) 4 dams; 8 fetuses	GDs 1–17 (GO)	3	BC, EA, OF	Hepatic	3			No alteration in maternal hepatic lipid levels
					Develop		3		Significant increase in cholesterol levels in fetal livers
Lee et al. 2015b									
PFOS									
60	Mouse (C57BL/6) 15 M,F	Daily 3 months (G)	0, 0.43, 2.15, 10.75	FX	Neuro	0.43	2.15		Impaired spatial learning and memory
Long et al. 2013									
PFOS									
61	Mouse (BALB/c) 28 M	3 weeks	0, 2.5, 5 and 10	BW, OF, OW	Bd wt		10 M		~15% reduction in body weight during the recovery period
					Immuno	2.5 M	5 M		~36% decrease in spleen index during recovery; ~15% inhibition in Con A-induced T-cell proliferation during treatment; 32% increase in CD3+ cells after recovery; ~60% increase in CD3+CD8+ cells and ~56% increase in CD3+CD4+ cells on week 2; 15% inhibition in Con A-induced T-cell proliferation during recovery
Lv et al. 2015									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
62	Mouse (C57BL/6J-Apc+/+) 20–21 F	GDs 1–17 (GW)	0, 0.01, 0.1, 3.0	BC, BW, FI, HE, OW	Develop	0.1	3		Decrease in number of successful births
Ngo et al. 2014									
PFOS									
63	Mouse (C57BL/6/Bk1) 6 F	GDs 1–21 ad lib	0, 0.3		Develop		0.3		Decreased locomotion, muscle strength, and motor coordination in adult offspring
Onishchenko et al. 2011									
PFOS, potassium salt									
64	Mouse (B6C3F1) 5M, 5F	28 days (G)	0, 0.000166, 0.00166, 0.00331, 0.0166, 0.0331, 0.166	OW, FX	Immuno	0.000166 M	0.00166 M		Suppressed response to sRBC (~60%)
Peden-Adams et al. 2008									
PFOS potassium salt									
65	Mouse (B6C3F1) 5 M	28 days (F)	0.20	NS	Bd wt		0.2		21% reduction in body weight
					Immuno	0.2			No alterations in thymic lymphocyte phenotypes, response to sRBC, or IgM antibodies to LPS
Qazi et al. 2010b									
PFOS									
66	Mouse (CD-1) 5 F	GDs 1–17 1 time/day (GW)	0, 5, 10		Develop	5			Peroxisome proliferation in fetal liver at ≥5 mg/kg/day
Rosen et al. 2009									
PFOS potassium salt									
67	Mouse (CD-1) 60–80 F	GDs 1–17 (GW)	0, 1, 5, 10, 15, 20	BW, OW, BI, DX	Bd wt	20			
					Hepatic	20			Increase in absolute and relative liver weight and decreased serum triglycerides at ≥5 mg/kg/day
					Endocr	15	20		Decreased total T4 on GD 6

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Develop	1	5	20	Increased incidences of sternal defects at ≥5 mg/kg/day; reduced percentage of live fetuses (9%) at 20 mg/kg/day
Thibodeaux et al. 2003									
PFOS potassium salt									
68	Mouse (CD-1) 4 M	21 days 1 time/day (GO)	0, 1, 5, 10	BW, BI, OW, HP	Bd wt	5	10		~15% reduced body weight
					Hepatic	10			Increased absolute liver weight at ≥5 mg/kg/day
					Repro	5	10		~17% reduced serum testosterone, ~38% reduced epididymal sperm count
Wan et al. 2011									
PFOS potassium salt									
69	Mouse (CD-1) 6 F (dams)	GD 3 to PND 21 or GD 3 to PND 63 (GO)	0, 0.3, 3	BW, OF, OW	Hepatic	3			~24% increase in relative liver weight in dams at 3 mg/kg/day
					Develop	3			Increase in relative liver weight at 3 mg/kg/day in male and female pups on PND 21 (~20–32%), in male STD-fed adults (~11%), and in male HDF-fed adults (~33%)
					Develop		0.3		In PND 63 offspring fed a high fat diet, increased serum glucose levels at ≥0.3 mg/kg/day in females (~40%) and 3 mg/kg/day in males (~8%); increased serum insulin in males (~109%) and females (~85%); increased response to oral glucose tolerance test, increased HOMA-IR index, and 33% increased relative liver weight at 3 mg/kg/day
					Other noncancer	3 F			No significant alteration in fasting serum insulin or glucose levels; significant increase in HOMA-IR index at 0.3 and 3 mg/kg/day
Wan et al. 2014b									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
70	Mouse (C57BL/6) 10 M	30 days (GO)	0, 2.5, 5, 10	BW, HP, OF, OW	Bd wt		10		31% reduction in body weight (correlated with 68% reduction in feed consumption)
					Hepatic		2.5		Increased liver weight (35%) and serum AST(~12%) and GGT levels (~98%) at ≥2.5 mg/kg/day; increases in ALT (~45%) and ALP (~36%) at ≥5 mg/kg/day; cytoplasmic vacuolation, focal or flake-like necrosis, and hepatocellular hypertrophy observed, but no incidence data provided
					Renal	10			
Xing et al. 2016									
PFOS									
71	Mouse (ICR) 5 F	GDs 0–17 GDs 0–18 (GW)	0, 1, 10, 20		Hepatic	20			60% increased absolute liver weight at ≥10 mg/kg/day
					Develop		1	20	GDs 0–17: 15.8% increased sternal defects in fetuses at ≥1 mg/kg/day; 8.8% decrease in number of live fetuses at 20 mg/kg/day
					Develop			10	GDs 0–18: decreased survival (55.2%) at 10 mg/kg/day on PND 4, decreased neonatal BW, intracranial blood vessel dilatation, lung atelectasis
Yahia et al. 2008									
PFOS potassium salt									
CHRONIC EXPOSURE									
72	Rat (Sprague-Dawley) 70 M,F	104 weeks ad lib (F)	0, 0.025, 0.10, 0.25, 1.04	CS, BW, FC, GN, HP, BI	Bd wt	0.25 F	1.04 F		14% reduction in final body weight
					Resp	1.04			
					Cardio	1.04			
					Gastro	1.04			
					Hemato	1.04			
					Musc/skel	1.04			

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	0.25 M	1.04 M		Hepatocellular hypertrophy at ≥0.1 mg/kg/day; single cell necrosis and cystic degeneration at 1.04 mg/kg/day
					Renal	1.04			
					Dermal	1.04			
					Ocular	1.04			
					Endocr	1.04			
					Immuno	1.04			No histological alterations
					Neuro	1.04			No histological alterations
					Repro	1.04			No histological alterations
Butenhoff et al. 2012b; Thomford 2002b									
PFOS potassium salt									

[a]The number corresponds to entries in Figure 2-9.
[b]Used to derive an intermediate-duration oral MRL of $2x10^{-6}$ mg/kg/day based on the predicted TWA serum PFOA level of 29.7 µg/mL at the NOAEL dose and an empirical clearance model to estimate a HED. The $NOAEL_{HED}$ of 0.000515 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) and a modifying factor of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.

ad lib = *ad libitum*; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BC = biochemistry; BH = behavioral; BI = biochemical changes; BW or Bd wt = body weight; C = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FX = fetal toxicity; FI = food intake; FX = fetal toxicity; G = gavage; Gastro = gastrointestinal; GD = gestation day; GGT = gamma-glutamyl transferase; GN = gross necropsy; GO = gavage in oil vehicle; GW = gavage in water vehicle; HDL = high-density lipoprotein; HE or Hemato = hematological; HED = human equivalent dose; HOMA IR = Homeostatic Model Assessment of Insulin Resistance; HP = histopathology; Immuno = immunotoxicological; LD = lactation day; LD50 = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; LPS = lipopolysaccharide; M = male(s); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NK = natural killer; NOAEL = no observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; PFOS = perfluorooctane sulfonic acid; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; sRBC = sheep red blood cell; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; TT4 = total thyroxine; TWA = time-weighted average; W = water

Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Acute (≤14 days)

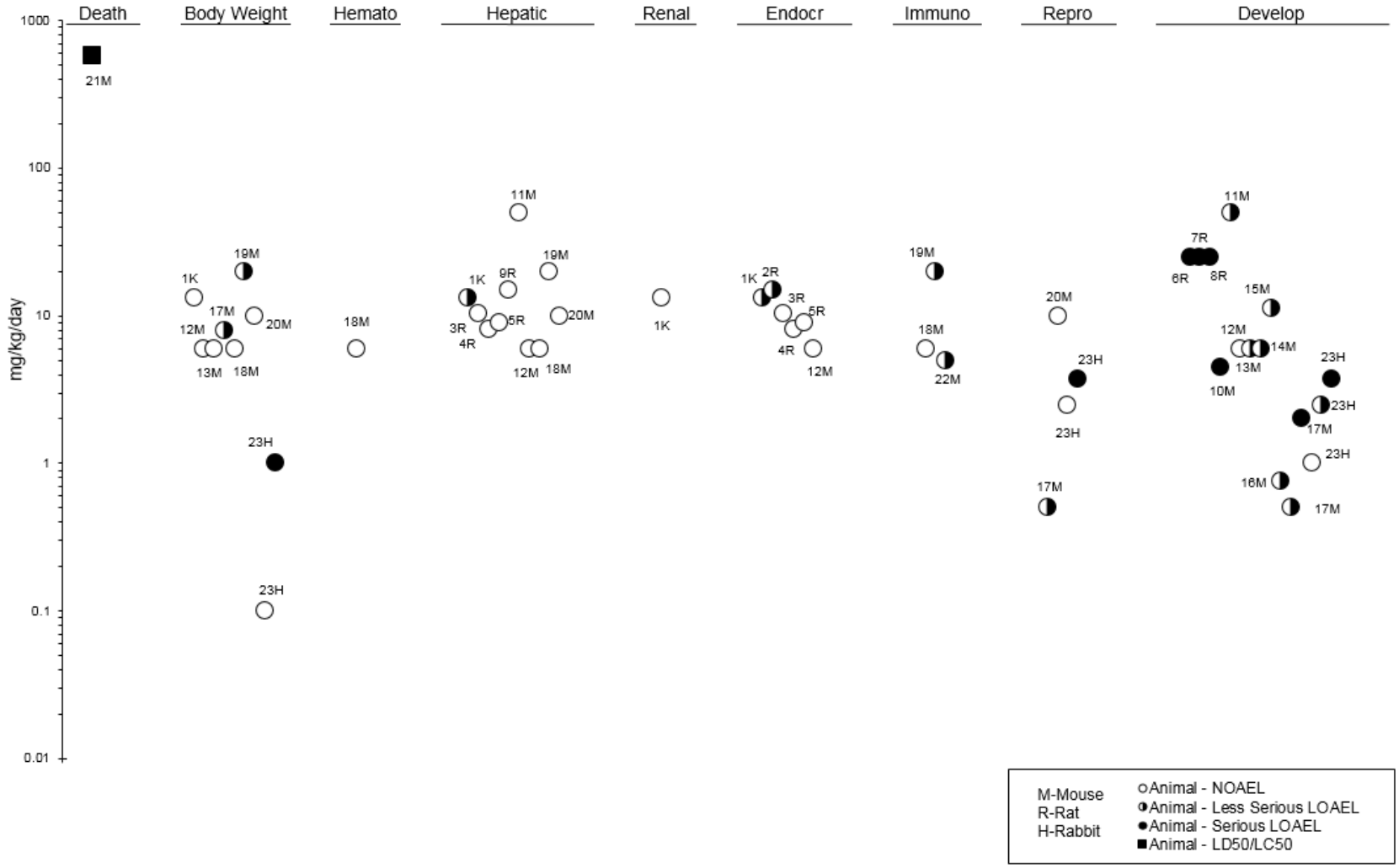


Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Intermediate (15–364 days)

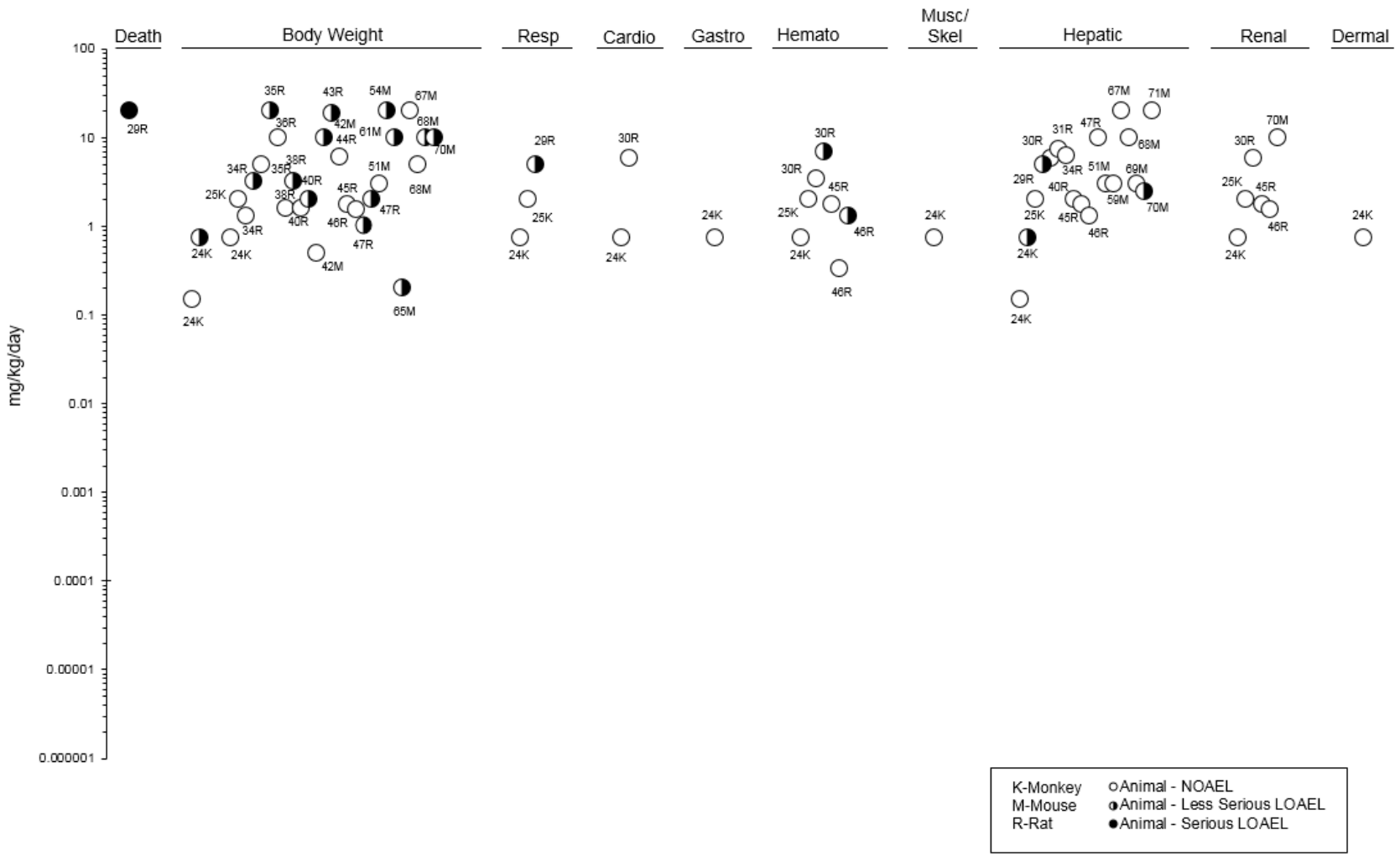


Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Intermediate (15–364 days)

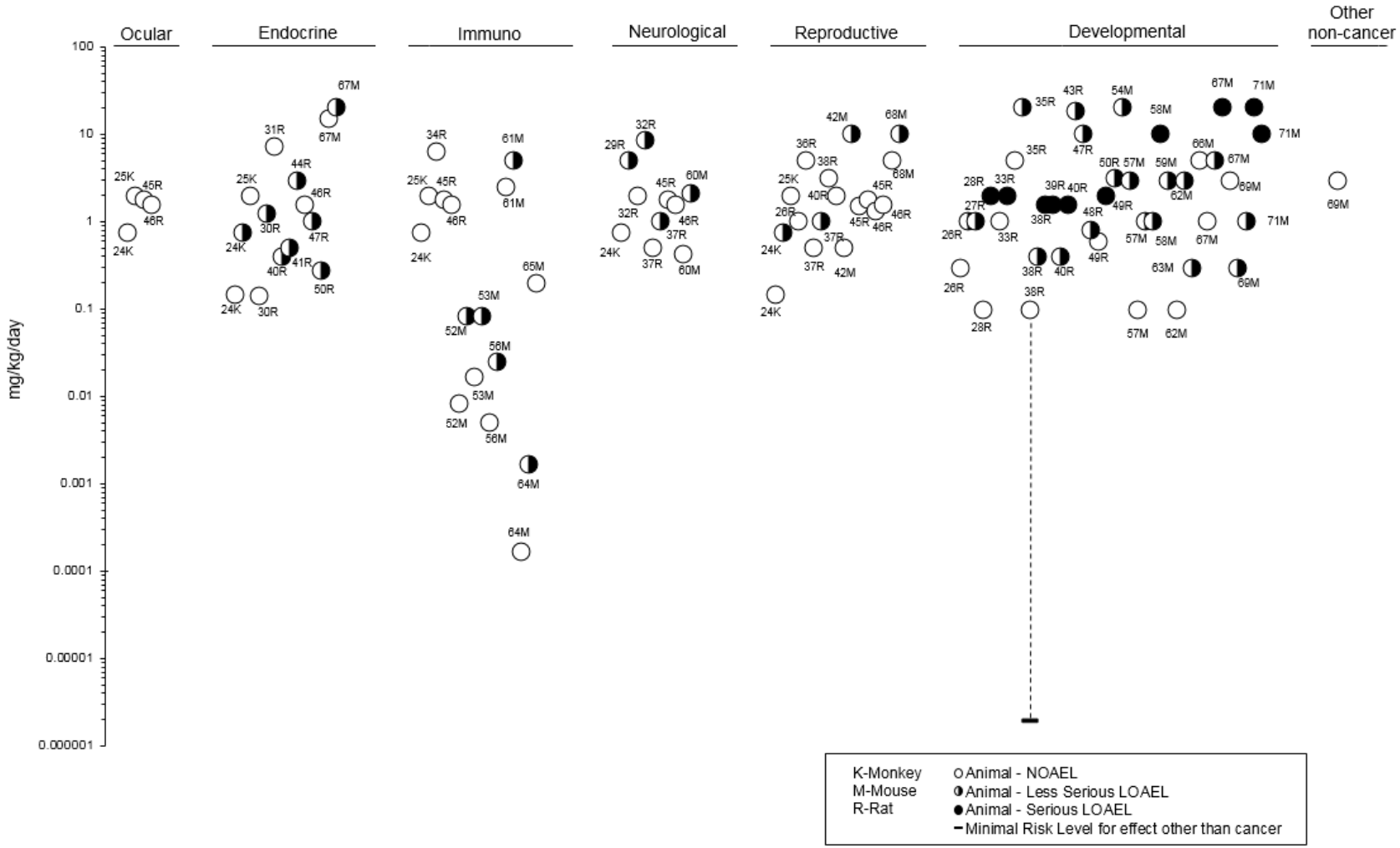


Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Chronic (≥365 days)

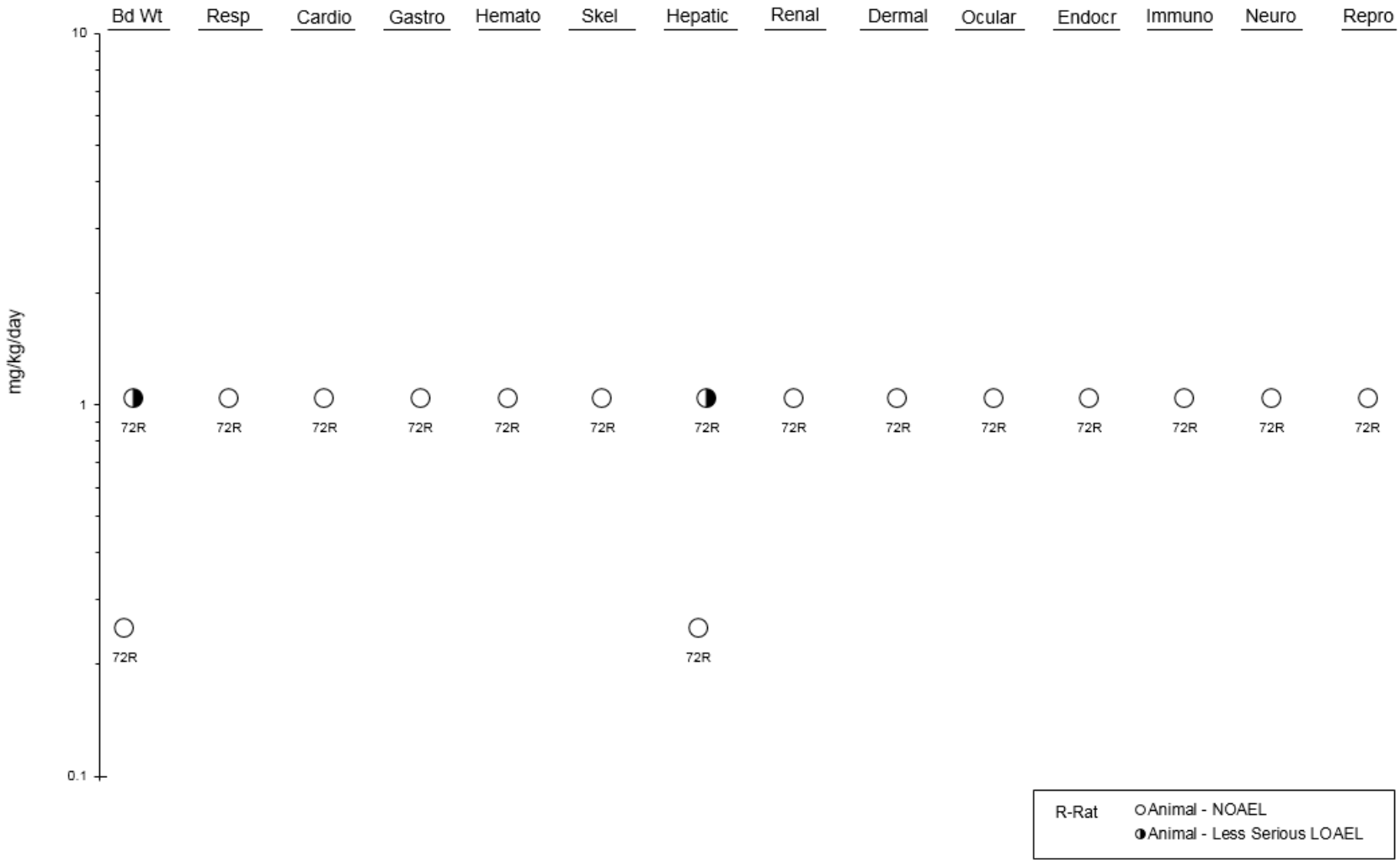


Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
PFHxS									
1	Mouse (SV129 WT and PPARα null) 4 M	7 days (G)	0, 10 PFHxS	BW, HP	Bd wt	10			
					Hepatic	10			Hepatocellular hypertrophy, steatosis, and increased hepatic triglyceride levels
Das et al. 2017									
2	Mouse (NMRI) 14–18 M,F	PND 10 Once (GO)	0, 0.61, 6.1, 9.2 PFHxS		Develop	6.1	9.2		Altered spontaneous behavior and habituation in adults exposed as neonates
Viberg et al. 2013									
PFNA									
3	Rat (Sprague-Dawley) 10 M	14 days (GW)	0, 1, 3, 5 PFNA	BC, BW, OW	Bd wt	1	3		Decreases in body weight at 3 and 5 mg/kg/day (18 and 39%)
					Immuno		1		24% increase in relative thymus weight at 1 mg/kg/day; 20% decrease in thymus weight at 3 or 5 mg/kg/day, increases in thymic cortex:medulla ratios, alterations in cytokine levels at ≥3 mg/kg/day
Fang et al. 2009									
4	Rat (Sprague-Dawley) 10 M	14 days (GW)	0, 1, 3, 5 PFNA	BC, OW	Immuno	3	5		Decreased relative spleen weight and increases in cytokine levels
Fang et al. 2010									
5	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 0.2, 1, 5 PFNA	BC	Hepatic	5			Decreased HDL levels at ≥1 mg/kg/day
					Other noncancer (glucose)	0.2	1		Increased serum glucose levels (1.11-fold at 1 mg/kg/day and 1.16-fold at 5 mg/kg/day)
Fang et al. 2012a									
6	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 0.2, 1, 5 PFNA	HP	Hepatic	5			Hepatocellular vacuolation and lipid accumulation at 5 mg/kg/day
Fang et al. 2012b									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
7	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 1, 3, 5 PFNA	BC, HP	Repro	3	5		85.4% decrease in serum testosterone and 105% increase in estradiol levels at 5 mg/kg/day; atrophy of seminiferous tubule epithelium
Feng et al. 2009									
8	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 1, 3, 5 PFNA	HP	Repro	3	5		Large vacuoles between testicular Sertoli cells and spermatogonia
Feng et al. 2010									
9	Rat (Wistar)	14 days (GO) 8 or 10M	0, 0.0125, 0.25, 5 PFNA	BI, BW, OW, GN, HP	Bd wt		5		Decreased body weight; magnitude of effect was not reported
					Endocr		5		Decreased androstenedione and testosterone concentrations (data not shown)
Hadrup et al. 2016									
10	Mouse (SV129 WT and PPARα null) 4 M	7 days (G)	0, 10 PFNA	BW, HP	Bd wt	10			
					Hepatic	10			Hepatocellular hypertrophy, steatosis, and increased hepatic triglyceride levels
Das et al. 2017									
11	Mouse (BALB/c) 6 M	14 days (G)	0, 1, 3, 5 PFNA	FX	Immuno		1		Decreases in the percentages of F4/80+ and CD49b+ cells in the spleen; no alteration in the response of splenic lymphocytes to ConA at ≤5 mg/kg/day
Fang et al. 2008									
12	Mouse (CD-1) 5 M,F	14 days (F)	0, 0.5, 1.8, 5.3, 54, 537 PFNA	LE, OW	Death			54	100% mortality before day 14
					Hepatic	5.3			50–70% increase in absolute liver weight at ≥0.5 mg/kg/day
Kennedy 1987									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
13	Mouse (BALB/c) 8 M	14 days (G)	0, 0.2, 1, 5 PFNA	BW, OW, BC	Bd wt	1	5		~25% decrease in body weight
					Hepatic	1	5		Increases in liver weight (~160%) and increases in hepatic triglyceride (~66%) and cholesterol levels (~26%) at ≥0.2 mg/kg/day; decreases in serum triglyceride (~67%) and cholesterol levels (~32%) and increases in serum ALT (~900%) and AST (~280%) levels at 5 mg/kg/day
Wang et al. 2015a									
PFDA									
14	Rat (Wistar) 5–12 M	Once (GO)	0, 50 PFDA	BW, FX	BW			50	8% weight loss was observed 10-days post-exposure
					Neuro	50			No alteration in performance on novel object recognition test
Kawabata et al. 2017									
15	Rat (Wistar) 25 M	1 week (F)	0, 1.2, 2.4, 4.7, 9.5 PFDA	BE, OW, EA	Bd wt	4.7		9.5	~32% weight loss
					Hepatic	9.5			Increases in liver weight at ≥2.4 mg/kg/day; increases in hepatic cholesterol at 9.5 mg/kg/day
Kawashima et al. 1995									
16	Mouse (C57BL/6N) 4 F	Once (GO)	0, 40, 80, 100, 120, 160 PFDA	OW, EA	Hepatic	160			Increase in hepatic lipids and liver weight 2 days post-exposure at 40 mg/kg/day
Brewster and Birnbaum 1989									
17	Mouse (C57BL/6N) 12–14 F	GDs 6–15 (GO)	0, 0.03, 0.1, 0.3, 1, 3, 6.4, 12.8 PFDA	MX, DX, BW, OW	Bd wt	3	6.4	12.8	No weight gain at 6.4 mg/kg/day and weight loss at 12.8 mg/kg/day (net change of -2.4)
					Develop	0.3	1	12.8	18–22% decreases in fetal weight per litter at ≥1 mg/kg/day; decreases in live fetuses per litter at 12.8 mg/kg/day (4.6 versus 7.2 in controls)
Harris and Birnbaum 1989									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
18	Mouse (C57BL/6N) 10 F	Once (GO)	0, 20, 40, 80, 160, 320 PFDA	BW, OW, GN, HP	Death			120	LD_{50} in 30-day observation period
					Bd wt	40	80		12% decreased body weight 30 days post-exposure
					Cardio	80			No histological alterations in the heart 30 days post-exposure; decreased relative heart weight at 80 mg/kg/day
					Hepatic	80			Increases in liver weight and pancellular hypertrophy at ≥20 mg/kg/day 30 days post-exposure
					Renal	80			No histological alterations 30 days post-exposure
					Endocr	40	80		2-fold increase in T3 and 4-fold increase in T4 levels 30 days post-exposure
					Immuno	40	80	160	28% decrease in relative spleen weight at 80 mg/kg/day; atrophy and lymphoid depletion in thymus and spleen at 160 mg/kg/day
Harris et al. 1989									
19	Mouse (CD-1) 10 M	Once on PND 10 (G)	0, 0.72, 10.8 PFDA	CS, DX	Develop	10.8			No alteration in spontaneous activity or habituation at 2–4 months of age
Johansson et al. 2008									
20	Mouse (C57BL/6N) 4 M	10 days (F)	0, 78 PFDA	BW, OW, EA	Bd wt			78	33% weight loss
					Hepatic	78			36% increase in liver weight
Permadi et al. 1992, 1993									
PFBA									
21	Rat (Sprague-Dawley) 3 M,F	5 days 1 time/day (GW)	0, 18, 58, 184 PFBA	CS, BW, OW, HE, BI, GN, HP	Bd wt	184			
					Resp	184			
					Cardio	184			
					Gastro	184			
					Hemato	184			
					Musc/skel	184			
					Hepatic	184			
					Renal	184			

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Endocr	184			
					Immuno	184			No histological alterations
					Neuro	184			No histological alterations
					Repro	184			No histological alterations
3M 2007a									
22	Rat (Sprague-Dawley) 3 M	14 days ad lib (F)	0, 20 PFBA	OW, EA	Hepatic	20			Biochemical and ultrastructural evidence of peroxisome proliferation
Ikeda et al. 1985									
23	Mouse (C57BL/6N) 4 M	10 days (F)	0, 78 PFBA	BW, OW, EA	Bd wt	78			
					Hepatic	78			63% increase in absolute liver weight
Permadi et al. 1992, 1993									
PFDoDA									
24	Rat (Wistar) 5–12 M	Once (GO)	0, 5, 20, 50 PFDoDA	BW, FX	BW		50		44% decrease in body weight gain (measured 10-days post-exposure)
					Neuro		50		Impaired performance on novel object recognition test
Kawabata et al. 2017									
25	Rat (Wistar) 10 M	Once (GO)	0, 50 PFDoDA	FX	Neuro	50			No alterations in open field activity
Kawabata et al. 2017									
26	Rat (Wistar) 8 M	Once (GO)	0, 50 PFDoDA	FX	Neuro	50			No alterations in tests of working memory or depressive behavior
Kawabata et al. 2017									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
27	Rat (Sprague-Dawley) 10 M	14 days 1 time/day (GW)	0, 1, 5, 10 PFDoDA	BW, OW, BI	Bd wt	1	5		25% reduction in final body weight
					Hepatic	10			35% increase in total serum cholesterol at 10 mg/kg/day
					Repro	1	5		Decreased serum testosterone (38%) and estradiol (~38%), and ultrastructural alterations in testes at ≥5 mg/kg/day; decreased testicular weight at 10 mg/kg/day (22%)
Shi et al. 2007									
28	Rat (Sprague-Dawley) 10 M	14 days 1 time/day (GW)	0, 1, 5, 10 PFDoDA	OW, BI, HP	Hepatic	10			Increased liver weight, increased hepatic triglyceride and cholesterol levels at ≥5 mg/kg/day; increased serum triglyceride levels at 10 mg/kg/day
Zhang et al. 2008									
FOSA									
29	Rat (Sprague-Dawley) 15 M	Once (G)	0, 5 FOSA	BW, OW	Bd wt	5			
					Hepatic	5			No alterations in liver weight
Seacat and Luebker 2000									
PFHxA									
30	Mouse (CD1) 20 F	GDs 6–18 (GW)	0, 100, 350, 500 PFHxA	CS, DX	Develop	100	350		12.5% decrease in birth weight and delayed eye opening at ≥350 mg/kg/day; increased pup mortality (PND 0–3) and decreased pup survival at 500 mg/kg/day
Iwai and Hoberman 2014									
31	Mouse (CD1) 20 F	GDs 6–18 (GW)	0, 7, 35, 175 PFHxA	CS, DX	Develop	35	175		Increase in stillborn pups and 12.5% decrease in birth weight
Iwai and Hoberman 2014									
32	Rat (Crl:CD (SD)) NS F	Once (GW)	175, 550, 1,550, 5,000 NaPFHx	LE, CS, BW	Death			1,750	1/4 died at 1,750 mg/kg/day; 3/3 died at 5,000 mg/kg/day
					Neuro			175	Abnormal gait, salivation, ataxia, lethargy
Loveless et al. 2009									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
INTERMEDIATE EXPOSURE									
PFHxS									
33	Rat (Sprague-Dawley) 15 M,F	42–56 days 1 time/day (GW)	0, 0.3, 1, 3, 10 PFHxS	CS, BW, MX, DX, OW, OF, BI, HE, GN, HP, FI	Bd wt	10			
					Resp	10			
					Cardio	10			
					Gastro	10			
					Hemato	10 F	0.3 M		6% increase in prothrombin time in males at ≥0.3 mg/kg/day; decreases in hemoglobin (4%), hematocrit (3%), and RBC levels (2.4%) in males at ≥3 mg/kg/day
					Hepatic	10			Increased liver weight; centrilobular hepatocellular hypertrophy in males at ≥3 mg/kg/day
					Renal	3 M	10 M		31% increase in BUN in males
					Endocr	1[b] M 10 F	3 M		Hypertrophy and hyperplasia of thyroid follicular cells in males
					Immuno	10			No histological alterations
					Neuro	10			No histological alterations
					Repro	10			No histological alterations or effects on fertility
					Develop	10 F			
Butenhoff et al. 2009a (results also reported in Hoberman and York 2003)									
34	Rat (Wistar) 8 F	GDs 7–22 (GO)	0, 25, 45 PFHxS	CS, BW, BC, DX, OW	Bd wt	45			
					Endocr		25		60% decreases in serum T4 levels in dams
					Develop		25		40% decrease in serum T4 levels in pups on PND 16
Ramhøj et al. 2018									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
35	Rat (Wistar) 16–20F	GDs 7–22 (GO)	0, 0.05, 5, 25 PFHxS	CS, BW, BC, DX, OW	Bd wt	25			
					Endocr	0.05	5		20–30% decreases in serum T4 levels in dams
					Develop	0.05	5		20% decrease in serum T4 levels in pups on PND 16/17
Ramhøj et al. 2018									
36	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 6 PFHxS	BW, FI, BC, OW	Bd wt	6			
					Hepatic	6			Decreased plasma triglyceride, cholesterol, non-HDL cholesterol, and HDL cholesterol levels; increased hepatic triglyceride levels, increased liver weight
Bijland et al. 2011									
37	Mouse (CD-1) 30 M, 30 F	M: 42 days F: 14 days premating, mating, gestation, lactation (G)	0, 0.3, 1, 3 PFHxS	CS, BW, FI, HE, BC, GN, HP, FX, RX, DX	Bd wt	3			
					Resp	3			
					Cardio	3			
					Gastro	3			
					Hemato	3			
					Musc/skel	3			
					Hepatic	1	3		Single cell necrosis and microvascular fatty changes at 3 mg/kg/day; centrilobular hepatocellular hypertrophy at ≥0.3 mg/kg/day in F0 mice and at 3 mg/kg/day in F1 mice
					Renal	3			
					Endocr	3			
					Neuro	**3**			
					Repro	3			
					Develop	0.3	1		Decrease in number of pups per litter with no change in pup to implantation site ratio
Chang et al. 2018									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
PFNA									
38	Rat (Sprague-Dawley) 18 dams; 10–12 M,F pups/litter	GDs 1–20 (GW)	5 PFNA	BW, OF, OW	Bd wt		5		Reduced maternal body weight; approximately 33% on GD 7 and 10% on GD 20
					Develop		5		Decreased birth weight in female pups only (approximately 11%); increased systolic blood pressure in 10-week-old male and female offspring; reduced nephron endowment
Rogers et al. 2014									
39	Mouse (CD-1) 8–10 F	GDs 1–17 (GW)	0, 1, 3, 5, 10 PFNA	BC, DX, FX, MX, OW	Bd wt			10	43% maternal weight loss at GD 13
					Hepatic	10			Increases in absolute and relative liver weights in dams on GD 17 and on post-weaning day 28 at ≥1 mg/kg/day
					Develop	1[c]	3	5	Delayed postnatal development [eye opening, preputial separation and vaginal opening] and decreased body weight gain persisting in males up to PND 287 at ≥3 mg/kg/day; decreased postnatal survival between PND 2 and 10 with 80% mortality at ≥5 mg/kg/day; full litter resorptions at 10 mg/kg/day
Das et al. 2015									
40	Mouse (Parkes) 14 M	90 days (GW)	0, 0.2, 0.5 PFNA	BW, OW, BC HP, RX	Bd wt	0.5			
					Hepatic	0.5			33% decrease in serum cholesterol
					Repro	0.2	0.5		Decreased sperm motility, viability, and number; degenerative changes in seminiferous tubules; decreased litter size
Singh and Singh 2018									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
41	Mouse (129S1/ Svlm) 8 F	GDs 1–18 (G)	0, 0.83, 1.1, 1.5, 2.0 PFNA	BW, OW, DX	Bd wt	2.0			No alterations in maternal body weight or gestational weight gain
					Hepatic	2.0			Increase in dam liver weight at ≥0.83 mg/kg/day
					Develop	0.83	1.1		Decreased number of live births (36 and 31%) and pup survival at 1.1 and 2.0 mg/kg/day, but not 1.5 mg/kg/day; decreased number of live pups per litter and decreased pup body weight gain in females at 2 mg/kg/day; increased pup liver weight at ≥0.83 mg/kg/day
Wolf et al. 2010									
42	Mouse (PPARα knockout) 8 F	GDs 1–18 (G)	0, 0.83, 1.1, 1.5, 2.0 PFNA	BW, OW, DX	Bd wt	2.0			No alterations in maternal body weight or gestational weight gain
					Hepatic	2.0			Increases in liver weight at ≥1.5 mg/kg/day non-pregnant adults, but not in the dams
					Develop	2.0			No alterations in the number of live pups per litter, birth weight, pup survival, day of eye opening, or pup body weight gain; no increases in pup liver weight were observed
Wolf et al. 2010									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
PFDA									
43	Rat (Sprague-Dawley) 8 F	28 days	0, 0.125, 0.25, 0.5, 1, 2 PFDA	BW, HE, OW, HP, IX	Bd wt	0.5	1		Decreased body weight gain (21%)
					Resp	0.5			
					Gastro	0.5			
					Hemato	0.125	0.25		Decreased MCH and MCHC
					Hepatic	0.25	0.5		Single cell necrosis
					Hepatic	0.5			
					Renal	0.5			
					Endocr	0.5			
					Immuno	0.125	0.25		Decreased phagocytosis by fixed tissue macrophages in the liver
Frawley et al. 2018									
44	Mouse (B6C3F1/N) 8 F	1 time/week 4 weeks	0, 0.325, 0.625, 1.25, 2.5, 5 PFDA	BW, HE, OW, HP, IX	Bd wt	5			
					Resp	5			
					Gastro	5			
					Hemato	5			
					Hepatic	5			
					Renal	5			
					Endocr	5			
					Immuno	0.625	1.25		Decreases in splenic T-cells, T-cell subsets, and macrophages
Frawley et al. 2018									
PFUnA									
45	Rat (Crl:CD[SD]) 12 M,F (main); 5 M,F (other)	41–46 days (GO)	0, 0.1, 0.3, 1.0 PFUnA	BH, BW, CS, HP, OF, OW, UR	Bd wt	0.3	1.0		Decreased body weight (~10%) in males during exposure and recovery and in satellite females during dosing (~23% on day 40) and recovery (~10%)
					Hemato	0.3	1.0		Main study males: decreased MCV (5%), MCH (5%), APTT (25%), and fibrinogen (33%) and increased platelet counts (7%); satellite males: increased WBC (52%) and decreased APTT (16%) and fibrinogen (19%); main study females: increased MCV (10%) and MCH (10%) and decreased fibrinogen (32%)

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	1.0			Increased absolute and relative liver weight and centrilobular hypertrophy at 1.0 mg/kg/day
					Renal	0.3	1.0		Increased BUN (61%) and ALP (140%); decreased total protein (11%) and albumin (7%) in main group males
					Develop	0.3 F	1.0 F		Decreased body weight in pups on PNDs 0 and 4 (13–19% in males and 12–16% in females)
Takahashi et al. 2014									
PFBS									
46	Rat (Sprague-Dawley)	28 days 1 time/day (GO)	0, 100, 300, 900 PFBS	CS, BW, FI, HE, BI, GN, HP, OF	Bd wt	900			
					Resp	900			
					Cardio	900			
					Gastro	900			
					Hemato	900			
					Musc/Skel	900			
					Hepatic	900			Increased absolute and relative liver weight at 900 mg/kg/day
					Renal	900			
					Ocular	900			
					Endocr	900			
					Immuno	900			No histological alterations
					Neuro	900			No histological alterations
					Repro	900			No histological alterations
3M 2001									
47	Rat (Sprague-Dawley) 10 NS	90 days (G)	0, 60, 200, 600 PFBS	LT, BW, OW, GN, HP, BC, CS, BI, BH, HE	Resp	600			
					Cardio	600			
					Gastro	200	600		Necrosis of individual squamous cells in forestomach and hyperplasia and hyperkeratosis of limiting ridge
					Hemato	60 M	200 M		Decreased hemoglobin (4.9%) and hematocrit (5.2%)
					Musc/skel	600			

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	600			
					Renal	200	600		Hyperplasia of medullary and papillary tubules and medullary ducts and focal papillary edema
					Endocr	600			
					Neuro	600			
Lieder et al. 2009a (data also reported in York 2003b)									
48	Rat (Sprague-Dawley)	P0: starting 70 days prior to mating; F1: starting at weaning (G)	0, 30, 100, 300, 1,000 PFBS	BW, OW, FI, GN, HP, FX, MX, DX, TG	Bd wt	1,000			
					Hepatic	1,000			Increased liver weight in males at ≥300 mg/kg/day
					Renal	100	300		Medullary/papillary tubular and ductal hyperplasia in P0 and F1
					Repro	1,000			
					Develop	1,000			
Lieder et al. 2009b (data also reported in York 2003c)									
49	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 30 PFBS	BW, FI, BC, OW	Bd wt	30			
					Hepatic	30			Decreased plasma triglyceride levels
Bijland et al. 2011									
50	Mouse (ICR) 30 F	GDs 1–20 (G)	0, 50, 200, 500 PFBS	BW, BC, OW, DX	Bd wt	500			
					Endocr	50	200		Decreased maternal total thyroxine, free thyroxine, and total triiodothyronine and increased TSH
					Develop	50	200		Decreases in pup body weight; delays in eye opening, vaginal opening, and first estrous; decreases in ovarian follicles; decreases in uterine endometrial and myometrial thickness; alterations in reproductive hormone levels; decreases in total T4 and total T3 and increases in TSH
Feng et al. 2017									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
51	Rat (Crl:CD(SD) 25 F	GDs 6–20 (GW)	0, 100, 300, 1,000 PFBS	BW FI DX	Bd wt	300	1,000		31% decrease in maternal body weight gain
					Develop	300	1,000		Decreases in fetal body weight (9%) and delays in hindlimb ossification
York 2002									
52	Rat (Crl:CD(SD) 8 F	GDs 6–20 (GW)	0, 100, 300, 1,000, 2,000 PFBS	BW FI DX	Bd wt	1,000	2,000		12% decrease in maternal body weight
					Develop	1,000	2,000		12–13% decrease in fetal body weights
York 2003a									
PFBA									
53	Rat (Sprague-Dawley) 10 M,F	28 days 1 time/day	0, 6, 30, 150 PFBA	CS, BW, OW, FI, BI, HE, GN, HP, OF	Bd wt	150			
					Resp	150			
					Cardio	150			
					Gastro	150			
					Hemato	150			
					Musc/skel	150			
					Hepatic	150			Increased absolute and relative liver weight and decreased serum cholesterol in males at ≥30 mg/kg/day; hepatocellular hypertrophy in males at 150 mg/kg/day
					Renal	150			
					Dermal	150			
					Ocular	150			
					Endocr	6 M	30 M		Hyperplasia/hypertrophy of follicular epithelium of the thyroid
					Immuno	150			No histological alterations
					Neuro	30 M	150 M		Delayed pupillary reflex
					Repro	150			No histological alterations
Butenhoff et al. 2012a; van Otterdijk 2007a									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
54	Rat (Sprague-Dawley) 10 M,F	90 days 1 time/day	0, 1.2, 6, 30 PFBA	CS, BW, FI, OW, BI, HE, GN, HP	Bd wt	30			
					Resp	30			
					Cardio	30			
					Gastro	30			
					Hemato	6	30 M		Reduced erythrocyte counts (3.8%), hemoglobin (5.7%), and hematocrit (4.5%)
					Musc/skel	30			
					Hepatic	30			Diffuse panlobular hepatocyte hypertrophy at 30 mg/kg/day in males
					Renal	30			
					Dermal	30			
					Ocular	30			
					Endocr	6 M	30 M		Hypertrophy/hyperplasia of follicular epithelium of the thyroid gland
					Immuno	30			No histological alterations
					Neuro	30			No histological alterations
					Repro	30			No histological alterations
Butenhoff et al. 2012a; van Otterdijk 2007b									
55	Mouse (CD-1)	18 days GDs 1–17 1 time/day (GW)	0, 35, 175, 350 PFBA	BW, MX, DX	Hepatic	350			Significant increase in absolute and relative liver weight at ≥175 mg/kg/day
					Develop		35		Eye opening delayed approximately 1 day
Das et al. 2008									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
PFDoDA									
56	Rat (Crl:CD (SD)) 12 M, 17 F	42–47 days (G)	0, 0.1, 0.5, 2.5 PFDoDA	CS, BW, BC, HE, OW, HP, NX, RX, DX	Death			2.5 F	58% mortality
					Bd wt	0.5	2.5		Decreased body weight gain (20–30%)
					Cardio	2.5			
					Gastro	2.5			
					Hemato	0.5	2.5		Decreased mean corpuscular volume and reticulocytes and increased mean corpuscular hemoglobin concentration in males
					Hepatic	0.5 F	2.5 F		Single cell necrosis in females; hepatocellular hypertrophy in males and females at 2.5 mg/kg/day; bile duct proliferation at 2.5 mg/kg/day in females in recovery group
					Renal	2.5			
					Endocr	0.5	2.5		Pancreas: edema in the interstitium in females and decrease in zymogen granules in males; thymus: atrophy of the cortex in females; adrenals: atrophy of the cortex in males
					Neuro	0.5	2.5		Decreased forelimb grip in males and motor activity in females during recovery period
					Repro	0.5	2.5 F		Hemorrhage at the implantation site; continuous diestrus in nonmated rats
					Develop	0.5	2.5		Decreases in pup body weight (only one litter had live pups)
Kato et al. 2015									
57	Rat (Sprague-Dawley) 8 F	28 days PNDs 24–72 (GW)	0, 0.5, 1.5, 3 PFDoDA		Endocr	1	3		40% reduced serum estradiol in pubertal females
Shi et al. 2009b									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
58	Rat (Sprague-Dawley) 6 M	110 days GW	0, 0.02. 0.05, 0.2, 0.5 PFDoDA	BW, BI, OW, HP	Bd wt	0.5			
					Repro	0.05	0.2		Decreased serum testosterone (44%)
Shi et al. 2009a									
PFHxA									
59	Rat (Crl:CD (SD)) 10 M, 10 F	90 days (GW)	0, 10, 50, 200 PFHxA	CS, BW, FI, HE, BC, UR, OP, NX, OW, GN, HP	Bd wt	200			
					Resp	200			
					Cardio	200			
					Gastro	200			
					Hemato	50	200		Slight decreases in RBC, hemoglobin, and hematocrit and increases in reticulocytes
					Musc/skel	200			
					Hepatic	200			Centrilobular hepatocellular hypertrophy at 200 mg/kg/day
					Renal	200			
					Ocular	200			
					Endo	200			
					Neuro	200			
					Repro	200			
Chengelis et al. 2009b									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
60	Rat (CRL:CD(SD)) 10–15 M, 10–15 F	32–44 days (GW)	0, 50, 150, 315 (TWA dose), 450 (4 days) **PFHxA**	CS, BW, FI, HE, BC, RX, DX, OW, GN, HP	Death			450	8/30 deaths (includes rats sacrificed *in extremis*) during first 4 days of exposure
					Bd wt	315			
					Gastro	315			Stomach erosions/ulceration in dying or sacrificed *in extremis* rats administered 450 mg/kg/day for 4 days
					Hemato	50 M 315 F	150 M		Decreased hemoglobin levels; increased reticulocytes at 315 mg/kg/day
					Hepatic	315			Increased relative liver weight at ≥150 mg/kg/day; hepatocellular hyperplasia and decreased serum cholesterol observed at 315 mg/kg/day
					Renal	315			
					Immuno	150 F 315 M	315 F		Thymic atrophy in 3/9 females
					Repro	315			
					Develop	315			
Kirkpatrick 2005									
61	Rat (CRL:CD (SD)) 30 M, 30 F	92–93 days (GW)	0, 20, 100, 500 NaPFHx	CS, BW, FI, OP, BC, HE, UR, OW, GN, HP, NX	Bd wt	200 M	500 M		19% decrease in body weight gain
					Resp	20	100		Degeneration/atrophy of nasal olfactory epithelium at ≥100 mg/kg/day and respiratory metaplasia at 500 mg/kg/day
					Hemato	100	500		Decreased RBC, hemoglobin, and hematocrit; increased reticulocytes; erythroid hypertrophy in bone marrow and extramedullary hematopoiesis in spleen
					Hepatic	500			Hepatocellular hypertrophy in males at ≥100 mg/kg/day and females at 500 mg/kg/day
					Ocular	500			
					Endocr	100 F	500 F		Thyroid follicular epithelial hypertrophy
					Neuro	500			
Loveless et al. 2009									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
62	Rat (CRL:CD (SD) 20M, 20F	110–126 days; 70 days prior to mating and during mating gestation, lactation (GW)	0, 20, 100, 500 NaPFHx	CS, BW, FI, RX, DX	Bd wt	20 M 100 F	100 M 500 F		Decreased weight gain (12%); decreased maternal weight gain during GDs 0–7 and increased maternal weight gain during lactation
					Repro	500			
					Develop	100	500		Decreased pup body weight (17–18%) during lactation period
Loveless et al. 2009									
63	Rat (CRL:CD (SD) 22 F	GDs 1–20 (GW)	0, 20, 100, 500 NaPFHx	BW, FI, DX	Bd wt	100	500		Decreased maternal weight gain (19%)
					Develop	100	500		Decreased fetal weight (10%)
Loveless et al. 2009									
CHRONIC EXPOSURE									
PFHxA									
64	Rat (Sprague-Dawley) 60 or 70 M,F	104 weeks (GW)	M: 0, 2.5, 15, 100 F: 0, 5, 30, 200 PFHxA	BC, BW, CS, GN, HP, LE, OP, OW, UR	Death			200 F	36, 43, 33, and 22% survival rate in females at 0, 5, 30, and 200 mg/kg/day, respectively
					Bd wt	100 M 200 F			
					Hemato	100 M	200 F		8.1% reduction in mean RBC count and 5.2% reduction in hemoglobin at 51 weeks; 23.6 and 53.6% increase in reticulocyte counts at weeks 25 and 51, respectively
					Hepatic	100 M 30F	200 F		Males: 42% decrease in triglycerides, 19% decrease in free fatty acids in males at 100 mg/kg/day; hepatocellular necrosis; 66% increase in triglycerides, 44% decrease in non-HDL cholesterol in females at 200 mg/kg/day

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Renal	100 M	200 F		Mild renal tubular degeneration and mild to severe papillary necrosis; increased mean urine volume (109%) and reduced specific gravity (0.96%)
					Neuro	100 M 200 F			
Klaunig et al. 2015									

[a]The number corresponds to entries in Figure 2-10.
[b]Used to derive an intermediate-duration oral MRL of $2x10^{-5}$ mg/kg/day for PFHxS based on a measured serum PFHxS level of 89.12 µg/mL at the NOAEL dose and an empirical clearance model to estimate a HED. The $NOAEL_{HED}$ of 0.0047 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) and a modifying factor of 10 for database deficiencies.
[c]Used to derive an intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day for PFNA based on a measured serum PFNA level of 8.91 µg/mL at the NOAEL dose and an empirical clearance model to estimate a HED. The $NOAEL_{HED}$ of 0.001 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) and a modifying factor of 10 for database deficiencies.

ad lib = *ad libitum*; ALT = alanine aminotransferase; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BC = biochemistry; BH = behavioral; BI = biochemical changes; BUN = blood urea nitrogen; BW or Bd wt = body weight; Cardio = cardiovascular; CI = confidence interval; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FOSA = perfluorooctane sulfonamide; FX = fetal toxicity; G = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; GO = gavage in oil vehicle; GW = gavage in water vehicle; HDL = high-density lipoprotein; HE or Hemato = hematological; HP = histopathology; Immuno = immunotoxicological; IX = immunotoxicity; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; Musc/skel = musculoskeletal; MX = maternal toxicity; NaPFHx = sodium perfluorohexanoate; Neuro = neurological; NOAEL = no observed-adverse-effect level; NS = not specified; NS = neurotoxicity; OF = organ function; OP = ophthalmology; OW = organ weight; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid; PPARα = peroxisome proliferator-activated receptor-α; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; T3 = triiodothyronine; T4 = thyroxine; TG = teratogenicity; TSH =thyroid stimulating hormone; TWA = time-weighted average; UR = urinalysis

Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Acute (≤14 days)

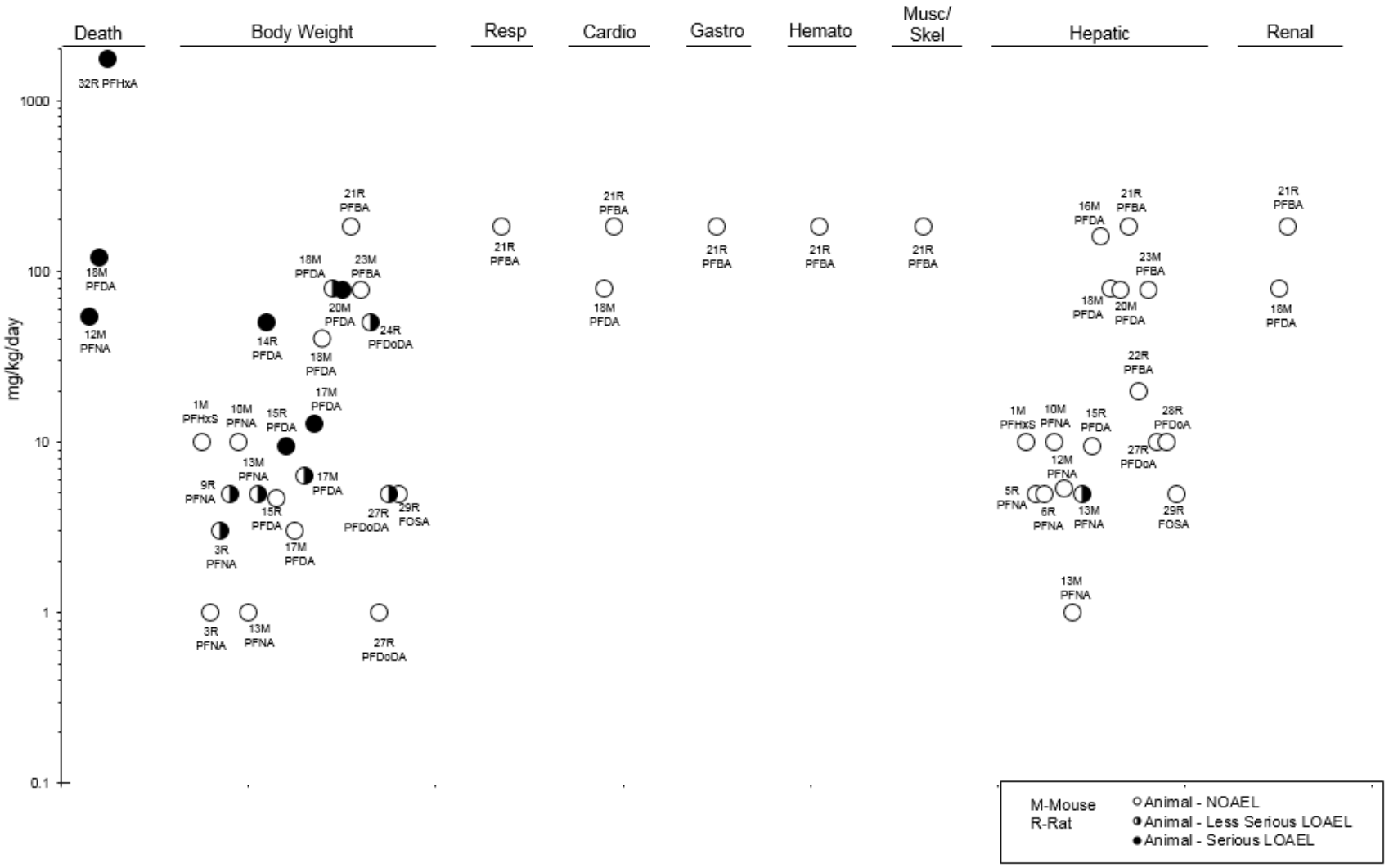


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Acute (≤14 days)

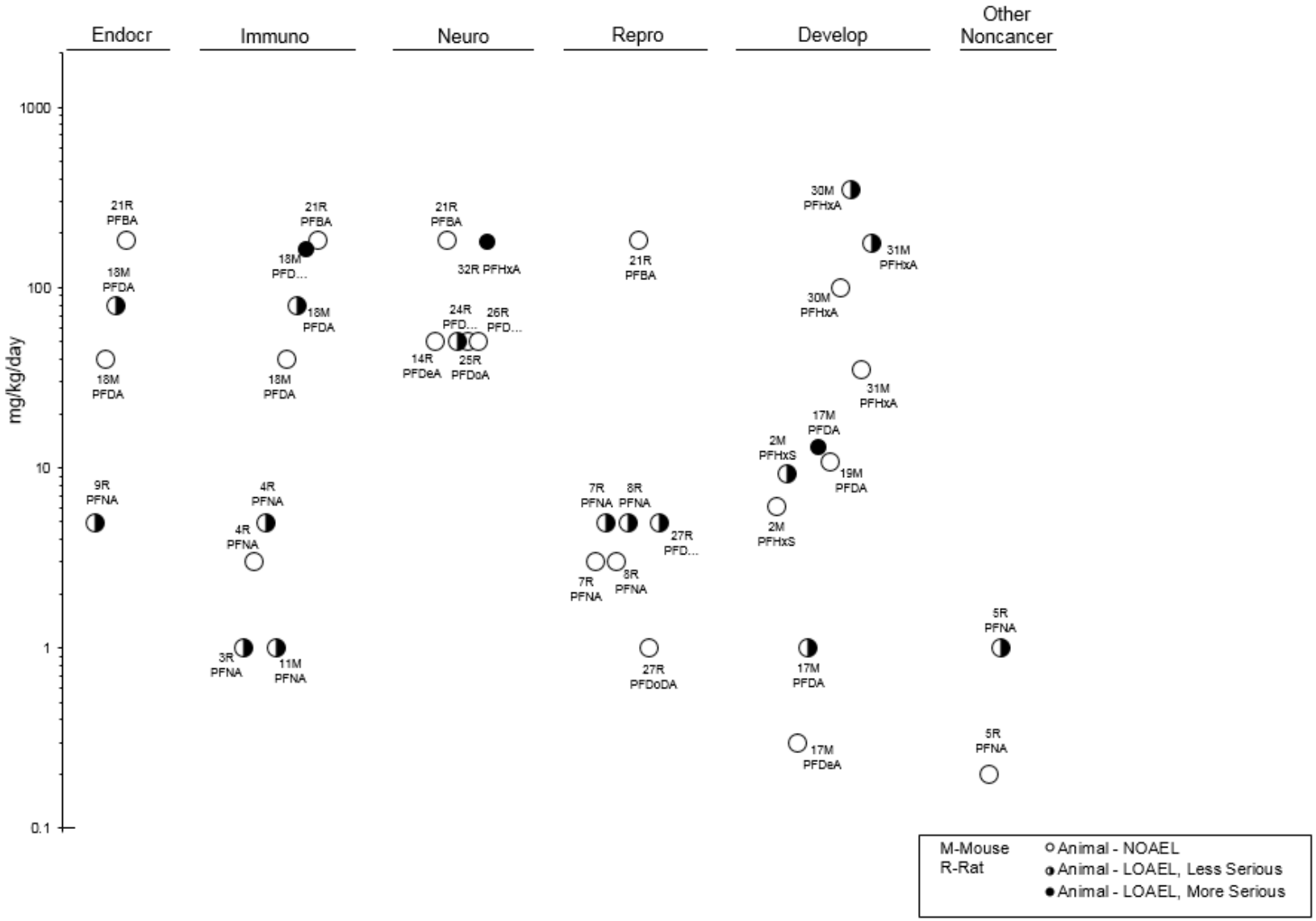


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Intermediate (15–364 days)

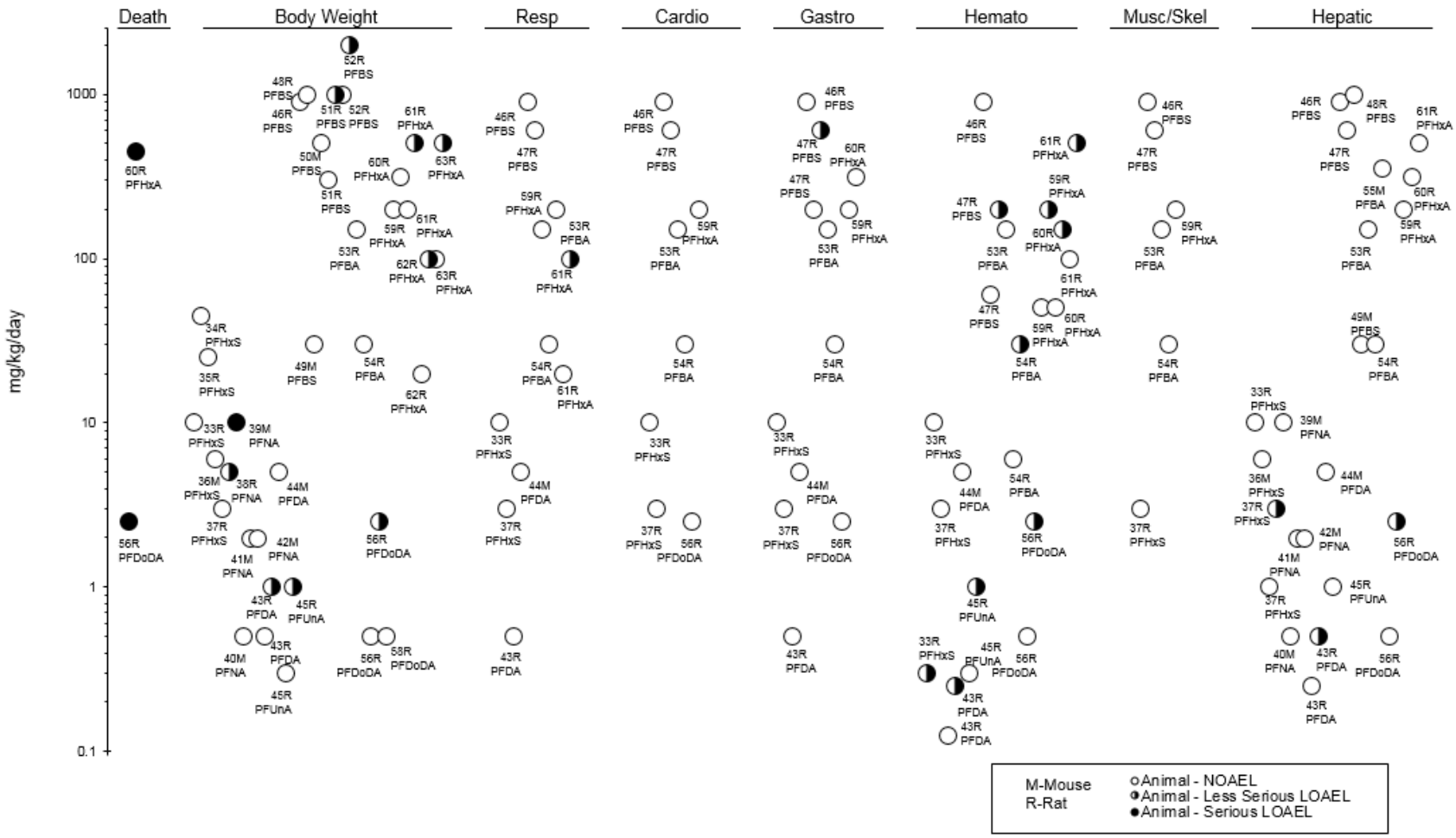


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Intermediate (15–364 days)

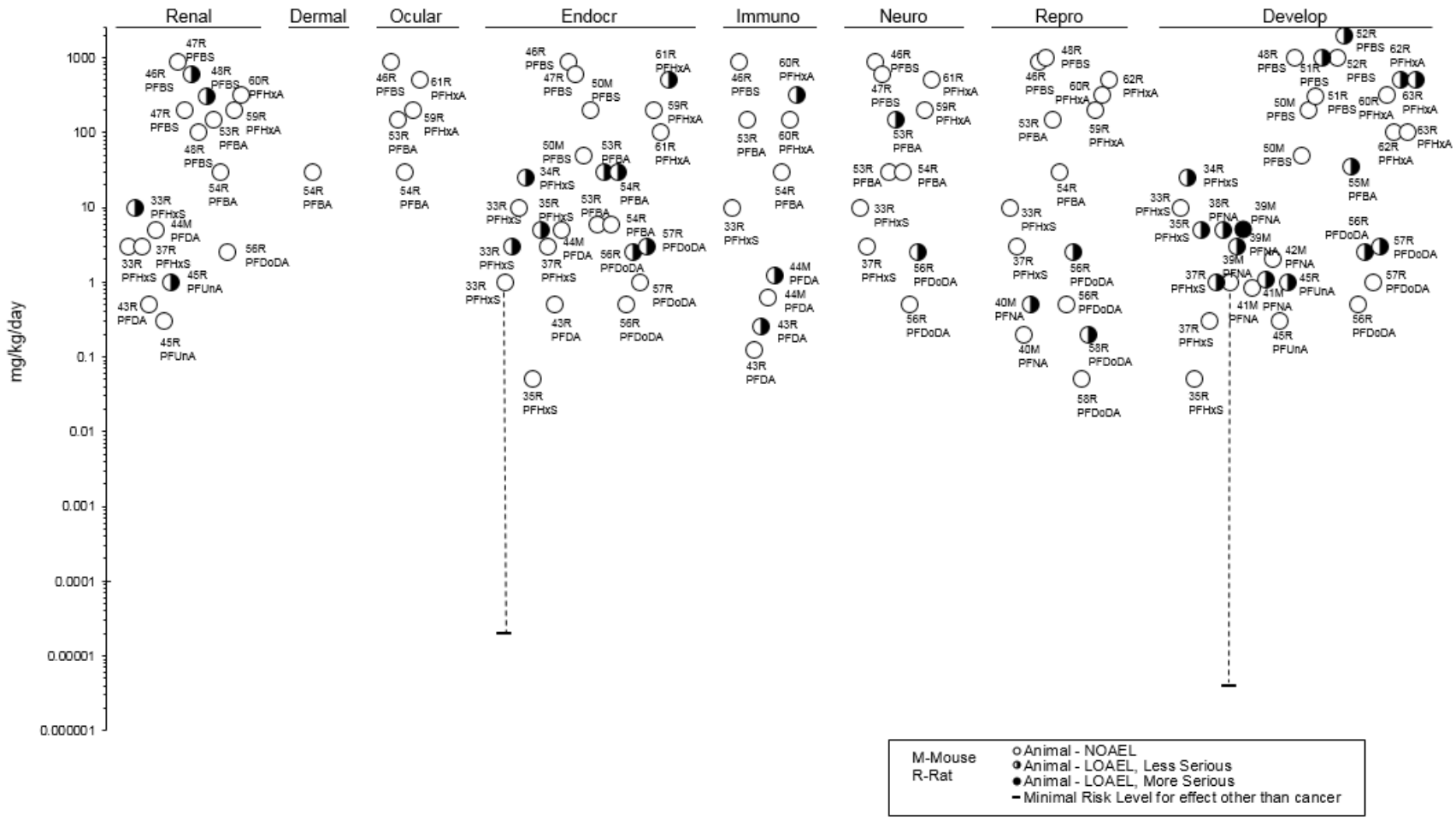


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Chronic (≥365 days)

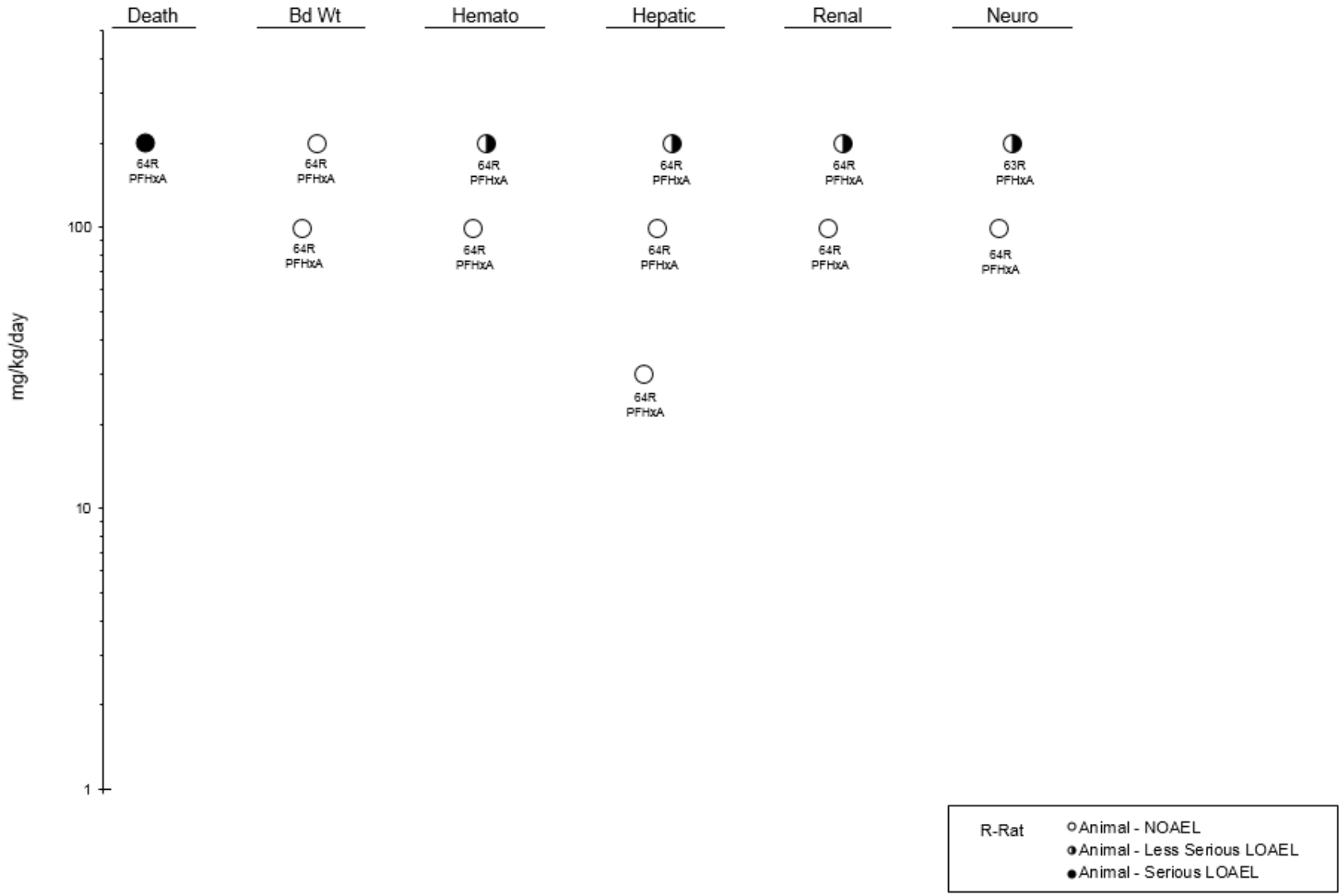


Table 2-6. Levels of Significant Exposure to PFOA – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
ACUTE EXPOSURE								
Rat (CD) 15 M,F	Once	3,000, 5,000, 7,500 mg/kg	CS, LE	Death			7,000 M	14-day LD_{50}
				Bd wt			3,000 M F	5–7% transient weight loss
				Dermal	3,000 M F	5,000 M F		Mild skin irritation
Kennedy 1985								
APFO								
LD_{50} in females was >7,500 mg/kg								
Rat (CD) 60 M	2 weeks 6 hours/day 5 days/week	0, 20, 200, 2,000 mg/kg/day	CS, BW, HE, BI, GN, HP	Bd wt	20 M		200 M	14% weight loss
				Resp	2,000 M			
				Cardio	2,000 M			
				Gastro	2,000 M			
				Hemato	2,000 M			
				Hepatic		20 M		Foci of coagulative necrosis
				Renal	2,000 M			
				Dermal	20 M	200 M	2,000 M	Skin irritation; acute necrotizing dermatitis
				Ocular	2,000 M			
				Endocr	2,000 M			
				Immuno	2,000 M			
				Neuro	2,000 M			
				Repro	2,000 M			
Kennedy 1985								
APFO								
The immunological NOAEL is for histopathology of the spleen, thymus, and lymph nodes. The neurological NOAEL is for histopathology of the brain. The reproductive NOAEL is for histopathology of the testes.								
Mouse (BALB/c) 35 F	4 days 1 time/day	0, 0.25, 2.5, 6.25, 12.5, 25, 50 mg/kg/day	BW, OW, OF	Bd wt	50 F			
				Hepatic	2.5 F	6.25 F		52% increase in absolute liver weight
Fairley et al. 2007								
PFOA								

Table 2-6. Levels of Significant Exposure to PFOA – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
Mouse (BALB/c) 35 F	4 days 1 time/day	0, 12.5, 18.8, 25, 50 mg/kg/day	BW, OW, OF	Immuno	12.5	18.8		Increased serum IgE following ovalbumin challenge
Fairley et al. 2007								
PFOA								
Rabbit (albino) 6 NS	Once (NS)	100 mg	CS	Ocular		100		Moderate eye irritation
Griffith and Long 1980								
APFO								
Rabbit (albino) 6 NS	24 hours (NS)	500 mg	CS	Dermal	500			
Griffith and Long 1980								
APFO								
Rabbit (New Zealand) 17 M	Once	1,500, 3,000, 5,000, 7,500 mg/kg	CS, LE	Death		4,300		14-day LD_{50}
Kennedy 1985								
APFO								

APFO = ammonium perfluorooctanoate; BI = biochemical changes; BW or Bd wt = body weight; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); Gastro = gastrointestinal; GN = gross necropsy; HE or Hemato = hematological; HP = histopathology; Immuno = immunotoxicological; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; PFOA = perfluorooctanoic acid; Repro = reproductive; Resp = respiratory

2.2 DEATH

Overview. There are limited data regarding the lethality of perfluoroalkyls in humans; the available data primarily come from cohort mortality studies in workers; data were only available for PFOA and PFOS. These studies did not find increases in deaths from all causes associated with PFOA and PFOS, although some increases in disease-specific mortalities were observed. Laboratory animal studies have measured LC_{50} and LD_{50} values and reported deaths following inhalation, oral, or dermal exposure to perfluoroalkyls. Increases in mortality have also been observed in repeated-exposure studies. These data are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and Figures 2-6, 2-7, 2-8, 2-9, and 2-10. No laboratory animal data were available for PFHxS, PFUnA, PFHpA, PFBS, PFBA, or FOSA.

PFOA

Epidemiological Studies. Five occupational exposure studies at two PFOA manufacturing facilities have examined the possible associations between PFOA exposure and increases in mortality from all causes and have not found associations (Gilliland and Mandel 1993; Leonard 2006; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012). Some increases in disease-specific mortality have been observed; these data are discussed in subsequent sections of this chapter (Sections 2.5, 2.8, 2.10, 2.18, and 2.19).

Laboratory Animal Studies. Limited data are available regarding death in animals following inhalation exposure to perfluoroalkyls. Exposure of male and female rats to 18,600 mg/m^3 ammonium perfluorooctanoate (APFO) dusts for 1 hour did not result in deaths during exposure or during a 14-day observation period (Griffith and Long 1980); APFO is the ammonium salt of PFOA. An LC_{50} of 980 mg/m^3 was reported in male CD rats exposed head-only to APFO dusts for 4 hours (Kennedy et al. 1986). Deaths occurred at all exposure levels (380–5,700 mg/m^3) and all deaths occurred within 48 hours of exposure. Rats dying during exposure had hyperinflated lungs. A similar LC_{50} value of 820 mg/m^3 was calculated for male CD rats exposed nose-only to APFO dusts for 4 hours (Kinney et al. 1989). Unlike the Kennedy et al. (1986) study, one death was observed at 590 mg/m^3 and no deaths occurred at 620 mg/m^3. In a developmental study with APFO, whole-body exposure of 12 pregnant rats to 25 mg/m^3, 6 hours/day during GDs 6–15 resulted in three deaths on GDs 12, 13, and 17 compared with no deaths in groups exposed to $\leq$10 mg/m^3 (Staples et al. 1984). The cause of death was not reported.

Oral LD_{50} values of 680 and 430 mg/kg were reported for male and female albino rats, respectively, administered single gavage doses of APFO and observed for 14 days (Griffith and Long 1980); all animals at the highest dose of 2,150 mg/kg died on day 1. Nonlethal signs observed included ptosis, piloerection, hypoactivity, decreased limb tone, ataxia, and corneal opacity. All signs were intermittent and there was no apparent dose-response relationship. In a 28-day dietary study with APFO in rats, all rats (males and females) in groups receiving approximately 1,000–1,130 mg/kg/day APFO died before the end of the first week (Griffith and Long 1980). In a similar study in mice, all mice receiving doses of approximately 180–195 mg/kg/day died before the second week of the study (Griffith and Long 1980). In this study, doses of approximately 54–58 mg/kg/day APFO were lethal to 4/5 male and 5/5 female mice before the 4^{th} week of the study.

In a 90-day gavage study, treatment of Rhesus monkeys with 100 mg/kg/day APFO by gavage resulted in the death of an unspecified number of animals (group size was 10/sex) on week 2 (Griffith and Long 1980). Doses of approximately 30 mg/kg/day were lethal to one male and two females during weeks 7–12. All animals that died in the 30 and 100 mg/kg/day groups had anorexia, emesis, black stool, pale face and gums, swollen face and eyes, hypoactivity, and prostration. Microscopic examination of tissues showed marked diffuse lipid depletion in the adrenals, slight to moderate hypocellularity of the bone marrow, moderate atrophy of the lymphoid follicles of the spleen, and moderate atrophy of the lymphoid follicles of the lymph nodes. No deaths occurred at 10 mg/kg/day. Deaths were also reported in intermediate-duration studies in Cynomolgus monkeys (Butenhoff et al. 2002). One monkey exposed to 30/20 mg/kg/day PFOA (12 days of exposure to 30 mg/kg/day, 10 days with no exposure, 23 weeks of exposure to 20 mg/kg/day) was sacrificed in moribund condition; the animal had a body weight loss of 12.5%, was notably hypoactive, and was cold to the touch (Butenhoff et al. 2002). The investigators noted that the death was likely due to the high toxicity of the 30 mg/kg/day dose. It is unclear if these deaths were compound-related; one monkey had pulmonary necrosis with a severe acute recurrence of pulmonary inflammation and the cause of morbidity for the second monkey was likely hyperkalemia. Neither effect was observed in the surviving animals.

The dermal LD_{50} values for APFO were 7,000 mg/kg in male CD rats and >7,500 mg/kg in female rats (Kennedy 1985). The protocol consisted of application of PFOA (as an aqueous paste) to a clipped area of the skin, which immediately was covered with gauze pads and wrapped with rubber sheeting around the trunk. The contact period was 24 hours, at which time the application site was washed with water and the rats were observed for clinical signs for 14 days. Using the same protocol, the dermal LD_{50} in male rabbits was 4,300 mg/kg (Kennedy 1985). Rabbits treated with 1,500 mg/kg showed skin irritation with

formation of a large crusty area at the application site. No deaths occurred at 1,500 mg/kg. Rabbits treated with 3,000 mg/kg were lethargic and a single death occurred 7 days after treatment. At 5,000 mg/kg, deaths occurred in 3–4 days. These rabbits also showed nasal discharge, pallor, diarrhea, weakness, severe weight loss, and severe skin irritation along with areas of necrosis.

PFOS

Epidemiological Studies. One occupational exposure study evaluated the potential of PFOS to increase lethality; the study did not find increases in deaths from all causes in workers at a PFOS manufacturing facility (Alexander et al. 2003). Alterations in disease-specific mortality are discussed in subsequent sections of this chapter.

Laboratory Animal Studies. Unpublished information summarized by the Organization for Economic Co-operation and Development (OECD) (2002) indicates that an LC_{50} of 5,200 mg/m^3 was calculated for PFOS in male and female Sprague-Dawley rats exposed to airborne concentrations of PFOS dusts from 1,890 to 45,970 mg/m^3 for 1 hour. All rats exposed to 24,090 mg/m^3 died by day 6.

Unpublished information summarized by OECD (2002) indicate that LD_{50} values of 233 and 271 mg/kg were calculated for male and female CD rats, respectively, following administration by gavage of single doses of up to 1,000 mg/kg of powdered PFOS suspended in an acetone/oil mixture and observed for 14 days. All rats (5/sex/dose group) dosed with ≥464 mg/kg PFOS died before the end of the study. The signs most frequently observed were hypoactivity, decreased limb tone, and ataxia. Gross necropsy showed stomach distension and signs of irritation of the glandular mucosa, and lung congestion. OECD (2002) also reported that a different study estimated that the acute oral LD_{50} for PFOS by gavage in water in Sherman-Wistar albino rats was >50 and <1,500 mg/kg. An oral LD_{50} value of 579 mg/kg/day was reported for male C57/BL/6 mice administered single gavage doses of PFOS and observed for 14 days (Xing et al. 2016). Mortality occurred within 3 hours of dosing, and moribund mice displayed signs of neurotoxicity (abdominal breathing, hind limb spasticity, tics, and urinary incontinence).

In a 26-week study, 2/6 male Cynomolgus monkeys administered 0.75 mg/kg/day PFOS via a capsule died or were sacrificed due to morbidity (Seacat et al. 2002). The cause of death in one monkey was pulmonary inflammation; the cause of morbidity in the second monkey was not determined, but the animal did have hyperkalemia.

PFNA

Laboratory Animal Studies. A LC_{50} of 820 mg/m^3 was identified in rats exposed to airborne PFNA for 4 hours (Kinney et al. 1989). In a 14-day dietary exposure study, all mice administered approximately 54 mg/kg/day PFNA died before the study period ended; no deaths occurred at 5.3 mg/kg/day (Kennedy 1987).

PFDA

Laboratory Animal Studies. An LD_{50} of 120 mg/kg was estimated for PFDA in female C57BL/6N mice administered single doses between 20 and 320 mg/kg/day PFDA by gavage in corn oil and observed for 30 days (Harris et al. 1989). All mice receiving 160 or 320 mg/kg were dead by 14 days; no mice died at ≤80 mg/kg PFDA. Early death was associated with mural thrombosis in the left ventricle of the heart. Without providing any details, George and Andersen (1986) reported that the 30-day oral LD_{50} for PFDA in male Fischer-344 rats was 57 mg/kg.

PFDoDA

Laboratory Animal Studies. Increases in mortality were observed in pregnant rats administered 2.5 mg/kg/day for 14 days prior to mating and throughout gestation; 4/12 dams between GD 18 and 22 and another 3 dams were sacrificed during the period due to morbidity (Kato et al. 2015). No deaths were observed in males or nonpregnant females exposed to 2.5 mg/kg/day (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. In a single exposure gavage study, deaths occurred in rats administered 1,750 or 5,000 mg/kg sodium perfluorohexanoate (NaPFHx) (Loveless et al. 2009). Decreased survival was observed in female Sprague-Dawley rats administered 200 mg/kg/day PFHxA via gavage in a 104-week study (Klaunig et al. 2015). There was no significant effect on survival rates of males. Mortality and morbidity were observed in male and female rats administered 450 mg/kg/day PFHxA via gavage for 4 days (Kirkpatrick 2005). The cause of death was determined to be renal papillary necrosis and/or stomach erosion/ulceration.

2.3 BODY WEIGHT

Overview. Epidemiological studies have examined the possible associations between *in utero* and/or early life exposure to perfluoroalkyls and body weight, body mass index (BMI; measure of body fat based on body weight and height), etc. Other studies have examined possible associations between serum perfluoroalkyl levels in older children or adults and body weight, adiposity markers, and the risk of being overweight or obese. The results of the epidemiological studies are summarized in Table 2-7, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 1. No epidemiological data were available for PFHpA, PFBS, PFBA, PFDoDA, or PFHxA. Animal studies have evaluated changes in body weight, including maternal body weight, in response to inhalation, oral, or dermal exposure to perfluoroalkyls; these data are summarized in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and Figures 2-6, 2-7, 2-8, 2-9, and 2-10. No laboratory animal studies examining body weight were identified for PFHpA.

Overall, the evidence from epidemiological studies does not suggest an association between *in utero* and/or early life exposure to perfluoroalkyls and alterations in growth (body weight or length), body composition (e.g., BMI), or the risk of being overweight or obese in children for PFOA, PFOS, PFHxS, or PFNA. Conclusions cannot be drawn for PFDA, PFUnA, PFDoDA, or FOSA because of the small number of studies (less than 5 studies for each compound) examining potential body weight endpoints. A small number of studies examined potential associations between PFOA and body weight effects in adults and only one study examined PFOS, PFHxS, PFNA, and PFDA associations; these data were considered inadequate for assessing potential associations in adults.

Studies in laboratory animals exposed to PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoDA, or PFHxA have consistently shown decreases in body weight or decreases in body weight gain. Studies with PFOA suggest that the decrease in body weight gain does not appear to be associated with alterations in food consumption and the mechanism may involve PPARα as studies in PPARα null have not found decreases in body weight gain. The small number of studies examining PFHxS, PFBS, PFBA, and FOSA have not reported decreases in body weight; although decreases in maternal body weight gain were observed for PFBS.

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Barry et al. 2014 Community (C8) (n=8,764 20–40-year-olds)	164.6 and 194.3 ng/mL (estimated early life [first 3 years] median PFOA)	Overweight or obesity at age 20–40 years	OR 0.9 (0.7–1.1), males OR 0.9 (0.7–1.1), females
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	2.43 ng/mL (maternal geometric mean serum PFOA)	Body weight at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body length at 1, 4, 6, and 12 months of age	NS (p>0.05)
Andersen et al. 2010 General population (n=1,010 infants)	5.21 ng/mL (maternal median PFOA)	Body weight (age 5 and 12 months)	**Inverse association (p<0.05)*, boys NS (p>0.05), girls**
		BMI (age 5 and 12 months)	**Inverse association (p<0.05)*, boys NS (p>0.05), girls**
		Height (age 5 and 12 months)	NS (p>0.05), boys NS (p>0.05), girls
Andersen et al. 2013 General population (n=811 children aged 7 years)	5.25 ng/mL (maternal median PFOA)	BMI	NS (p>0.05)
		Waist circumference	NS (p>0.05)
Braun et al. 2016a, 2016b General population (n=204 children)	5.3 ng/mL (maternal median PFOA)	Changes in BMI scores between 2 and 8 years of age	**Association (p=0.03)***
		Overweight/obesity risk	RR 1.54 (0.77–3.07), 3rd tertile
Cao et al. 2018 General population (n=337 infants)	1.59 ng/mL (mean cord serum PFOA)	Body weight at 19 months	NS (p=0.57)
		Length at 19 months	NS (p=0.16)
		Head circumference at 19 months	NS (p=0.94)
de Cock et al. 2014 General population (n=89 infants aged 1–11 months)	0.9402 ng/mL (cord blood mean PFOA)	Weight	NS (p=0.350)
		Height	NS (p=0.045)
		BMI	NS (p=0.813)
		Head circumference	NS (p=0.774)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Halldorsson et al. 2012 General population (n=665 20-year-olds)	3.7 and 5.8 ng/mL (maternal median PFOA and 4th quartile median)	BMI	**Association (p=0.001)*, females**
		Waist circumference	**Association (p=0.006)*, females**
		Overweight risk	**RR 3.1 (1.4–6.9)*, females 4th quartile**
		High waist circumference	**RR 3.0 (1.3–6.8)*, females 4th quartile**
Hartman et al. 2017 General population (n=359 girls aged 9 years)	3.7 ng/mL (maternal median PFOA)	Total body fatness	NS (p=0.20)
		Trunk fatness	NS (p=0.05)
		BMI	NS (p=0.05)
Høyer et al. 2015b General population (n=1,122 children aged 5–9 years; n=531 for Greenland cohort and n=491 for Ukraine cohort)	2.2–5.1 and 1.1–9.8 ng/mL (maternal 3rd tertile PFOA for Greenland and Ukraine cohorts)	Overweight Greenland cohort Ukraine cohort	 RR 1.23 (0.87–1.74), 3rd tertile RR 0.78 (0.47–1.29), 3rd tertile
		Waist-to-height ratio >0.5 Greenland cohort Ukraine cohort	 RR 1.18 (0.80–1.74), 3rd tertile RR 1.11 (0.48–2.57), 3rd tertile
Karlsen et al. 2017 General population (n=444 children)	1.37 ng/mL (maternal geometric mean serum PFOA)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.14 (0.92–1.4)
		Risk of being overweight 5 years	**RR 1.50 (1.01–2.24, p<0.05)***
Karlsen et al. 2017 General population (n=444 children aged 5 years)	2.22 ng/mL (child geometric mean serum PFOA)	BMI score	**Inverse association (p<0.05)***
		Risk of being overweight	RR 0.68 (0.38–1.22)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group)	Risk of being overweight	OR 0.98 (0.90–1.13)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	4.5 ng/mL (median serum PFOA)	Weight loss	NS (p=0.73, trend)
		Weight regain Males Females	NS (p=0.16, trend) NS (p=0.78, trend) **Association (p=0.007, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 NS (p=0.48, trend) **Association (p=0.03, trend)***
Manzano-Salgado et al. 2017b General population (n=1,230 children)	2.32 ng/mL (maternal geometric mean serum PFOA)	Weight gain until 6 months of age	β 0.04 (-0.04–0.12)
		BMI at 4 years of age	β 0.04 (-0.04–0.13)
		BMI at 7 years of age	β 0.03 (-0.08–0.13)
		Waist circumference at 4 years of age	β 0.00 (-0.09–0.10)
		Waist circumference at 7 years of age	β -0.02 (-0.11–0.06)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	5.6 ng/mL (maternal median plasma PFOA in early childhood group)	BMI	β 0.09 (-0.02–0.19),
		Waist circumference	**β 0.31 (0.04–0.57)*, boys and girls** **β 0.50 (0.06–0.93)*, boys only** β 0.14 (-0.18–0.47), girls only
		Risk of being overweight	RRR 1.05 (0.87–1.26)
		Risk of being obese	RRR 1.03 (0.80–1.32)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	5.6 ng/mL (maternal median plasma PFOA in late childhood group)	BMI	β 0.13 (-0.10–0.35)
		Total fat mass index	β 0.13 (0.02–0.29)
		Waist circumference	β 0.20 (-0.39–0.80)
		Risk of being overweight	RRR 1.02 (0.88–1.29)
		Risk of being obese	RRR 1.10 (0.88–1.37)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Timmermann et al. 2014 General population (n=499 8–10-year-old children)	9.3 ng/mL (mean PFOA)	Adiposity markers	NS (p>0.05), per 10 ng/mL PFOA increase
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	2.37 and 2.34 ng/mL (median maternal PFOA for boys and girls)	Growth during childhood	NS (p>0.05)
PFOS			
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	9.04 ng/mL (maternal geometric mean serum PFOS)	Body weight at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body length at 1, 4, 6, and 12 months of age	NS (p>0.05)
Andersen et al. 2010 General population (n=1,010 infants)	33.8 ng/mL (maternal median PFOS)	Body weight (age 5 months)	NS (p>0.05), boys NS (p>0.05), girls
		Body weight (age 12 months)	**Inverse association (p<0.05)*, boys** NS (p>0.05), girls
		BMI (age 5 months)	NS, boys NS (p>0.05), girls
		BMI (age 12 months)	**Inverse association (p<0.05)*, boys** NS (p>0.05), girls
		Height (age 5 and 12 months)	NS (p>0.05), boys NS (p>0.05), girls
Andersen et al. 2013 General population (n=811 children aged 7 years)	33.8 ng/mL (maternal median PFOS)	BMI	NS (p>0.05)
		Waist circumference	NS (p>0.05)
Braun et al. 2016a, 2016b General population (n=204 children)	13 ng/mL (maternal median PFOS)	Changes in BMI scores between 2 and 8 years of age	NS (p>0.23)
		Overweight/obesity risk	RR 1.08 (0.59–1.95), 3rd tertile

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Cao et al. 2018 General population (n=337 infants)	1.43 ng/mL (mean cord serum PFOS)	Body weight at 19 months	NS (p=0.72)
		Length at 19 months	NS (p=0.91)
		Head circumference at 19 months	NS (p=0.63)
Halldorsson et al. 2012 General population (n=665 20-year-olds)	21.5 and 5.8 ng/mL (maternal median PFOS)	BMI	NS (p>0.56)
		Waist circumference	NS (p>0.56)
Høyer et al. 2015b General population (n=1,122 children aged 5–9 years; n=531 for Greenland cohort and n=491 for Ukraine cohort)	23.9–87.3 and 5.9–18.1 ng/mL (maternal 3rd tertile PFOS for Greenland and Ukraine cohorts)	Overweight Greenland cohort Ukraine cohort	 RR 0.84 (0.61–1.14), 3rd tertile RR 0.89 (0.57–1.37), 3rd tertile
		Waist-to-height ratio >0.5 Greenland cohort Ukraine cohort	 RR 1.22 (0.86–1.74), 3rd tertile RR 1.44 (0.62–3.31), 3rd tertile
Hartman et al. 2017 General population (n=359 girls aged 9 years)	19.7 ng/mL (maternal median PFOS)	Total body fatness	NS (p=0.12)
		Trunk fatness	**Inverse association (p=0.02)**
		BMI	**Inverse association (p=0.03)***
Karlsen et al. 2017 General population (n=444 children)	8.04 ng/mL (maternal geometric mean serum PFOS)	BMI score, 18 months	**Association (p<0.05)**
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	**RR 1.29 (1.01–1.64)***
		Risk of being overweight 5 years	RR 1.01 (0.58–1.75)
Karlsen et al. 2017 General population (n=444 children aged 5 years)	4.68 ng/mL (child geometric mean serum PFOS)	BMI score	NS (p>0.05)
		Risk of being overweight	RR 0.68 (0.36–1.29)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group)	Risk of being overweight	OR 1.00 (0.90–1.07)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	24.5 ng/mL (median serum PFOS)	Weight loss	NS (p=0.27, trend).
		Weight regain Males Females	**Association (p=0.009, trend)*** NS (p=0.34, trend) **Association (p=0.001, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p<0.001, trend)*** **Association (p<0.001, trend)***
Maisonet et al. 2012 General population (n=447 girls)	19.6 ng/mL (median maternal PFOS)	Body weight at 20 months (adjusted for birth weight)	**Significant trend (p<0.0001) when adjusted for birth weight and height**
Manzano-Salgado et al. 2017b General population (n=1,230 children)	5.80 ng/mL (maternal geometric mean serum PFOS)	Weight gain until 6 months of age	β -0.02 (-0.11–0.07)
		BMI at 4 years of age	β 0.04 (-0.05–0.13)
		BMI at 7 years of age	β 0.03 (-0.08–0.14)
		Waist circumference at 4 years of age	β -0.03 (-0.13–0.07)
		Waist circumference at 7 years of age	β 0.00 (-0.09–0.09)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	24.8 ng/mL (maternal median plasma PFOS in early childhood group)	BMI	**β 0.04 (0.05–0.12)*, boys and girls** β 0.02 (-0.11–0.15), boys only β 0.04 (-0.08–0.16), girls only
		Waist circumference	β 0.05 (-0.17–0.27)
		Risk of being overweight	RRR 1.07 (0.92–1.24)
		Risk of being obese	RRR 0.97 (0.76–1.23)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	24.7 ng/mL (maternal median plasma PFOS in late childhood group)	BMI	β 0.16 (-0.04–0.36)
		Total fat mass index	β 0.11 (-0.03–0.25)
		Waist circumference	β 0.34 (-0.19–0.87),
		Risk of being overweight	RRR 1.15 (0.95–1.40)
		Risk of being obese	RRR 1.12 (0.99–1.47)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Timmermann et al. 2014 General population (n=499 8–10-year-old children)	41.5 ng/mL (mean PFOS)	Adiposity markers	NS (p>0.05), per 10 ng/mL PFOS increase
PFHxS			
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	0.62 ng/mL (maternal geometric mean serum PFHxS)	Body weight at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body weight (longitudinal analysis)	**β -5.270 (-9.591 to -0.950)***
		Body length at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body length (longitudinal analysis)	**β 4.516 (1.368–7.664)***
Braun et al. 2016a, 2016b General population (n=204 children)	1.4 ng/mL (maternal median PFHxS)	Changes in BMI scores between 2 and 8 years of age	NS (p>0.23)
		Overweight/obesity risk	RR 1.48 (0.75–2.96), 3rd tertile
Cao et al. 2018 General population (n=337 infants)	0.16 ng/mL (mean cord serum PFHxS)	Body weight at 19 months	NS (p=0.96)
		Length at 19 months	NS (p=0.31)
Hartman et al. 2017 General population (n=359 girls aged 9 years)	1.6 ng/mL (maternal median PFHxS)	Total body fatness	NS (p=0.47)
		Trunk fatness	NS (p=0.77)
		BMI	NS (p=0.37)
Karlsen et al. 2017 General population (n=444 children)	0.19 ng/mL (maternal geometric mean serum PFHxS)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.12 (0.97–1.30)
		Risk of being overweight 5 years	RR 1.11 (0.77–1.59)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karlsen et al. 2017 General population (n=444 children aged 5 years)	0.34 ng/mL (child geometric mean serum PFHxS)	BMI score	NS (p>0.05)
		Risk of being overweight	RR 0.73 (0.44–1.23)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group)	Risk of being overweight	OR 1.04 (0.97–1.11)
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	3.6 ng/mL (median serum PFHxS)	Weight loss	NS (p=0.45, trend).
		Weight regain Males Females	NS (p=0.49, trend**)** NS (p=0.17 trend) **Association (p=0.009, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p=0.04, trend)*** **Association (p=0.02, trend)***
Maisonet et al. 2012 General population (n=447 girls)	1.6 ng/mL (maternal median PFHxS)	Body weight at 20 months	NS (p=0.4375 for trend)
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.61 ng/mL (maternal geometric mean serum PFHxS)	Weight gain until 6 months of age	β -0.06 (-0.15–0.02)
		BMI at 4 years of age	β -0.02 (-0.10–0.07)
		BMI at 7 years of age	β -0.04 (-0.14–0.06)
		Waist circumference at 4 years of age	B -0.04 (0.14–0.15)
		Waist circumference at 7 years of age	NS β -0.04 (-0.12–0.04)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	2.4 ng/mL (maternal median plasma PFHxS in early childhood group)	BMI	β 0.01 (-0.05–0.06)
		Waist circumference	β 0.03 (-0.10–0.16)
		Subscapular and triceps skinfold thickness	**β 0.16 (0.01–0.31)*, boys and girls** β 0.15 (-0.09–0.38), boys only β 0.18 (-0.03–0.38, girls only
		Risk of being overweight	RRR 1.03 (0.94–1.13)
		Risk of being obese	RRR 1.02 (0.89–1.17)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	2.3 ng/mL (maternal median plasma PFHxS in late childhood group)	BMI	β 0.04 (-0.08–0.17)
		Total fat mass index	β 0.11 (-0.03–0.25)
		Waist circumference	β 0.11 (-0.22–0.43)
		Subscapular to triceps skinfold thickness ratio	β 0.02 (-0.02–0.06), boys and girls β -0.50 (-1.70–0.71), boys only **β 1.61 (0.58–2.65)*, girls only**
		Risk of being overweight	RRR 1.04 (0.92–1.17)
		Risk of being obese	RRR 1.07 (0.94–1.22)
PFNA			
Braun et al. 2016a, 2016b General population (n=204 children)	0.9 ng/mL (maternal median PFNA)	Changes in BMI scores between 2 and 8 years of age	NS (p>0.23)
		Overweight/obesity risk	RR 1.26 (0.64–2.48), 3rd tertile
Cao et al. 2018 General population (n=337 infants)	0.13 ng/mL (mean cord serum PFNA)	Body weight at 19 months	NS (p=0.88)
		Length at 19 months	NS (p=0.15)
		Head circumference at 19 months	NS (p=0.62)
Halldorsson et al. 2012 General population (n=665 20-year-olds)	0.3 ng/mL (maternal median PFNA)	BMI	NS (p>0.56)
		Waist circumference	NS (p>0.56)
Hartman et al. 2017 General population (n=359 girls aged 9 years)	0.5 ng/mL (maternal median PFNA)	Total body fatness	NS (p=0.26)
		Trunk fatness	NS (p=0.97)
		BMI	NS (p=0.68)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karlsen et al. 2017 General population (n=444 children)	0.67 ng/mL (maternal geometric mean serum PFNA)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.02 (0.79–1.31)
		Risk of being overweight 5 years	RR 1.15 (0.67–1.98)
Karlsen et al. 2017 General population (n=444 children aged 5 years)	1.12 ng/mL (child geometric mean serum PFNA)	BMI score	**Inverse association (p<0.05)***
		Risk of being overweight	RR 0.67 (0.45–1.00)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group)	Risk of being overweight	OR 1.01 (0.92–1.13)
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	1.5 ng/mL (median serum PFNA)	Weight loss	NS (p=0.28, trend).
		Weight regain Males Females	**Association (p=0.01, trend)*** NS (p=0.48 trend) **Association (p=0.006, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p<0.001, trend)*** **Association (p=0.03, trend)***
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.61 ng/mL (maternal geometric mean serum PFNA)	Weight gain until 6 months of age	β 0.0 (-0.07–0.09)
		BMI at 4 years of age	β 0.05 (-0.03–0.13)
		BMI at 7 years of age	β 0.06 (-0.04–0.16)
		Waist circumference at 4 years of age	β 0.02 (-0.07–0.10)
		Waist circumference at 7 years of age	β -0.02 (-0.07–0.10)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	0.6 ng/mL (maternal median plasma PFNA in early childhood group)	BMI	β 0.02 (-0.07–0.12)
		Waist circumference	β 0.01 (-0.23–0.22),
		Risk of being overweight	RRR 1.12 (0.96–1.30)
		Risk of being obese	RRR 0.97 (0.75–1.27)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	0.6 ng/mL (maternal median plasma PFNA in late childhood group)	BMI	β 0.17 (-0.03–0.36)
		Total fat mass index	β 0.08 (-0.07–0.23)
		Waist circumference	β 0.31 (-0.19–0.82)
		Subscapular and triceps skinfold thickness	**β 0.62 (0.01–1.22)*, boys and girls** β 0.13 (0.74–1.01), boys only **β 1.01 (0.16–1.86)*, girls only**
		Subscapular to triceps skinfold thickness ratio	**β 1.78 (0.57–2.98)*, boys and girls** β 1.23 (-0.58–3.03), boys only **β 2.17 (0.52–3.83)*, girls only**
		Risk of being overweight	RRR 1.06 (0.85–1.32)
		Risk of being obese	RRR 1.21 (0.99–1.47)
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	1.55 and 1.58 ng/mL (median maternal PFNA for boys and girls)	Growth during childhood	NS (p>0.05)
PFDA			
Cao et al. 2018 General population (n=337 infants)	0.12 ng/mL (mean cord serum PFDA)	Body weight at 19 months	NS (p=0.57)
		Length at 19 months	NS (p=0.18)
		Head circumference at 19 months	NS (p=0.94)
Karlsen et al. 2017 General population (n=444 children)	0.26 ng/mL (maternal geometric mean serum PFDA)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.14 (0.91–1.43)
		Risk of being overweight 5 years	RR 1.02 (0.61–1.70)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karlsen et al. 2017 General population (n=444 children aged 5 years)	0.33 ng/mL (child geometric mean serum PFDA)	BMI score	**Inverse association (p<0.05)***
		Risk of being overweight	**RR 0.64 (0.46–0.90)***
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group)	Risk of being overweight	OR 0.98 (0.93–1.03)
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	0.37 ng/mL (median serum PFDA)	Weight loss	NS (p=0.45, trend).
		Weight regain Males Females	NS (p=0.16, trend) NS (p=0.75 trend) **Association (p=0.03, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p=0.01, trend)*** **Association (p=0.05, trend)***
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.46 and 0.43 ng/mL (median maternal PFDA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
PFUnA			
Cao et al. 2018 General population (n=337 infants)	0.06–0.11 ng/mL (2nd quartile cord serum PFUnA)	Body weight at 19 months	NS (p=0.88)
		Length at 19 months	**β 1.19 (-0.68–3.07, p<0.05)*, 2nd quartile**
		Head circumference at 19 months	NS (p=0.60)
Koshy et al. 2017 General population (World Trade Center Health Registry, n=180 children; n=222 children in comparison group)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCH group and comparison group)	Risk of being overweight	**OR 0.95 (0.91–0.99)***

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	3.52 and 3.31 ng/mL (median maternal PFUnA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
PFDoDA			
Cao et al. 2018 General population (n=337 infants)	0.04 ng/mL (mean cord serum PFDoDA)	Body weight at 19 months	NS (p=0.74)
		Head circumference at 19 months	NS (p=0.97)
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.37 and 0.37 ng/mL (median maternal PFDoDA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
FOSA			
Halldorsson et al. 2012 General population (n=665 20-year-olds)	1.1 ng/mL (maternal median FOSA)	BMI	NS (p>0.56)
		Waist circumference	NS (p>0.56)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 1 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BMI = body mass index; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR = relative risk; WTCHR = World Trade Center Health Registry

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PFOA

Epidemiological Studies. Mixed results were found in studies of monitoring infant growth from 1 to 12 months of age. Andersen et al. (2010) found an inverse association between maternal serum PFOA and body weight and BMI in male infants at 5 and 12 months of age; no associations were found in girls. Other studies of infants less than 19 months of age did not find associations between maternal serum PFOA (Alkhalawi et al. 2016; Manzano-Salgado et al. 2017b) or cord blood PFOA (Cao et al. 2018; de Cock et al. 2014) levels and weight, length, head circumference, or BMI. One study of children (Braun et al. 2016a) found an association between changes in BMI scores between ages 2 and 8 years and maternal PFOA levels; however, there was no increase in the risk of being overweight or obese. Another study of young children (median age 3.2 years) found an association between maternal PFOA and waist circumference (Mora et al. 2017); when the children were segregated by sex, the association was only found in boys. This study did not find associations between maternal PFOA and waist circumference when the children were older (median age 7.7 years). Other studies in children (2–11 years of age) found no associations between maternal PFOA or cord blood PFOA and growth during childhood (Wang et al. 2016), risk of being overweight or obese (Andersen et al. 2013; Braun et al. 2016a; Høyer et al. 2015b; Mora et al. 2017), waist circumference (Manzano-Salgado et al. 2017b), BMI (Hartman et al. 2017; Karlsen et al. 2017; Manzano-Salgado et al. 2017b; Mora et al. 2017), body fatness (Hartman et al. 2017), or risk of having a waist-to-height ratio of >0.5 (Høyer et al. 2015b). In a study of children aged 8–10 years, no associations were found between plasma PFOA levels and markers of adiposity (BMI, skinfold thickness, waist circumference, adiponectin levels, and leptin levels) (Timmermann et al. 2014). Similarly, in a study of children in the World Trade Center Health Registry, no association was found between serum PFOA and risk of being overweight (Koshy et al. 2017). In contrast, a study of 5-year-old children found an inverse association between the child's serum PFOA levels and BMI score, but no association with the risk of being overweight (Karlsen et al. 2017). Overall, the available epidemiological data do not suggest a connection between serum PFOA levels and body weight or risk of being overweight/obese in children.

Two studies in adults have not found associations between PFOA and body weight gain. A general population study of 20-year-old females found associations between maternal PFOA levels and BMI and waist circumferences, and increases in the risk of being overweight and having a high waist circumference (Halldorsson et al. 2012); these associations were not observed in males. No increases in the risk of being overweight or obese were observed in male or female C8 participants (20–40 years of age) when estimated early life PFOA exposure was used as the exposure metric (Barry et al. 2014). In a

study of participants in a weight loss study, no association between weight loss and PFOA levels was found; however, PFOA was associated with weight gain in females, but not males (Liu et al. 2018a). PFOA was also associated with a lower increase in resting metabolic rate in all participants during the weight regain period of the study.

Laboratory Animal Studies. Male rats that survived a 4-hour inhalation exposure to 380 mg/m^3 APFO dusts lost weight for 1–2 days after exposure, but resumed normal weight gain thereafter (Kennedy et al. 1986). Male rats exposed via inhalation intermittently to 84 mg/m^3 APFO dusts for 2 weeks lost approximately 7% of their body weight by day 5 of exposure (250 g at start of study, 237 g on day 5) (Kennedy et al. 1986), but recovered by day 16 after exposure ceased. Nose-only exposure of male CD rats to 590 mg/m^3 ammonium perfluorononanoate dusts for 4 hours resulted in 18 and 36% reductions in body weight 5 and 12 days after exposure, respectively (Kinney et al. 1989). Inhalation exposure to 67 mg/m^3 had no significant effect on body weight. In a developmental study, inhalation exposure of pregnant rats to 25 mg/m^3 APFO dusts during GDs 6–15 induced a 37% reduction in maternal body weight gain relative to controls during the exposure period (Staples et al. 1984); in a pair-fed group, the reduction of weight gain during the same period was 61% relative to *ad libitum* controls.

Reductions in body weight or body weight gain are typical, although not particularly sensitive, responses of rodents to oral exposure to perfluoroalkyls. In many cases, this effect is not associated with reduced food intake, and in some cases, exposed animals have shown an increase in relative food consumption (grams of food/grams of body weight) relative to controls. For example, administration of 50 mg/kg/day APFO for 7 days resulted in 17% weight loss; a similar decrease was observed in a pair-fed group (Pastoor et al. 1987). In mice, doses of approximately 25–30 mg/kg/day PFOA in the food for 7 days reduced terminal body weight by >10% relative to controls without a significant reduction in food intake (Xie et al. 2003; Yang et al. 2000, 2002a, 2002b). However, administration of the same dose to PPARα-null mice did not cause a reduction in weight gain, suggesting that the effect on body weight is a specific effect of peroxisome proliferators possibly due to increased fat utilization (Yang et al. 2002b). In general, body weight recovered once treatment ceased.

Intermediate-duration oral studies in rats have also reported reduced body weight gain with doses ≥10 mg/kg/day APFO (Butenhoff et al. 2004b; Griffith and Long 1980). In the former study, mean absolute food consumption was decreased, but mean relative food consumption was increased. In a 2-year bioassay, body weight gain in rats dosed with 15 mg/kg/day PFOA was reduced >10% relative to controls at the 1-year mark and at termination (Biegel et al. 2001). Similar observations have been made

in mice dosed with approximately ≥18 mg/kg/day APFO for 28 days (Griffith and Long 1980), or 10 mg/kg 5 days/week for 4 weeks (Yang et al. 2009), and in pregnant mice dosed with ≥10 mg/kg/day APFO during GDs 1–17 (Lau et al. 2006). A study comparing wild-type mice and PPARα knockout mice (DeWitt et al. 2016) found a decrease in body weight gain in the wild-type mice, but not in the knockout mice. A 90-day and a 26-week study in monkeys also reported significant reductions in body weight gain or weight loss associated with decreased food consumption at dose levels in the range of 20–30 mg/kg/day APFO (Butenhoff et al. 2002; Griffith and Long 1980), but a 4-week study in monkeys dosed with 20 mg/kg/day PFOA did not (Thomford 2001).

Transient weight loss was reported in rats applied 3,000 mg/kg APFO to the shaven skin for 24 hours (Kennedy 1985). In the 2-week study, rats in the 200 and 2,000 mg/kg/day groups lost weight during the treatment period (14 and 24%, respectively, on test day 10), but body weights were comparable to controls after 42 days of recovery. No changes in body weight were reported in mice applied up to 50 mg/kg/day PFOA daily for 4 days on the dorsal surface of the ears (Fairley et al. 2007).

PFOS

Epidemiological Studies. General population studies have evaluated body weight, height, and BMI in infants, children, and adults to assess whether there were associations between growth and maternal serum PFOS levels. Andersen et al. (2010) found that maternal PFOS levels were inversely related to body weight and BMI in 12-month-old male infants; no associations were found in females at 12 months of age or in males and females at 5 months of age. The magnitude of the effect on body weight in the boys was small, 9 g per 1 ng/mL increase in maternal serum PFOS level. Other studies have not found associations between maternal PFOS or cord blood PFOS and body weight, length, or head circumference in infants <2 years of age (Alkhalawi et al. 2016; Cao et al. 2018; Manzano-Salgado et al. 2017b). Hartman et al. (2017) also found an inverse association between maternal serum PFOS and trunk body fatness in 9-year-old girls, but no associations with total body fatness or BMI. Karlsen et al. (2017) found associations between maternal PFOS levels and BMI and risk of being overweight at 18 months of age, but not at 5 years of age. Maisonet et al. (2012) found that at 20 months of age, girls whose mothers had serum PFOS levels in the 3rd tertile weighed 438 g more than those in the first tertile. Studies in children (Andersen et al. 2013; Braun et al. 2016a; Høyer et al. 2015b; Koshy et al. 2017; Manzano-Salgado et al. 2017b; Mora et al. 2017) or young adults (Halldorsson et al. 2012) did not find associations between maternal PFOS levels and BMI, waist circumference, and/or risk of being overweight. No associations between plasma PFOS and markers of adiposity (BMI, skinfold thickness, waist circumference,

adiponectin levels, and leptin levels) were found in a study of children aged 8–10 years (Timmermann et al. 2014). Similarly, a study of 5-year-old children found no association between child serum PFOS levels and BMI score or risk of being overweight (Karlsen et al. 2017). Overall, the epidemiological studies do not suggest a connection between serum PFOS and body weight or the risk of being overweight/obese.

In a study of weight loss programs, PFOS did not influence weight loss, but was associated with greater weight regain in women (Liu et al. 2018a). PFOS was also associated with greater declines in resting metabolic rate in all participants during the weight loss period of the study and lower increases in resting metabolic rate during the weight regain period.

Laboratory Animal Studies. Dietary treatment of rats with 15 mg/kg/day PFOS (only dose level tested) for 7 days did not significantly alter body weight (Haughom and Spydevold 1992). Oral treatment of pregnant rats with 25 mg/kg/day PFOS on GDs 2–5 or 6–9 resulted in maternal weight loss during treatment, whereas treatment on GDs 10–13, 14–17, or 17–20 resulted in significant reductions in maternal weight gain (Grasty et al. 2003). In pregnant mice, oral dosing with up to 6 mg/kg/day PFOS on GDs 6–18 or 12–18 did not significantly affect body weight (Fuentes et al. 2006, 2007b). Decreases in maternal body weight were observed in rats administered 20 mg/kg/day on GDs 12–18 (Li et al. 2016). Pregnant rabbits appeared to be more sensitive as oral doses of 1 mg/kg/day on GDs 6–20 caused a 21% reduction in weight gain during treatment without altering food consumption (Case et al. 2001).

Alterations in body weight have also been observed following intermediate- or chronic-duration exposure. Reductions in body weight gain of >10% have been reported in intermediate-duration studies in rats dosed with ≥2 mg/kg/day PFOS associated with reductions in mean absolute and relative food consumption (Luebker et al. 2005a, 2005b). In a developmental toxicity study, treatment of pregnant rats with ≥2 mg/kg/day PFOS on GDs 2–20 resulted in significant reductions in body weight gain, which were associated with significant reductions in mean absolute food and water consumption (Thibodeaux et al. 2003). In a 4-week study, treatment of Cynomolgus monkeys with up to 2 mg/kg/day, administered via a capsule, did not affect body weight gain (Thomford 2002a). In a 26-week study in Cynomolgus monkeys, the highest dose of PFOS tested, 0.75 mg/kg/day, produced a 13.5% reduction in final body weight, at which time the mean concentration of PFOS in serum was 172 µg/mL (Seacat et al. 2002). In a 2-year dietary study in rats, final mean body weight of females that received doses of approximately 1.04 mg/kg/day PFOS was 14% lower than controls; this could have been due, in part, to a tendency of decreased food consumption during weeks 28 through 104 of the study (Butenhoff et al. 2012b;

Thomford 2002b). No significant effect (<10% difference with controls) was seen in females dosed with ≤0.25 mg/kg/day PFOS.

PFHxS

Epidemiological Studies. Nine studies have evaluated the influence of *in utero* PFHxS exposure on childhood growth and found no associations between maternal PFHxS levels and body weight in infants <2 years of age (Alkhalawi et al. 2016; Cao et al. 2018; Maisonet et al. 2012; Manzano-Salgado et al. 2017b), body fatness or BMI at 9 years of age (Hartman et al. 2017), BMI or waist circumference at 3 or 7 years of age (Mora et al. 2017), changes in BMI scores between 2 and 8 years of age (Braun et al. 2016a), BMI at 18 months or 5 years of age (Karlsen et al. 2017), BMI at 4 or 7 years of age (Manzano-Salgado et al. 2017b), or the risk of childhood overweight/obesity (Braun et al. 2016a; Karlsen et al. 2017; Mora et al. 2017). Similarly, no associations were found between serum PFHxS levels in 5-year-old children and their BMI score or risk of being overweight (Karlsen et al. 2017) or between serum PFHxS and risk of being overweight in children in the World Trade Center Healthy Registry (Koshy et al. 2017). Alkhalawi et al. (2016) found no associations between maternal PFHxS levels and infant body weight or length at 1, 4, 6, or 12 months of age; however, longitudinal analysis of growth during this period showed an inverse association for body weight and an association for length.

In a clinical trial of weight loss programs, PFHxS was not associated with weight loss during the first 6 months of the study, but was associated with weight regain in females during the last 18 months of the study (Liu et al. 2018a). PFHxS was also associated with greater declines in resting metabolic rate in all participants during the weight loss period and lower increases in resting metabolic rate during the weight regain period.

Laboratory Animal Studies. Administration of PFHxS by gavage for 40–60 days did not significantly affect body weight in rats at ≤10 mg/kg/day PFHxS (Butenhoff et al. 2009a) or mice at ≤3 mg/kg/day (Chang et al. 2018); the mean terminal body weights were within 10% of the body weight of the control group (Butenhoff et al. 2009a).

PFNA

Epidemiological Studies. Several studies have examined the influence of maternal serum PFNA levels on childhood growth. These studies did not find associations between maternal PFNA levels and growth

during childhood (Cao et al. 2018; Wang et al. 2016), BMI (Braun et al. 2016a; Halldorsson et al. 2012; Hartman et al. 2017; Karlsen et al. 2017; Manzano-Salgado et al. 2017b; Mora et al. 2017), body fatness (Hartman et al. 2017), or overweight/obesity risk (Braun et al. 2016a; Karlsen et al. 2017; Mora et al. 2017). However, when the child's serum PFNA levels at age 5 years were used as the exposure biometric, an inverse association was found for BMI, but not for the risk of being overweight (Karlsen et al. 2017). Koshy et al. (2017) found no associations between serum PFNA and the risk of being overweight in children enrolled in the World Trade Center Health Registry.

PFNA was associated with greater weight regains in a study of participants in a 2-year weight loss clinical trial, but was not associated with weight loss during the first 6 months of the study (Liu et al. 2018b). PFNA also affected resting metabolic rate in all participants; it was associated with a greater decline during the weight loss period of the study and a lower increase during the weight regain period.

Laboratory Animal Studies. Decreases in body weight gain have been observed in rats administered ≥3 mg/kg/day for 14 days (Fang et al. 2009, 2010; Hadrup et al. 2016) and in mice administered 5 mg/kg/day for 14 days (Wang et al. 2015a). The NOAEL for body weight effects was 1 mg/kg/day for both species. In intermediate-duration developmental toxicity studies, decreases in body weight were observed at 5 mg/kg/day in rats (Rogers et al. 2014) and weight loss was observed in mice at 10 mg/kg/day (Das et al. 2015). No alterations in maternal weight gain were observed in mice at 2.0 mg/kg/day (Wolf et al. 2010).

PFDA

Epidemiological Studies. Four studies examined the effect of PFDA levels on childhood growth. Cao et al. (2018) did not find associations between cord blood PFDA and body weight, length, or head circumference in 19-month-old infants. Wang et al. (2016) reported decreases in weight and height in girls associated with increasing maternal serum PFDA levels. Inverse associations between serum PFDA levels in 5-year-old children and BMI and the risk of being overweight were reported by Karlsen et al. (2017). When using maternal serum PFDA levels (measured 2 weeks after childbirth) as the biomarker of exposure, no associations were found with BMI or the risk of being overweight in children aged 18 months or 5 years (Karlsen et al. 2017). In a study of children in the World Trade Center Health Registry, no association between serum PFDA and risk of being overweight was found (Koshy et al. 2017).

In a study of adult participants in a 2-year weight loss clinical trial, PFDA was not associated with weight loss but was associated with weight regain in females during the last 18 months of the study (Liu et al. 2018a). PFDA also was associated with greater declines in resting metabolic rate during the weight loss period and lower increases in resting metabolic rate during the weight regain period of the study.

Laboratory Animal Studies. Ten days following administration of a single gavage dose of 50 mg/kg, weight loss was observed in rats (Kawabata et al. 2017). In a 1-week study, exposure to 9.5 mg/kg/day PFDA in the diet resulted in a 32% weight loss in rats (Kawashima et al. 1995); the NOAEL was 4.7 mg/kg/day. Rats administered 1 mg/kg/day PFDA for 28 days exhibited a 21% decrease in body weight gain (Frawley et al. 2018).

Body weight of female C57BL/6N mice administered a single gavage dose of 80 mg/kg PFDA was reduced 12% relative to controls 30 days post dosing (Harris et al. 1989); no significant effect was seen at 40 mg/kg PFDA. In a developmental study, pregnant mice dosed with 6.4 mg/kg/day PFDA on GDs 6–15 gained 92% less weight (adjusted for the weight of the gravid uterus) on GDs 6–18 than controls; mice dosed with 12.8 mg/kg/day lost weight (Harris and Birnbaum 1989). Weight loss was also observed in C57BL/6N mice exposed to 78 mg/kg/day PFDA in the diet for 10 days (Permadi et al. 1992, 1993).

PFUnA

Epidemiological Studies. Cao et al. (2018) found an association between cord blood PFUnA levels and length at 19 months of age, but found no associations for body weight or head circumference. Wang et al. (2016) found an inverse association between maternal serum PFUnA levels and weight and height in girls. Koshy et al. (2017) also found an inverse association between the serum PFUnA levels and the risk of being overweight in children enrolled in the World Trade Center Health Registry.

Laboratory Animal Studies. Decreases in body weight gain (10% in males and 23% in females) were observed in rats exposed to 1.0 mg/kg/day in a 41–46-day developmental toxicity study (Takahashi et al. 2014).

PFBS

Laboratory Animal Studies. No significant alterations in body weight gain were observed in Sprague-Dawley rats administered ≤900 mg/kg/day PFBS via gavage for 28 days (3M 2001) or in Sprague-

Dawley rats administered ≤1,000 mg/kg/day PFBS via gavage for at least 70 days (Lieder et al. 2009b). Two studies did report decreases in maternal body weight gain in rats administered 1,000 or 2,000 mg/kg/day (York 2002, 2003a).

PFBA

Laboratory Animal Studies. Alterations in body weight do not appear to be a sensitive outcome of PFBA exposure in rats or mice. No alterations in body weight gain were observed in Sprague-Dawley rats administered 184 mg/kg/day PFBA via gavage for 5 days (3M 2007a), C57BL/6 mice exposed to 78 mg/kg/day PFBA in the diet for 10 days (Permadi et al. 1992, 1993), Sprague-Dawley rats administered 150 mg/kg/day PFBA via gavage for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or Sprague-Dawley rats administered 30 mg/kg/day PFBA via gavage for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b).

PFDoDA

Epidemiological Studies. Cao et al. (2018) found no association between cord blood PFDoDA and body weight or head circumference at 19 months of age. In contrast, Wang et al. (2016) found an inverse association between maternal serum PFDoDA levels and growth (weight and height) in girls.

Laboratory Animal Studies. Dosing of Sprague-Dawley rats with 5 mg/kg/day PFDoDA by gavage for 14 days resulted in a 25% reduction in final body weight relative to a control group or 7% loss of body weight compared with the starting body weight (Shi et al. 2007). Decreases in body weight gain (measured 10 days postexposure) were also observed in rats administered a single gavage dose of 50 mg/kg PFDoDA (Kawabata et al. 2017). Gavage administration of 2.5 mg/kg/day for 42 days resulted in approximately 30% decreases in male rats; the decreases in body weight gain persisted during a 14-day recovery period (Kato et al. 2015). An approximately 20% decrease in body weight gain was also observed in pregnant and nonpregnant females similarly exposed to 2.5 mg/kg/day (Kato et al. 2015). The decreases in body weight gain were accompanied by decreases in food intake in males and females. In a longer duration study (110 days), no alterations in body weight gain were observed in rats administered 0.5 mg/kg/day (Shi et al. 2009a).

PFHxA

Laboratory Animal Studies. Gavage administration of up to 315 mg/kg/day did not result in alterations in body weight gain in rats exposed for 32–44 days (Kirkpatrick 2005), 90 days (Chengelis et al. 2009b), 92–93 days (Loveless et al. 2009), or 2 years (Klaunig et al. 2015). A 19% decrease in body weight gain was observed in rats administered 500 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009) and a 19% decrease in maternal body weight gain was observed in rats administered 500 mg/kg/day on GDs 1–20 (Loveless et al. 2009). In contrast to these findings, a 110–126-day study found a 12% decrease in male rats administered 100 mg/kg/day NaPFHx (Loveless et al. 2009).

FOSA

Epidemiological Studies. Halldorsson et al. (2012) did not find associations between maternal serum FOSA levels and BMI or waist circumference in 20-year-olds.

Laboratory Animal Studies. No alterations in body weight were observed in Sprague-Dawley rats following a single gavage dose of 5 mg/kg FOSA in 2% Tween 80 vehicle (Seacat and Luebker 2000).

2.4 RESPIRATORY

Overview. A small number of epidemiological studies have examined the potential of PFOA to damage the respiratory tract; detailed descriptions of these studies are presented in Table 2 in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*. Epidemiological studies examining respiratory endpoints were not identified for the other perfluoroalkyls. These studies were primarily conducted in PFOA workers or in residents of nearby communities. The possible associations between perfluoroalkyl exposure and asthma are discussed along with other immune effects in Section 2.14. Studies in laboratory animals have examined the potential for perfluoroalkyls to induce histological lesions in the lungs following inhalation (see Tables 2-1 and 2-2) or oral exposure (see Tables 2-3, 2-4, and 2-5). No laboratory animal studies examining potential respiratory tract effects were identified for PFUnA, PFHpA, PFDoDA, or FOSA.

Epidemiological studies examining respiratory effects are only available for PFOA. No alterations in lung function were observed in workers at a PFOA facility but increases in respiratory illnesses were observed in residents living near the PFOA facility.

Inhalation exposure to PFOA, PFOS, or PFNA dusts have resulted in nasal discharge, rales, and/or labored breathing in laboratory animals. Oral exposure studies in laboratory animals have not found consistent evidence of histological alterations for PFOA, PFOS, PFHxS, PFBS, or PFBA. An oral study with PFHxA reported nasal lesions in rats, however, a second study did not find these effects at higher doses.

PFOA

Epidemiological Studies. There are limited data on the potential of PFOA to damage the respiratory tract. Pulmonary function tests and chest roentgenograms conducted on workers potentially exposed to PFOA at the Washington Works fluoropolymers production facility were within normal limits (Sakr et al. 2007b); the serum PFOA levels ranged from 5 to 9,550 ng/mL. Another study of workers at this facility did not find an association between estimated cumulative serum PFOA levels and the risk of chronic obstructive pulmonary disease (Steenland et al. 2015). In contrast, a study of residents living near this facility found an increase in the risk of chronic bronchitis (standard prevalence ratio [SPR] of 3.60, 95% confidence interval [CI] 2.92–4.44) and shortness of breath (SPR 2.05, 95% CI 1.70–2.46) (Anderson-Mahoney et al. 2008); it is noted that results were based on health surveys, and some of the subjects also worked at the facility. Summaries of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 2.

Laboratory Animal Studies. Inhalation exposure of male and female rats to 18,600 mg/m^3 APFO dusts for 1 hour induced a red nasal discharge and dry rales (Griffith and Long 1980). Necropsy conducted 14 days after exposure showed bilateral mottling of the lungs in 8 out of 10 rats. Head-only exposure for 4 hours to 380 mg/m^3 APFO dusts, a concentration that was lethal to some rats, produced pulmonary edema, which disappeared within 1 week of exposure (Kennedy et al. 1986). Examination of the lungs and trachea from rats exposed head-only to up to 84 mg/m^3 APFO dusts 6 hours/day, 5 days/week for 2 weeks showed no significant gross or microscopic alterations (Kennedy et al. 1986). Male CD rats exposed nose-only to ≥590 mg/m^3 ammonium perfluorononanoate dusts for 4 hours exhibited lung noise and labored breathing during exposure and throughout a 12-day recovery period (Kinney et al. 1989).

Oral dosing of male and female CD rats with ≤110 mg/kg/day APFO did not induce gross or microscopic changes in the lungs (Griffith and Long 1980; Perkins et al. 2004). Dosing for 2 years with 15 mg/kg/day APFO increased the incidence of lung hemorrhage in males (3M 1983; Butenhoff et al. 2012c). The

incidences were 10/50, 14/50, and 22/50 for groups receiving doses of 0, 1.5, and 15 mg/kg/day, respectively. Pair-wise comparison between controls and high-dose groups revealed a statistically significant difference ($p<0.05$). The investigators suggested that pulmonary lesions were not related to PFOA based on lower incidence of interstitial pneumonia in the 15 mg/kg/day males. In a study in monkeys administered up to 20 mg/kg/day APFO, administered via a capsule, for 26 weeks, no signs of respiratory problems were observed during the study and no gross or microscopic alterations in the lungs and trachea were observed at termination (Butenhoff et al. 2002).

No gross or microscopic alterations were found in the lung and trachea from male CD rats following application of up to 2,000 mg/kg/day APFO as an aqueous paste to an area of the shaven back (approximately 15% of the total body surface) 6 hours/day, 5 days/week for 2 weeks (Kennedy 1985).

PFOS

Laboratory Animal Studies. Unpublished data summarized by OECD (2002) indicate that inhalation exposure of rats to concentrations of PFOS dust between 1,890 and 45,970 mg/m^3 for 1 hour induced dry rales and other breathing disturbances.

Dosing of Cynomolgus monkeys with up to 2 mg/kg/day PFOS, administered in a capsule, for 4 weeks had no effect on the gross or microscopic morphology of the lungs (Thomford 2002a). Administration of doses of up to 0.75 mg/kg/day of PFOS (potassium salt) administered via a capsule to Cynomolgus monkeys for 26 weeks did not produce any gross or microscopic alterations in the lungs or the trachea (Seacat et al. 2002). Dosing rats with up to 1.04 mg PFOS/kg/day in the diet for 104 weeks did not induce significant gross or microscopic alterations in the lungs or trachea (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Laboratory Animal Studies. Examination of the respiratory tract of rats administered ≤10 mg/kg/day PFHxS or mice administered ≤3 mg/kg/day by gavage in a reproductive study (40–60 days of dosing) showed no treatment-related effects (Butenhoff et al. 2009a; Chang et al. 2018).

PFNA

Laboratory Animal Studies. Labored breathing during and after a 4-hour nose-only exposure to 590 mg/m^3 PFNA dust was reported in rats (Kinney et al. 1989).

PFDA

Laboratory Animal Studies. No histological alterations were observed in the respiratory tract of rats administered 0.5 mg/kg/day for 28 days or mice administered 5 mg/kg once a week for 4 weeks (Frawley et al. 2018).

PFBS

Laboratory Animal Studies. Administration of PFBS at gavage doses of ≤900 mg/kg/day for 28 days (3M 2001) or 600 mg/kg/day for 90 days (Lieder et al. 2009a) had no significant effect on the gross or microscopic morphology of the lungs or trachea in rats; no increases in nasal lesions were observed in the 90-day study (Lieder et al. 2009a).

PFBA

Laboratory Animal Studies. Administration of PFBA to rats by gavage in doses ≤184 mg/kg/day for 5 days (3M 2007a), ≤150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or ≤30 mg/kg/day for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b) did not cause morphological alterations in the respiratory tract.

PFHxA

Laboratory Animal Studies. Degeneration/atrophy of the nasal olfactory epithelium was observed in rats administered via gavage 100 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009); at 500 mg/kg/day, respiratory metaplasia was observed in the nasal cavity. A second study did not report histological alterations in the nasal cavity of rats administered up to 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b).

2.5 CARDIOVASCULAR

Overview. Epidemiological and laboratory animal studies have evaluated the toxicity of perfluoroalkyls to the cardiovascular system. The epidemiological studies evaluated several cardiovascular outcomes including ischemic heart disease, cerebrovascular disease, stroke, cardiovascular disease, myocardial infarction, hypertension, and pregnancy-induced hypertension. The results of these studies are summarized in Table 2-8, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 3. The available occupational, community, and general population studies have not consistently found increases in the risk of heart disease or stroke that were associated with serum PFOA levels. Considerably less epidemiological data are available for other perfluoroalkyls; general population studies for PFOS, PFHxS, PFNA, PFDA, PFHpA, and PFDoDA have not consistently found increases in the risk of cardiovascular disease, although single studies for PFUnA, PFBS, PFHxA, and FOSA have found associations. Most of the available epidemiological studies did not find an association between serum PFOA and hypertension. A small number of studies (three or less for each compound) have examined potential associations with hypertension for other perfluoroalkyls. These studies found associations (PFBA), no associations (PFHxS, PFDA, PFUnA, PFHpA, PFBS, PFDoDA), or mixed results (PFOS, PFNA).

Several studies have evaluated the possible associations between serum perfluoroalkyls and pregnancy-induced hypertension and pre-eclampsia. Pregnancy-induced hypertension, also referred to as gestational hypertension, is the onset of hypertension after the 20th week of pregnancy. Pre-eclampsia is pregnancy-induced hypertension accompanied by signs of damage to another organ system, such as the kidney or liver; elevated levels of protein in the urine are often present. While the two diseases are distinct, they can be inaccurately reported in studies that relied on self-reporting or use of birth certificates (birth certificates often only have an option for pregnancy-induced hypertension; thus, pre-eclampsia may be reported as pregnancy-induced hypertension). Due to possibility of misreporting, ATSDR has opted to group these two outcomes together. Although mixed results were found in studies of highly exposed community residents, the strongest methodological study (Darrow et al. 2013) found an increased risk of pregnancy-induced hypertension that was associated with serum PFOA levels. Increases in the risk of pregnancy-induced hypertension associated with serum PFOS levels were also found in two community studies. General population studies have not found associations between serum PFHxS or PFDA and pre-eclampsia; one study on PFUnA found an inverse association.

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Leonard 2006 Occupational (n=6,027)	5–9,550 ng/mL (PFOA range)	Heart disease deaths	SMR 110 (98–123)
		Cerebrovascular disease deaths	SMR 86 (60–120)
		Ischemic heart disease deaths	SMR 109 (96–124)
Lundin et al. 2009 Occupational (n=3,993)	2,600–5,200 ng/mL (range of definite exposure group)	Heart disease deaths	SMR 0.7 (0.5–1.3)
		Cerebrovascular disease deaths	SMR 1.6 (0.5–3.7)
		Ischemic heart disease deaths	SMR 0.8 (0.5–1.4)
		Cerebrovascular disease risk	**HR 4.6 (1.3–17.0)* workers with definite exposure of ≥6 months HR 2.1 (1.0–4.6)* workers with definite exposure ≥5 years**
Raleigh et al. 2014 Occupational (n=9,027)	Cumulative PFOA exposure	Ischemic heart disease deaths	**SMR 0.84 (0.74–0.95)***
		Cerebrovascular disease	SMR 0.81 (0.61–1.05)
		Ischemic heart disease risk	HR 0.89 (0.66–1.21), 4th quartile
		Cerebrovascular disease risk	HR 0.98 (0.53–1.81), 4th quartile
Sakr et al. 2009 Occupational (n=4,747)	NR	Ischemic heart disease risk	NS (p=0.16 for trend)

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA exposure	Coronary artery disease risk	NS (p=0.78 for trend), no lag NS (p=0.75 for trend), 10-year lag
		Hypertension	NS (p=0.95 for trend), no lag NS (p=0.54 for trend), 10-year lag
		Stroke	NS (p=0.35 for trend), no lag NS (p=0.64 for trend), 10-year lag
Steenland and Woskie 2012 Occupational (n=1,084)	7,800 ng/mL-years (mean estimated cumulative PFOA exposure)	Ischemic heart disease deaths	SMR 0.93 (0.72–1.19), no lag
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Cardiovascular disease (self-reported)	**SPR 4.29 (3.47–5.29)***
		Angina (self-reported)	**SPR 8.07 (6.54–9.95)***
		Myocardial infarction	**SPR (1.91 (1.40–2.62)***
		Stroke	**SPR 2.17 (1.47–3.21)***
		Hypertension	SPR 1.18 (0.97–1.43)
Darrow et al. 2013 Community (C8) (n=1,330)	6.9–<11.1 ng/mL (2nd PFOA quintile)	Pregnancy-induced hypertension	**OR 2.39 (1.05–5.46)* (2nd quintile)**
Nolan et al. 2009 Community (C8) (n=1,555 women)	NR	Pregnancy-induced hypertension LHWA residents Partial LHWA residents	 OR 1.2 (0.7–2.0), unadjusted OR 0.8 (0.5–1.4), unadjusted
Savitz et al. 2012a Community (C8) (n=11,737 pregnant women)	6.8–<16.6 ng/mL (2nd PFOA quartile, estimated)	Pre-eclampsia	**OR 1.2 (1.0–1.5)***
Savitz et al. 2012b Community (C8) (n=224 cases of pregnancy-induced hypertension)	21.0–717.6 ng/mL (5th PFOA quintile, estimated)	Pregnancy induced hypertension	OR 1.0 (0.7–1.3), 5th quintile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Savitz et al. 2012b Community (C8) (n=4,547 pregnant women)	21.0–717.6 ng/mL (5th PFOA quintile, estimated)	Pregnancy induced hypertension	OR 1.1 (0.8–1.5)
Simpson et al. 2013 Community (C8) (n=28,541; 11% also had occupational exposure)	>178–319 ng/mL (cumulative, estimated 2nd PFOA quintile)	Stroke	**OR 1.39 (1.11–1.76)*, 2nd quintile**
Stein et al. 2009 Community (C8) (n=5.262 pregnant women)	120.6–894.4 ng/mL (4th PFOA quartile)	Pre-eclampsia	OR 0.9 (0.5–1.8)
Winquist and Steenland 2014a Community (C8) (n=28,541; 11% also had occupational exposure)	≥3,579 ng/mL (cumulative, estimated 5th PFOA quintile)	Hypertension	HR 0.98 (0.91–1.06), 5th quintile
		Coronary artery disease	HR 1.07 (0.93–1.23), 5th quintile
Bao et al. 2017 General population (n=1,612 adults)	6.19 ng/mL (median serum PFOA in males and females); 6.59 and 5.08 ng/mL (median serum PFOA in males and females, respectively)	Risk of hypertension	OR 1.12 (0.97–1.30)
		Systolic blood pressure	β-0.06 mm Hg (-1.70–1.59), males **β 2.91 mm Hg (0.10–5.72)*, females** **β 1.69 mm Hg (0.25–3.13)*, combined**
		Diastolic blood pressure	**β 1.48 mm Hg (0.60–2.35)*, males** β 1.34 mm Hg (-0.14–3.05), females **β 2.12 mm Hg (1.33–2.90)*, combined**
Geiger et al. 2014a General population (NHANES) (n=1,655 adolescents)	>5.4 ng/mL (4th quartile PFOA)	Hypertension	OR 0.69 (0.41–1.17), 4th quartile
Huang et al. 2018 General population (NHANES, n=10,859 adults)	3.17 ng/mL (median serum PFOA)	Risk of cardiovascular disease	OR 1.25 (0.91–1.70), 4th quartile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	Association (p=0.03)*
		Arterial pulse wave velocity	NS (p=0.39)
Lin et al. 2013a, 2013b General population (n=644)	3.49 ng/mL (median PFOA)	Carotid intima media thickness	NS (p=0.285 for trend)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.58)
		Echogenicity of intima media complex	NS (p=0.80), males NS (p=0.25), females
Manzano-Salgado et al. 2017b General population (n=1,230 children)	2.32 ng/mL (maternal geometric mean PFOA)	Blood pressure at 4 years of age	β -0.06 (-0.16–0.04)
		Blood pressure at 7 years of age	β -0.02 (-0.11–0.07)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	4.2 and 4.0 ng/mL (median PFOA in cases and controls)	Coronary artery disease	OR 0.88 (0.50–1.55), 4th quartile
Melzer et al. 2010 General population (NHANES) (n=3,966 adults)	10.39 and 9.47 ng/mL (mean 4th quartile PFOA)	Coronary artery disease, angina, and/or heart attack	OR 1.08 (0.70–1.69, p=0.715), 4th quartile
Min et al. 2012 General population (NHANES) (n=2,208)	4.00 ng/mL (geometric mean PFOA)	Systolic blood pressure	**Association (p=0.0004)***
		Hypertension risk	**OR 1.71 (1.23–2.36)*, 4th quartile**
Shankar et al. 2012 General population (NHANES) (n=1,216)	4.0–5.6 and 4.4–6.1 ng/mL (females and males, 3rd PFOA quartile) >5.6 and >6.1 ng/mL (females and males, 4th PFOA quartile)	Cardiovascular disease	**OR 1.77 (1.04–3.02)*, 3rd quartile**
		Peripheral arterial disease	**OR 1.78 (1.03–3.08)*, 4th quartile**
		Coronary heart disease	**OR 2.24 (1.02–4.94)*, 4th quartile**
		Stroke	**OR 4.26 (1.84–9.89)*, 4th quartile**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014b General population (n=976 pregnant women)	2.78 ng/mL (median PFOA)	Pre-eclampsia	HR 0.89 (0.65–1.22), per ln unit
PFOS			
Darrow et al. 2013 Community (C8) (n=1,330)	12.1–<15.9 ng/mL (3rd PFOS quintile)	Pregnancy-induced hypertension	**OR 2.71 (1.33–5.52)* (3rd quintile)**
Stein et al. 2009 Community (C8) (n=5,262 pregnant women)	23.2–83.4 ng/mL (4th PFOS quartile)	Pre-eclampsia	**OR 1.6 (1.2–2.3)***
Bao et al. 2017 General population (n=1,612 adults)	24.22 ng/mL (median serum PFOS in males and females); 27.39 and 14.05 ng/mL (median serum PFOS in males and females, respectively)	Risk of hypertension	OR 1.08 (0.90–1.29), males **OR 1.63 (1.24–2.13)*, females** **OR 1.24 (1.08–1.44)*, combined**
		Systolic blood pressure	B 1.50 mm Hg (-0.17–3.18), males **β 6.65 mm Hg (4.32–8.99)*, females** **β 4.84 mm Hg (3.55–6.12)*, combined**
		Diastolic blood pressure	β 0.45 mm Hg (-0.47–4.36), males **β 2.86 mm Hg (1.51–4.20)*, females** **β 2.70 mm Hg (1.98–3.42)*, combined**
Geiger et al. 2014a General population (NHANES) (n=1,655 adolescents)	>25.5 ng/mL (4th PFOS quartile)	Hypertension	OR 0.77 (0.37–1.61), 4th quartile
Huang et al. 2018 General population (NHANES, n=10,859 adults)	12.40 ng/mL (median serum PFOS)	Risk of cardiovascular disease	OR 1.25 (0.92–1.69), 4th quartile
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.06)
		Arterial pulse wave velocity	NS (p=0.51)

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lin et al. 2013a, 2013b General population (n=644)	8.65 ng/mL (median PFOS)	Carotid intima media thickness	**Association (p<0.001 for trend)***
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.72)
		Echogenicity of intima media complex	NS (p=0.40), males NS (p=0.56), females
Manzano-Salgado et al. 2017b General population (n=1,230 children)	5.80 ng/mL (maternal geometric mean PFOS)	Blood pressure at 4 years of age	β 0.00 (-0.09–0.10)
		Blood pressure at 7 years of age	β -0.05 (-0.15–0.06)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	22.8 and 22.0 ng/mL (median PFOS in cases and controls)	Coronary artery disease	OR 1.07 (0.60–1.92), 4th quartile
Melzer et al. 2010 General population (NHANES) (n=3,966 adults)	57.73 and 50.96 ng/mL (mean 4th quartile PFOS)	Coronary artery disease, angina, and/or heart attack	OR 0.91 (0.570–1.64, p=0.745), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	12.87 ng/mL (median PFOS)	Pre-eclampsia	HR 1.13 (0.84–1.52), per ln unit
PFHxS			
Bao et al. 2017 General population (n=1,612 adults)	0.71 ng/mL (median serum PFHxS in males and females)	Risk of hypertension	OR 0.99 (0.95–1.03)
		Systolic blood pressure	β 0.10 mm Hg (-0.30–0.51)
		Diastolic blood pressure	β 0.12 mm Hg (-0.11–0.35)
Huang et al. 2018 General population (NHANES, n=10,859 adults)	1.60 ng/mL (median serum PFHxS)	Risk of cardiovascular disease	OR 0.96 (0.68–1.37), 4th quartile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.69)
		Arterial pulse wave velocity	NS (p=0.89)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.90)
		Echogenicity of intima media complex	NS (p=0.40), males NS (p=0.95), females
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.61 ng/mL (maternal geometric mean PFHxS)	Blood pressure at 4 years of age	β -0.01 (-0.10–0.09)
		Blood pressure at 7 years of age	β 0.04 (-0.04–0.13)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	1.6 ng/mL (median PFHxS in cases and controls)	Coronary artery disease	OR 0.95 (0.54–1.67), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.69 ng/mL (median PFHxS)	Pre-eclampsia	HR 0.91 (0.72–1.14), per ln unit
PFNA			
Bao et al. 2017 General population (n=1,612 adults)	1.96 ng/mL (median serum PFNA in males and females); 2.19 and 1.31 ng/mL (median serum PFNA in males and females, respectively)	Risk of hypertension	OR 1.08 (0.92–1.26), males **OR 1.49 (1.16–1.92)*, females** **OR 1.19 (1.04–1.36)*, combined**
		Systolic blood pressure	B -0.12 mm Hg (-1.62–1.39), males **β 5.70 mm Hg (3.55–7.85)*, females** **β** 3.01 mm Hg (1.79–4.23)***, combined**
		Diastolic blood pressure	**β 0.94 mm Hg (0.12–1.76)*, males** **β 2.74 mm Hg (1.51–3.97)*, females** **β 2.48 mm Hg (1.80–3.16)*, combined**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.98 ng/mL (median serum PFNA)	Risk of cardiovascular disease	OR 1.30 (0.99–1.72), 4th quartile **OR 1.42 (1.07–1.88)*, 4th quartile with adjustment for serum total proteins and eGFR**
		Risk of coronary heart disease	**OR 1.89 (1.29–2.76)*, 4th quartile**
		Risk of heart attack	**OR 1.51 (1.02–2.23)*, 3rd quartile**
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	**Association (p=0.04)***
		Arterial pulse wave velocity	NS (p=0.14)
Lin et al. 2013a, 2013b General population (n=644)	0.38 ng/mL (median PFNA)	Carotid intima media thickness	**Inverse association (p=0.014 for trend)***
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.76)
		Echogenicity of intima media complex	NS (p=0.66), males **Association (p=0.01)*, females**
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.66 ng/mL (maternal geometric mean PFNA)	Blood pressure at 4 years of age	β -0.01 (-0.10–0.08)
		Blood pressure at 7 years of age	β 0.00 (-0.08–0.09)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.5 ng/mL (median PFNA in cases and controls)	Coronary artery disease	OR 0.68 (0.39–1.20), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.54 ng/mL (median PFNA)	Pre-eclampsia	HR 0.90 (0.70–1.16), per ln unit

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Bao et al. 2017 General population (n=1,612 adults)	0.86 ng/mL (median serum PFNA in males and females)	Risk of hypertension	OR 0.96 (0.85–1.09)
		Systolic blood pressure	β -0.19 mm Hg (-1.39–1.02)
		Diastolic blood pressure	**β 0.81 mm Hg (0.08–1.54)*, males** β 0.61 mm Hg (-0.81–2.04), females **β 1.19 mm Hg (0.52–1.37)*, combined**
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.20 ng/mL (median serum PFDA)	Risk of cardiovascular disease	OR 1.32 (0.99–1.78), 4th quartile **OR 1.43 (1.06–1.92)*, 4th quartile with adjustment for serum total proteins and eGFR**
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.10)
		Arterial pulse wave velocity	NS (p=0.39)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.85)
		Echogenicity of intima media complex	NS (p=0.84), males NS (p=014), females
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.2 ng/mL (median PFDA in cases and controls)	Coronary artery disease	OR 0.92 (0.53–1.60), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.10 ng/mL (median PFDA)	Pre-eclampsia	HR 0.88 (0.75–1.04), per ln unit
PFUnA			
Bao et al. 2017 General population (n=1,612 adults)	0.5 ng/mL (median serum PFUnA in males and females)	Risk of hypertension	OR 0.95 (0.90–1.01)
		Systolic blood pressure	β -0.49 mm Hg (-1.04–0.05)
		Diastolic blood pressure	β -0.11 mm Hg (-0.41–0.20)

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.20 ng/mL (median serum PFUnA)	Risk of cardiovascular disease	**OR 1.58 (1.17–2.12)*, 2nd quartile**
		Risk of coronary heart disease	**OR 1.57 (1.00–2.46)*, 2nd quartile**
		Risk of angina pectoris	**OR 1.97 (1.09–3.55)*, 3rd quartile**
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.97)
		Arterial pulse wave velocity	NS (p=0.41)
Lin et al. 2013a, 2013b General population (n=644)	6.59 ng/mL (median PFUnA)	Carotid intima media thickness	NS (p=0.953 for trend)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.96)
		Echogenicity of intima media complex	NS (p=0.09), males NS (p=0.14), females
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.2 ng/mL (median PFUnA in cases and controls)	Coronary artery disease	OR 0.88 (0.51–1.51), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.17 ng/mL (median PFUnA)	Pre-eclampsia	**HR 0.78 (0.66–0.92)*, per ln unit**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHpA			
Bao et al. 2017 General population (n=1,612 adults)	0.01 ng/mL (median serum PFHpA in males and females); 0.01 and 0.01 ng/mL (median serum PFHpA in males and females, respectively)	Risk of hypertension	OR 1.02 (0.89–1.16)
		Systolic blood pressure	**β 1.84 mm Hg (0.43–3.25)*, males** β 0.34 mm Hg (-2.47–3.15), females **β 1.50 mm Hg (0.21–2.80)*, combined**
		Diastolic blood pressure	**β 0.79 mm Hg (0.02–1.55)*, males** β 0.14 mm Hg (-1.45–1.73), females β 0.66 mm Hg (0.05–1.40), combined
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.20 ng/mL (median serum PFHpA)	Risk of cardiovascular disease	OR 1.16 (0.71–1.91), 4th quartile
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.78)
		Echogenicity of intima media complex	NS (p=0.53), males NS (p=0.13), females
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.06 and 0.04 ng/mL (median PFHpA in cases and controls)	Coronary artery disease	**OR 2.58 (1.39–4.78)*, 3rd quartile** OR 1.73 (0.94–3.16), 4th quartile
PFBS			
Bao et al. 2017 General population (n=1,612 adults)	0.01 ng/mL (median serum PFBS in males and females)	Risk of hypertension	OR 0.94 (0.78–1.12)
		Systolic blood pressure	β -0.69 mm Hg (-2.49–1.11)
		Diastolic blood pressure	β -0.41 mm Hg (-1.42–0.60)
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.07 ng/mL (median serum PFBS)	Risk of cardiovascular disease	**OR 1.34 (1.05–1.723)*, 2nd quartile**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFBA			
Bao et al. 2017 General population (n=1,612 adults)	0.15 ng/mL (median serum PFBA in males and females); 0.17 and 0.12 ng/mL (median serum PFBA in males and females, respectively)	Risk of hypertension	**OR 1.09 (1.02–1.16)*, males** **OR 1.16 (1.04–1.29)*, females** **OR 1.10 (1.04–1.17)*, combined**
		Systolic blood pressure	**β 0.66 mm Hg (0.03–1.28)*, males** β 0.77 mm Hg (-0.27–1.80), females **β 0.80 mm Hg (0.25–1.34)*, combined**
		Diastolic blood pressure	β 0.09 mm Hg (-0.22–0.40), combined
PFDoDA			
Bao et al. 2017 General population (n=1,612 adults)	0.12 ng/mL (median serum PFDoDA in males and females); 0.17 and 0.12 ng/mL (median serum PFDoDA in males and females, respectively)	Risk of hypertension	OR 1.02 (0.93–1.11)
		Systolic blood pressure	β -0.74 (-1.71–0.22), males **β 1.89 mm Hg (0.21–3.56*, females** β 0.30 mm Hg (-0.56–1.16), combined
		Diastolic blood pressure	β 0.13 mm Hg (-0.40–0.66), males **β 1.02 mm Hg (0.07–1.97)*, females** **β 0.59 mm Hg (0.12–1.07)*, combined**
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.14 ng/mL (median serum PFDoDA)	Risk of cardiovascular disease	**OR 1.53 (1.14–2.04)*, 4th quartile**
		Risk of congestive heart failure	**OR 1.55 (1.07–2.25)*, 3rd quartile**
		Risk of angina pectoris	**OR 1.64 (1.06–2.54)*, 4th quartile**
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.02 ng/mL (median PFDoDA in cases and controls)	Coronary artery disease	OR 0.63 (0.35–1.11), 4th quartile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.07 ng/mL (median serum FOSA)	Risk of cardiovascular disease	**OR 1.29 (1.01–1.65)*, 2nd quartile**
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.35), males **Association (p=0.004)*, females** **Association (p=0.01)*, combined**
		Echogenicity of intima media complex	NS (p=0.84), males NS (p=0.78, females

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 3 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CHD = coronary heart disease; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; LHWA = Little Hocking Water Authority; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; SMR = standardized mortality ratio; SPR = standard prevalence ratio; WTCHR = World Trade Center Health Registry

Examination of the cardiovascular system in laboratory animals primarily consists of inhalation, oral, and dermal studies examining the heart for morphological alterations (see Tables 2-1, 2-3, 2-4, 2-5, and 2-6). No studies in laboratory animals were identified for PFNA, PFUnA, PFHpA, or FOSA.

The laboratory animal studies did not find increases in the incidence of histological alterations in the heart following exposure to PFOA, PFOS, PFHxS, PFDA, PFBS, PFBA, PFDoDA, or PFHxA.

PFOA

Epidemiological Studies—Heart Disease. Possible associations between PFOA exposure and increased risk of heart disease have been examined in cohort mortality studies of workers, community members living near a PFOA facility, and the general population. Occupational exposure studies have not found increases in deaths from all heart disease, cerebrovascular disease, or ischemic heart disease when compared to U.S. general populations, state populations, and/or a population of workers at other company facilities (Leonard 2006; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012). One occupational exposure study found an increase in the risk of cerebrovascular disease in workers with definite exposure for at least 6 months compared to an internal referent group (Lundin et al. 2009). However, other studies have not found increased risks of ischemic heart disease (Raleigh et al. 2014; Sakr et al. 2009), cerebrovascular disease (Raleigh et al. 2014), or coronary artery disease (Steenland et al. 2015). In another occupational exposure study, the investigators noted that electrocardiograms (EKGs) were within normal limits (Sakr et al. 2007b).

Studies of residents living near the Washington Works facility in West Virginia reported increased risks of self-reported cardiovascular disease (Anderson-Mahoney et al. 2008), angina (Anderson-Mahoney et al. 2008), myocardial infarction (Anderson-Mahoney et al. 2008), and stroke (Anderson-Mahoney et al. 2008; Simpson et al. 2013). It is noted that the Anderson-Mahoney et al. (2008) study did not measure serum PFOA levels; the incidences of self-reported diseases were compared to NHANES rates. Another community study of residents in this area did not find an increased risk of coronary artery disease (Winquist and Steenland 2014a). Seven general population studies have examined possible associations between serum PFOA and heart disease risks. A case-control study did not find increases in the risk of coronary artery disease in subjects with median serum PFOA levels of 4.2 ng/mL (cases) or 4.0 ng/mL (controls) (Mattsson et al. 2015). Utilizing the NHANES data set, Shankar et al. (2012) found increases in the risk of peripheral arterial disease, coronary heart disease, or stroke in participants with serum PFOA levels in the 4th quartile (>5.6 and >6.1 ng/mL in females and males, respectively) and for cardiovascular

disease in participants with serum PFOA levels in the 3rd and 4th quartiles (>4.0 and >4.4 ng/L for females and males, respectively). In contrast, two other NHANES studies did not find associations between serum PFOA and physician-diagnosed coronary artery disease, angina, and/or heart attack (Melzer et al. 2010) or total cardiovascular heart disease (Huang et al. 2018). Two general population studies did not find associations between serum PFOA levels and carotid intima media thickness (Lin et al. 2013a; Lind et al. 2017b). Another study did not find associations with arterial wall stiffness or arterial pulse wave velocity (Koshy et al. 2017).

Epidemiological Studies—Hypertension. Occupational, community, and general population exposure studies have investigated the possible association between PFOA and blood pressure, the risk of hypertension, and the risk of pregnancy-induced hypertension and/or pre-eclampsia. A study by Min et al. (2012) utilizing NHANES data found an increase in hypertension risk among participants with serum PFOA levels in the 4th quartile. Another general population study did not find an association between serum PFOA and the risk of hypertension, but did find associations between serum PFOA and systolic and diastolic blood pressure (Bao et al. 2017). In contrast, no increases in the risk of hypertension were observed in workers at the Washington Works facility (Steenland et al. 2015), adult community members living near this facility (Winquist and Steenland 2014a), or adolescent NHANES participants (Geiger et al. 2014a). Additionally, Manzano-Salgado et al. (2017b) did not find associations between maternal serum PFOA levels and blood pressure in children at ages 4 or 7 years. There is some epidemiological evidence suggesting that an elevated uric acid level is a risk factor for hypertension (Johnson et al. 2003; Sündstrom et al. 2005). Several occupational, community, and general population studies have found increases in uric acid levels and increased risks of hyperuricemia; these data are discussed in Section 2.10. Overall, the results of these studies are suggestive of a connection between serum PFOA and increased risk of hyperuricemia.

Several studies have examined the possible associations between PFOA and pregnancy-induced hypertension/pre-eclampsia. Four studies have evaluated the community living near the Washington Works facility using different approaches to assess PFOA exposure. Savitz et al. (2012a, 2012b) used residential history and environmental dispersion of PFOA to estimate serum PFOA levels over time. Stein et al. (2009) used serum PFOA levels measured in 2005–2006 to assess the risk of pre-eclampsia occurring prior to the blood sampling. Darrow et al. (2013) primarily used serum PFOA levels measured in 2005–2006 to assess the association with pregnancy-induced hypertension occurring after the blood samples were collected. Savitz et al. (2012a) found an increased risk of self-reported pre-eclampsia in C8 Health Project participants with elevated PFOA levels and Darrow et al. (2013) found significant

increases in the odds ratios (ORs) for self-reported pregnancy-induced hypertension in women with higher PFOA (≥6.9 ng/mL) levels. A third study of highly exposed residents reported a weak association between serum PFOA and self-reported pre-eclampsia in subjects whose serum PFOA levels were above the median (Stein et al. 2009); however, there was no dose-response gradient. Using birth record data and serum PFOA levels predicted from addresses, Savitz et al. (2012b) found no consistent associations between serum PFOA and the occurrence of pregnancy-induced hypertension in participants in the C8 Health Project. Similarly, Stein et al. (2009) did not find increases in the odds of self-reported pre-eclampsia among C8 Health Project participants categorized by serum PFOA levels. Another study of residents of this area did not find increases in the risk of pregnancy-induced hypertension among residents living in an area where PFOA-contaminated water was supplied by the Little Hocking Water Authority (Nolan et al. 2010). A general population study did not find an association between plasma PFOA and the risk of pre-eclampsia (Starling et al. 2014a).

Laboratory Animal Studies. A small number of laboratory animal studies have evaluated the cardiovascular toxicity of PFOA. These studies focused on potential histological alterations in the heart; none of the available studies evaluated endpoints related to hypertension. No histopathological alterations were seen in the heart from rats exposed intermittently head-only to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986). Administration of APFO in the diet at doses up to approximately 100–110 mg/kg/day to male and female CD rats or 10 mg/kg/day by gavage to Rhesus monkeys did not cause gross or microscopic alterations in the heart or aorta (Griffith and Long 1980). Similar negative findings were reported in Cynomolgus monkeys administered up to 20 mg/kg/day APFO by capsule for 26 weeks (Butenhoff et al. 2002) and in male and female Sprague-Dawley rats that received doses of up to 15 mg/kg/day APFO for 2 years (3M 1983; Butenhoff et al. 2012c). No morphological alterations were seen in the heart from male rats dermally exposed to ≤2,000 mg/kg APFO for 2 weeks (Kennedy 1985).

Summary. Cardiovascular toxicity as assessed by deaths from heart disease, risk of heart disease, and risk of hypertension has been evaluated in workers, community members living near a PFOA facility, and the general population. In general, occupational exposure studies have not found increases in the risks of deaths from heart disease or in the risks of ischemic heart disease, cerebrovascular disease, or coronary disease. Inconsistent results have been found in a small number of studies examining residents living in areas with high PFOA drinking water contamination or the general population. Studies of hypertension have also not found associations between serum PFOA and hypertension risk. However, studies of highly exposed residents provide some suggestive evidence of an association between serum PFOA and

increased risks of pregnancy-induced hypertension/pre-eclampsia. Studies in laboratory animals did not find histological alterations in the heart following acute-, intermediate-, or chronic-duration oral exposure.

PFOS

Epidemiological Studies—Heart Disease. Three studies have evaluated the possible association between PFOS and heart disease. Melzer et al. (2010) did not find an association between serum PFOS and the risk of physician-diagnosed coronary artery disease, angina, and/or heart attack among NHANES participants; Huang et al. (2018) did not find increases in the risk of cardiovascular disease among NHANES participants. In a case-control study (Mattsson et al. 2015), no alterations in the risk of coronary artery disease were observed. Lin et al. (2013a) found an association between serum PFOS levels and carotid intima media thickness in a general population study. When the subjects were divided into subpopulations, associations between PFOS and carotid intima media thickness were found for females, nonsmokers, subjects 12–19 years of age, BMI <24, and those with an apolipoprotein E genotype of E2 carrier or E3/E3. A second study of 70-year-old subjects did not find associations between serum PFOS and the intima media thickness of the common carotid artery (Lind et al. 2017b). Similarly, no alterations in arterial wall stiffness or pulse wave velocity were found in children enrolled in the World Trade Center Health Registry (Koshy et al. 2017).

Epidemiological Studies—Hypertension. An increased risk of hypertension associated with serum PFOS levels were observed in adults; when categorized by sex, the association was only found in females (Bao et al. 2017). The study also found associations for systolic and diastolic blood pressure in males and females combined and in females only. No increases in the risk of hypertension associated with serum PFOS levels were observed in adolescent NHANES participants (Geiger et al. 2014a). Similarly, no associations between maternal serum PFOS levels and blood pressure were found in children at ages 4 and 7 years (Manzano-Salgado 2017b). Two studies found increases in the risk of self-reported pregnancy-induced hypertension (Darrow et al. 2013) or self-reported pre-eclampsia (Stein et al. 2009) associated with serum PFOS levels among C8 participants. No increase in the risk of pre-eclampsia was observed in a general population study (Starling et al. 2014b).

Laboratory Animal Studies. Studies in laboratory animal studies have evaluated the cardiovascular toxicity of PFOS but have not evaluated endpoints related to hypertension. Administration of doses of up to 0.75 mg/kg/day PFOS (potassium salt) via capsule to Cynomolgus monkeys for 26 weeks did not cause any significant gross or microscopic alterations in the heart or aorta (Seacat et al. 2002). Rats that

received up to approximately 1.04 mg/kg/day of PFOS in the diet for 2 years had no significant gross or microscopic changes in the heart (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Epidemiological Studies. Eight general population studies examined possible cardiovascular outcomes associated with PFHxS exposure. No increases in the risk of coronary artery disease (Mattsson et al. 2015) or cardiovascular disease (Huang et al. 2018) were found. Serum PFHxS levels were not associated with arterial wall stiffness (Koshy et al. 2017) or carotid artery intima media thickness (Lind et al. 2017b). Studies examining blood pressure have not found associations in adults (Bao et al. 2017) or children (Manzano-Salgado et al. 2017b). Additionally, no association between serum PFHxS and pre-eclampsia were found (Starling et al. 2014b).

Laboratory Animal Studies. Dosing of rats with≤10 mg/kg/day PFHxS or mice with ≤3 mg/kg/day by gavage for 40–60 days did not cause morphological alterations in the heart (Butenhoff et al. 2009a; Chang et al. 2018).

PFNA

Epidemiological Studies. In a general population study, an inverse association between serum PFNA levels and carotid intima media thickness was observed (Lin et al. 2013a). The investigators suggested that this finding may be secondary to an interaction between higher serum PFOS levels and lower serum PFNA levels in the study population. Associations were only found in subjects with serum PFOS higher than the 50th percentile regardless of whether the serum PFNA was higher or lower than the 60th percentile. A second study did not find an association between serum PFNA and intima media thickness (Lind et al. 2017b), but did find an association with the echogenicity of the intima media complex, an indicator of early changes in the carotid artery. Koshy et al. (2017) also found an association between serum PFNA and arterial wall stiffness in children enrolled in the World Trade Center Health Registry. Increased risks of cardiovascular disease, coronary heart disease, and heart attack were found in NHANES participants (Huang et al. 2018). In contrast, another general population study did not find increases in the risk of coronary heart disease (Mattsson et al. 2015). An association between serum PFNA and hypertension risk and systolic and diastolic blood pressure was found in a general population study (Bao et al. 2017). Manzano-Salgado et al. (2017b) did not find associations between maternal

serum PFNA and blood pressure in children aged 4 or 7 years, and Starling et al. (2014b) did not find associations between serum PFNA and pre-eclampsia (Starling et al. 2014b).

PFDA

Epidemiological Studies. In a study of NHANES participants, Huang et al. (2018) found an increased risk of any type of cardiovascular disease among participants with the highest serum PFDA levels when the statistical analyses adjusted for serum total protein levels and estimated glomerular filtration rate; however, no associations were found for specific types of cardiovascular disease. In another general population study, Mattsson et al. (2015) found no association between serum PFDA and the risk of coronary artery disease. Studies examining carotid artery intima media thickness or arterial wall stiffness of the brachial artery did not find associations with serum PFDA levels (Koshy et al. 2017; Lind et al. 2017b). Although Bao et al. (2017) did not find an association between serum PFDA levels and the risk of hypertension or systolic blood pressure levels, associations were found in diastolic blood pressure levels in males only and in males and females combined. No association was found between serum PFDA and pre-eclampsia (Starling et al. 2014b).

Laboratory Animal Studies. Death in female C57BL/6N mice following administration of a single lethal dose of 160 or 320 mg/kg PFDA by gavage was associated with mural thrombosis of the left ventricle of the heart (Harris et al. 1989). Doses ≤80 mg/kg did not cause gross or microscopic alterations in the heart, assessed 30 days after dosing, but 80 mg/kg significantly decreased relative heart weight (Harris et al. 1989).

PFUnA

Epidemiological Studies. Serum PFUnA levels were associated with increased risks of any type of cardiovascular disease, coronary heart disease, and angina pectoris in NHANES participants (Huang et al. 2018). No associations between serum PFUnA levels and the risk of hypertension of systolic or diastolic blood pressure were observed (Bao et al. 2017). Starling et al. (2014b) found an inverse association between serum PFUnA levels and the risk of pre-eclampsia in pregnant women. No associations between serum PFUnA levels and carotid intima artery thickness (Lin et al. 2013a; Lind et al. 2017b) or brachial artery wall stiffness (Koshy et al. 2017) were observed in general population studies. Another general population study (Mattsson et al. 2015) did not find an increase in the risk of coronary artery disease associated with serum PFUnA levels.

PFHpA

Epidemiological Studies. Mattsson et al. (2015) found an increase in the risk of coronary artery disease in individuals with serum PFHpA levels in the 3rd quartile; however, the risk was not increased for those with serum levels in the 4th quartile. A study of NHANES participants did not find an association between the serum PFHpA levels and any type of cardiovascular disease or a specific type of heart disease (Huang et al. 2018). No associations between serum PFHpA and the thickness of the intima media of the common carotid artery were observed in a general population study of 70-year-old adults (Lind et al. 2017b). Bao et al. (2017) did not find an association between serum PFHpA levels and the risk of hypertension; the study did find associations for systolic and diastolic blood pressure levels in males only.

PFBS

Epidemiological Studies. Two general population studies have evaluated the potential associations between serum PFBS and cardiovascular effects. Huang et al. (2018) found increased risks of cardiovascular disease (all types combined) in NHANES participants with serum PFBS levels in the 2nd quartile and higher; however, no associations were found for specific disease types. Bao et al. (2017) did not find associations between serum PFBS levels and the risk of hypertension or systolic or diastolic blood pressure levels among adults.

Laboratory Animal Studies. No morphological alterations were reported in the heart or aorta from rats dosed with ≤900 mg/kg/day PFBS by gavage for 28 days (3M 2001) or ≤600 mg/kg/day PFBS for 90 days (Lieder et al. 2009a).

PFBA

Epidemiological Studies. Only one epidemiological study examined potential cardiovascular health outcomes. Bao et al. (2017) found increases in the risk of hypertension in male and female adults, which was associated with serum PFBA levels. Systolic blood pressure levels were also associated with serum PFBA levels in males and females combined or in males only; no associations were found for diastolic blood pressure.

Laboratory Animal Studies. PFBA administered to rats by gavage in doses of up to 184 mg/kg/day for 5 days, 150 mg/kg/day for 28 days, or 30 mg/kg/day for 90 days did not induce gross or microscopic alterations in the heart (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFDoDA

Epidemiological Studies. No increase in the risk of coronary heart disease associated with serum PFDoDA levels was found in a general population study (Mattsson et al. 2015). In contrast, Huang et al. (2018) found increased risks of cardiovascular disease (any type), congestive heart failure, or angina pectoris in NHANES participants with higher serum PFDoDA levels. Bao et al. (2017) reported associations between serum PFDoDA levels in systolic and diastolic blood pressure levels among women, but there was no association with the risk of hypertension.

Laboratory Animal Studies. No histological alterations were observed in male rats administered 2.5 mg/kg/day for 42 days (Kato et al. 2015).

PFHxA

Epidemiological Studies. An increased risk of cardiovascular disease (any type) was found in NHANES participants with higher serum PFHxA levels (Huang et al. 2018). A study of 70-year-old adults reported increases in the intima media thickness in the common carotid artery that was associated with serum PFHxA levels (Lind et al. 2017b).

Laboratory Animal Studies. No histological alterations were observed in the heart of rats administered up to 500 mg/kg/day NaPFHx for 90–93 days (Chengelis et al. 2009b; Loveless et al. 2009).

FOSA

Epidemiological Studies. Serum FOSA levels were associated with an increased risk of cardiovascular disease (any type) in a study of NHANES participants (Huang et al. 2018). Increases in the intima media thickness in the common carotid artery was associated with serum FOSA levels in a study of 70-year-old men and women (Lind et al. 2017b).

2.6 GASTROINTESTINAL

Overview. Available epidemiological data on the potential of perfluoroalkyls to induce gastrointestinal effects are limited to two studies of workers at a PFOS facility that found mixed results on the possible association between PFOS and colon polyps; summaries of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 4. Epidemiological studies examining potential gastrointestinal effects were not identified for the other perfluoroalkyls. Studies examining ulcerative colitis are discussed in Section 2.14, Immunological. Laboratory animal studies have examined the gastrointestinal tract for morphological alterations following inhalation, oral, or dermal exposure to PFOA (Tables 2-1, 2-3, and 2-6), oral exposure to PFOS (Table 2-4), and oral exposure to other perfluoroalkyls (Table 2-5); the NOAELs and LOAELs are presented in Figures 2-6, 2-8, and 2-9. No laboratory animal studies were identified for PFNA, PFUnA, PFHpA, or FOSA. Studies on PFOA and PFBS have reported some signs of gastrointestinal irritation following gavage administration. Most studies did not report histological alterations in the gastrointestinal tract following exposure to PFOA, PFOS, PFHxS, PFDA, PFBA, PFDoDA, or PFHxA.

PFOA

Laboratory Animal Studies. The available data in rats and monkeys do not suggest that the gastrointestinal tract is a sensitive target of toxicity, although two studies did report some signs of irritation. Stomach irritation was reported in male rats exposed head-only to ≥380 mg/m^3 APFO dusts for 4 hours (Kennedy et al. 1986). No histopathological alterations were seen in the stomach, small intestine, or large intestine from male rats exposed intermittently nose-only to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986).

No significant gross or microscopic alterations of the gastrointestinal tract were observed in male or female rats exposed to approximately 100–110 mg/kg/day APFO through the diet for 90 days (Griffith and Long 1980). Similar observations were reported in male and female rats exposed to 15 mg/kg/day APFO via the diet for 2 years (3M 1983; Butenhoff et al. 2012c). The same investigators also reported that emesis occurred in Rhesus monkeys exposed to lethal doses (30 and 100 mg/kg/day) of APFO by gavage for 90 days (Griffith and Long 1980). In another intermediate-duration study in which Cynomolgus monkeys were exposed to up to 20 mg/kg/day APFO administered via a capsule for

26 weeks, no treatment-related alterations in the gastrointestinal tract were observed at termination (Butenhoff et al. 2002).

Intermittent application of up to 2,000 mg/kg/day APFO to the skin of male rats for up to 2 weeks did not result in gross or microscopic alterations in the gastrointestinal tract (Kennedy 1985).

PFOS

Epidemiological Studies. There are limited data available on the potential of PFOS to induce gastrointestinal damage. A study of current, retired, or former workers employed for at least 1 year at a PFOS-based fluorochemical manufacturing facility in Decatur, Alabama found no association between self-reported incidence of gastric ulcer or colon polyps and having worked in a job with either low (estimated serum PFOS levels of 390–890 ng/mL) or high (estimated PFOS serum levels of 1,300–1,970 ng/mL) exposure to PFOS, as compared to workers with no direct workplace exposure (estimated serum PFOS levels of 110–290 ng/mL) (Grice et al. 2007). A second study of workers at the Decatur facility found an increase in the risk ratio episodes of care for benign colonic polyps in workers with high potential exposure to PFOS (Olsen et al. 2004a).

Laboratory Animal Studies. Unpublished data summarized by OECD (2002) indicate that distension of the small intestine was observed in rats exposed to lethal concentrations of airborne PFOS dusts (1,890–45,970 mg/m^3) for 1 hour. Treatment of rats with up to approximately 1.04 mg/kg/day PFOS via the diet for 2 years did not induce morphological alterations in the gastrointestinal tract (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Laboratory Animal Studies. No morphological alterations were observed in the gastrointestinal tract of rats administered ≤10 mg/kg/day or mice administered ≤3 mg/kg/day PFHxS via gavage for 40–60 days (Butenhoff et al. 2009a; Chang et al. 2018).

PFDA

Laboratory Animal Studies. Administration of 0.5 mg/kg/day PFDA to rats for 28 days or 5 mg/kg to mice for 4 weeks (once/week) did not result in histological alterations in the gastrointestinal tract (Frawley et al. 2018).

PFBS

Laboratory Animal Studies. Necrosis of individual squamous cells and hyperplasia and hyperkeratosis were observed in the limiting ridge of the forestomach of male and female rats administered 600 mg/kg/day PFBS via gavage for 90 days (Lieder et al. 2009a); these lesions were likely due to irritation from the repeated gavage administration with PFBS. In another study, no morphological alterations were observed in the gastrointestinal tract of rats administered ≤900 mg/kg/day PFBS via gavage for 28 days (3M 2001).

PFBA

Laboratory Animal Studies. Administration of PFBA to rats by gavage in doses of up to 184 mg/kg/day for 5 days, 150 mg/kg/day for 28 days, or 30 mg/kg/day for 90 days did not cause morphological alterations in the gastrointestinal tract (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFDoDA

Laboratory Animal Studies. No histological alterations were observed in the gastrointestinal tract of male rats receiving gavage administration of 2.5 mg/kg/day for 42 days or in male and female rats administered 42 mg/kg/day for 42 days and allowed to recover for 14 days (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Rat administered 200 mg/kg/day NaPFHx for 90 days did not exhibit histological alterations in the gastrointestinal tract (Chengelis et al. 2009b). Erosions/ulcerations were observed in the glandular or nonglandular stomach of rats receiving gavage doses of 450 mg/kg/day PFHxA for 4 days; all animals exhibiting these lesions died early or were sacrificed *in extremis*

(Kirkpatrick 2005). No gastrointestinal lesions were observed in rats administered a time-weighted average (TWA) dose of 315 mg/kg/day for 32–44 days (Kirkpatrick 2005).

2.7 HEMATOLOGICAL

Overview. A small number of epidemiological studies have evaluated hematological endpoints in workers exposed to PFOA or PFOS and in a community exposure study; these studies did not find alterations in hematological indices; epidemiological data were not identified for the other perfluoroalkyls. Details of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 5. Laboratory animal studies have evaluated potential alterations in hematological endpoints for a variety of perfluoroalkyls (Tables 2-1, 2-3, 2-4, 2-5, and 2-6). No studies examining hematological endpoints were identified for PFNA, PFHpA, or FOSA. Some laboratory animal studies have reported alterations in hematological indices following exposure to higher doses of PFOA, PFOS, PFHxS, PFDA, PFUnA, PFBS, PFBA, PFDoDA, or PFHxA.

PFOA

Epidemiological Studies. Information on effects on hematological parameters is available from a study of residents in the Little Hocking water district in southeastern Ohio where there was significant environmental exposure to PFOA via the water supply (Emmett et al. 2006b). No significant correlations between any of the hematology parameters evaluated (including hemoglobin, hematocrit, red blood cell indices, white cell count, and platelet count) and serum PFOA were observed, whether the analysis included all of the individuals as a group or separate analyses were done for adults or children. In an occupational study, the investigators reported no alterations in blood counts in workers, with a range of serum PFOA levels of 5–9,550 ng/mL (Sakr et al. 2007b). A second occupational exposure study found an inverse association between serum fluorine (used as a measure of PFOA exposure) and hemoglobin levels (Gilliland 1992); no alterations in mean corpuscular hemoglobin or volume were found. Although no associations were found for total leukocyte counts, an inverse association with lymphocyte count and association with monocyte counts was found.

Laboratory Animal Studies. No treatment-related hematological alterations were reported in male rats exposed intermittently nose-only to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986). The specific parameters evaluated included erythrocyte counts, hemoglobin concentration, hematocrit, and differential leukocyte counts.

No significant hematological alterations were reported in male and female rats orally dosed with approximately 100–110 mg/kg/day APFO in diet for 90 days (Griffith and Long 1980). Similar results were reported in Cynomolgus monkeys treated daily with up to 20 mg/kg/day APFO administered via a capsule (Butenhoff et al. 2002; Thomford 2001) or in Rhesus monkeys dosed daily by gavage with up to 30 mg/kg/day (Griffith and Long 1980). In a 2-year dietary study in rats dosed with 1.5 or 15 mg/kg/day APFO, hematology tests performed at various times during the study showed changes in treated groups consisting of decreases in red blood cell counts, hemoglobin concentration, and hematocrit that were not always dose-related or consistent among sexes and were within acceptable ranges for the rat (3M 1983; Butenhoff et al. 2012c).

Hematology tests (erythrocyte count, hemoglobin concentration, hematocrit, total and differential leukocyte count, and red cell indices) conducted in blood from rats following intermittent dermal exposure to ≤2,000 mg/kg/day APFO for 2 weeks showed inconsistent alterations or changes of unlikely biological significance (Kennedy 1985).

PFOS

Epidemiological Studies. Two occupational exposure studies (Olsen et al. 1998a, 2003a) have examined the potential association between serum PFOS and hematological parameters (including hematocrit, hemoglobin, red blood cells, white blood cells, and platelets) in workers at 3M facilities in Decatur, Alabama and Antwerp, Belgium; mean measured levels of serum PFOS ranged from 800 to 2,440 ng/mL. No consistent alterations in hematological parameters were observed at either facility or at the different measuring time points.

Laboratory Animal Studies. Treatment of male and female rats with approximately 1.5–1.8 mg/kg/day PFOS (potassium salt) in the diet for 4 weeks did not result in significant alterations in hematological parameters (Seacat et al. 2003). Oral dosing with 1.3–1.6 mg/kg/day for 14 weeks resulted in a significant increase (45%) in non-segmented neutrophils (Seacat et al. 2003). The biological significance of this finding was not discussed by the investigators. In a 4-week study, oral administration of up to 2 mg/kg/day PFOS to Cynomolgus monkeys had no effect on hematological parameters (Thomford 2002a). In Cynomolgus monkeys dosed with 0, 0.03, 0.15, or 0.75 mg/kg/day PFOS (potassium salt) administered via a capsule for 26 weeks and subjected to comprehensive hematological tests during the study, the only significant effect was a 9% decrease in hemoglobin in 0.75 mg/kg/day males at

termination (Seacat et al. 2002). The investigators considered this a treatment-related effect, but not biologically significant given that the value was within the published range and there was no evidence of blood in the stools. No significant hematological effects were reported in a 2-year study in rats dosed with approximately 1.04 mg/kg/day PFOS in the diet (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Laboratory Animal Studies. Treatment of male rats with doses ≥0.3 mg/kg/day PFHxS by gavage for at least 42 days significantly increased prothrombin time (Butenhoff et al. 2009a). Doses ≥1 mg/kg/day significantly decreased hemoglobin concentration, whereas ≥3 mg/kg/day decreased erythrocyte count and hematocrit; the decrease in hemoglobin (<5%) was not considered adverse at 1 mg/kg/day. Oral treatment of female rats with up to 10 mg/kg/day PFHxS did not significantly alter hematological parameters (Butenhoff et al. 2009a). No alterations in hematological parameters were observed in mice administered up to 3 mg/kg/day prior to mating and during mating, gestation, and lactation (Chang et al. 2018).

PFDA

Laboratory Animal Studies. Significant decrease in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were observed in rats administered 0.25 or 0.5 mg/kg/day for 28 days (Frawley et al. 2018). No other alterations in hematological parameters were observed. Hematological alterations were also not observed in mice receiving once weekly doses of 5 mg/kg PFDA for 4 weeks (Frawley et al. 2018).

PFUnA

Laboratory Animal Studies. Treatment of rats with 1.0 mg/kg/day PFUnA via gavage for 41–46 days resulted in significant hematological changes (Takahashi et al. 2014). Effects in males included decreased mean corpuscular volume (MCV) (5%), mean corpuscular hemoglobin (MCH) (5%), activated partial thromboplastin time (APTT) (16–25%), and fibrinogen (19–33%), and increased platelet counts (13%) and white blood cells (7%). In females, there were increases in MCV (10%) and MCH (10%) and a decrease in fibrinogen (32%). The NOAEL was 0.3 mg/kg/day.

PFBS

Laboratory Animal Studies. A 90-day exposure to PFBS resulted in significant decreases in hemoglobin and hematocrit levels in males orally administered 200 or 600 mg/kg/day, and a decrease in erythrocyte levels was observed in males administered 600 mg/kg/day; the NOAEL was 60 mg/kg/day (Lieder et al. 2009a). In contrast, no hematological alterations were observed in rats administered 900 mg/kg/day PFBS for 28 days (3M 2001).

PFBA

Laboratory Animal Studies. Administration of PFBA by gavage to rats in doses of up to 184 mg/kg/day for 5 days (3M 2007a) or up to 150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a) did not result in significant alterations in hematological parameters. Oral doses of 30 mg/kg/day, but not 6 mg/kg/day, for 90 days resulted in significant reductions in red blood cell counts, hemoglobin, and hematocrit, and an increase in red cell distribution width in male rats (Butenhoff et al. 2012a; van Otterdijk 2007b). This dose level also caused a reduction in MCH and reduced MCH concentration in male rats. The lower hemoglobin and hematocrit observed in males were still detected at the end of a 3-week recovery period. These hematological effects were considered minor and not evidence of an adverse effect on red blood cell turnover by the investigator based on lack of alterations in bone marrow or the spleen.

PFDoDA

Laboratory Animal Studies. Gavage administration of 2.5 mg/kg/day for 42 days resulted in decreases in mean corpuscular volume and reticulocytes and increases in mean corpuscular hemoglobin concentration in male rats (Kato et al. 2015). In animals allowed to recover for 14 days, decreases in red blood cells, hemoglobin, hematocrit, and leukocyte levels and increases in reticulocytes were observed. In females administered 2.5 mg/kg/day for 42 days and allowed to recover for 14 days, decreases in hemoglobin, hematocrit, and mean corpuscular hemoglobin and increases in neutrophil levels were observed (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Several studies in rats have identified the hematological system as a target of PFHxA toxicity. Decreases in red blood cell counts, hemoglobin levels, and/or hematocrit levels and increases in reticulocyte levels have been observed in rats administered 315 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005), 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b), 500 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009), or 200 mg/kg/day PFHxA for 104 weeks (Klaunig et al. 2015). A decrease in hemoglobin levels was also observed in rats administered 150 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005). Hematological alterations were not observed at doses ≤100 mg/kg/day. Hematological alterations were only observed in female rats in the Klaunig et al. (2015) study and only in males in the Kirkpatrick (2005) study; sex-specific differences were not observed in the Chengelis et al. (2009b) or Loveless et al. (2009) intermediate-duration studies.

2.8 MUSCULOSKELETAL

Overview. Several epidemiological studies have evaluated possible associations between perfluoroalkyls and bone mineral density, risk of bone fractures, and risk of osteoarthritis; the results of these studies are summarized in Table 2-9, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 6. Several cross-sectional community and general population studies have found associations between serum PFOA and the risk of osteoarthritis, particularly in participants under the age of 55 years. However, associations were not found in a study of mostly male workers. Mixed results were found in studies of PFOS, with studies finding a decreased risk of osteoarthritis, increased risk in women under 50 years of age, or no association. One general population study found increased risks of osteoarthritis associated with serum PFHxS and PFNA. The data provide some suggestive evidence of a relationship between serum perfluoroalkyls and osteoarthritis. Assessing whether there is an association between perfluoroalkyl exposure and osteoarthritis is complicated by the lack of mechanistic data to support this association and it is noted that there are a number of factors that contribute to the osteoarthritis risk, and that some of these factors may be affected by perfluoroalkyls, including elevations in uric acid levels. Epidemiological information on bone mineral density is limited to a study of women and a study of children both examining PFOA, PFOS, PFHxS, and PFNA; the database was not considered adequate for assessing possible associations. No epidemiological studies evaluating musculoskeletal outcomes were identified for PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, or FOSA. No morphological alterations were noted in bone or skeletal muscle in

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Steenland et al. 2015 Occupational (n=3,713 workers)	Estimated cumulative exposure	Osteoarthritis risk	NS (p=0.92 for trend), no lag NS (p=0.13 for trend), 10-year lag
Innes et al. 2011 Community (C8) (n=49,432 adults)	13.6–28.0 ng/mL (2nd PFOA quartile)	Osteoarthritis risk (physician diagnosed)	**OR 1.16 (1.03–1.31)*, 2nd quartile** **OR 1.22 (1.02–1.45)*, 2nd quartile participants <55 years of age**
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	3.7 ng/mL (mean PFOA)	Total femur neck mineral density	**β -0.017 (-0.033 to -0.001)*, women** β 0.001 (-0.025–0.022), men
		Osteoporosis risk (women)	**OR 1.84 (1.17–2.90; p=0.008)*, per ln-PFOA increase**
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	0.99 ng/mL (mean serum PFOA)	Bone mineral density	NS (p>0.05)
Lin et al. 2014 General population (NHANES) (n=2,339 participants)	4.70 and 3.31 ng/mL (geometric mean PFOA in males and females)	Total lumbar spine bone mineral density	NS (p>0.01), premenopausal women, postmenopausal women, men
		Total hip bone mineral density	NS (p>0.01), premenopausal women, postmenopausal women, men
		All fracture types	OR 0.98 (0.75–1.28), premenopausal women OR 1.53 (0.63–3.74), postmenopausal women OR 0.84 (0.67–1.07), men
		Hip fracture	OR 1.59 (0.57–4.46), premenopausal women OR 0.48 (0.06–4.16), postmenopausal women OR 0.64 (0.39–1.06), men

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Wrist fracture	OR 1.07 (0.65–1.77), premenopausal women OR 1.21 (0.46–3.13), postmenopausal women OR 1.12 (0.75–1.70), men
		Spine fracture	OR 1.83 (0.59–5.61), premenopausal women OR 0.84 (0.46–1.53), postmenopausal women OR 1.54 (0.85–2.79), men
Uhl et al. 2013 General population (NHANES) (n=1,888 male and 1,921 female adults)	>5.89 ng/mL (4th PFOA quartile)	Osteoarthritis risk (self-reported)	**OR 1.98 (1.24–3.19)*, 4th quartiles females** OR 0.82 (0.40–1.70), 4th quartile males **OR 4.95 (1.27–19.4)*, 4th quartile women 20–49 years of age** OR 1.33 (0.82–1.16), 4th quartile women 50–84 years of age
PFOS			
Innes et al. 2011 Community (C8) (n=49,432 adults)	≥29.4 ng/mL (4th PFOS quartile)	Osteoarthritis risk (physician diagnosed)	**OR 0.76 (0.68–0.85)*, 4th quartile**
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	12.7 ng/mL (mean PFOS)	Total femur neck mineral density	**β -0.016 (-0.029 to -0.002)*, women** **β -0.013 (-0.024 to -0.002)*, men**
		Osteoporosis risk (women)	OR 1.14 (0.68–1.94; p=0.619), per ln-PFOS increase
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	2.79 ng/mL (mean serum PFOS)	Bone mineral density	NS (p>0.05)
Lin et al. 2014 General population (NHANES) (n=2,339 participants)	19.23 and 12.09 ng/mL (geometric mean PFOS in males and females)	Total lumbar spine bone mineral density	**β -0.022 (-0.038 to -0.007)*, premenopausal women** NS (p>0.01), postmenopausal women NS (p>0.01), men

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Total hip bone mineral density	NS (p>0.01), premenopausal women, postmenopausal women, men
		All fracture types	OR 0.97 (0.75–1.24), premenopausal women OR 1.59 (0.88–2.86), postmenopausal women OR 0.92 (0.73–1.16), men
		Hip fracture	OR 1.12 (0.62–2.03), premenopausal women OR 0.83 (0.23–3.00), postmenopausal women OR 1.07 (0.76–1.52), men
		Wrist fracture	OR 1.04 (0.63–1.72), premenopausal women OR 1.22 (0.61–2.45), postmenopausal women OR 1.09 (0.72–1.66), men
		Spine fracture	OR 0.52 (0.15–1.86), premenopausal women OR 1.12 (0.26–4.78), postmenopausal women OR 1.27 (0.67–2.42), men
PFHxS			
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	2.5 ng/mL (mean PFHxS)	Total femur bone mineral density	**β -0.014 (-0.074 to -0.014)*, women** β -0.026 (-0.065–0.013), men
		Osteoporosis risk (women)	**OR 1.64 (1.14–2.38; p=0.008)*, per ln-PFHxS increase**
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	1.09 ng/mL (mean serum PFHxS)	Bone mineral density	NS (p>0.05)

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	1.9 ng/mL (mean PFNA)	Total femur bone mineral density	**β -0.040 (-0.077 to -0.003)*, women** β 0.007 (-0.031–0.045), men
		Osteoporosis risk (women)	**OR 1.45 (1.02–2.05; p=0.001)*, per ln-PFNA increase.**
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	0.24 ng/mL (mean serum PFNA)	Bone mineral density	**Inverse association (p<0.05)*** NS (p>0.05) after adjustment for multiple testing

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 6 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

NHANES = National Health and Nutrition Examination Survey; NS = not significant; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluoro-nonanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

laboratory animals following exposure to PFOA, PFOS, PFHxS, PFBS, PFBA, or PFHxA; these data are summarized in Tables 2-1, 2-3, 2-4, and 2-5 and Figures 2-6, 2-8, 2-9, and 2-10. No laboratory animal data were available for PFNA, PFDA, PFUnA, PFHpA, PFDoDA, or FOSA.

PFOA

Epidemiological Studies. Several studies have examined the possible association between serum PFOA levels and the risk of osteoarthritis; the possible mechanisms associated with these findings have not been elucidated. In an occupational study (80% male), no association between estimated cumulative serum PFOA levels and the risk of osteoarthritis was found (Steenland et al. 2015). Innes et al. (2011) examined adult participants in the C8 Health Project and found that the odds of reporting osteoarthritis were higher in participants with serum PFOA levels in the 2nd, 3rd, and 4th quartiles compared to participants in the 1st quartile. When segregated by age and BMI, the strongest associations between serum PFOA levels and osteoarthritis were found in subjects under 55 years of age and in nonobese (BMI <30) subjects. Increases in the risk of osteoarthritis associated with serum PFOA levels were observed in female NHANES participants (Uhl et al. 2013); there were no associations in men. When stratified by age, the associations were found in women 20–49 years of age, but not in older women (50–84 years old) (Uhl et al. 2013). An association between increases in risk of osteoporosis and serum PFOA levels was found in another study of female NHANES participants (Khalil et al. 2016). Two studies of adult NHANES participants found no associations between serum PFOA and bone mineral density of the total femur (Khalil et al. 2016), hip (Lin et al. 2014), or lumbar spine (Khalil et al. 2016; Lin et al. 2014); however, an inverse association was found in the neck portion of the femur in the Khalil et al. (2016) study. A study in obese children did not find an association between serum PFOA levels and measures of bone mineral density (Khalil et al. 2018). Additionally, Lin et al. (2014) did not find associations between serum PFOA levels and the risk of bone fractures (total fractures, hip fractures, wrist fractures, or spine fractures) in premenopausal women, postmenopausal women, or men.

Laboratory Animal Studies. In male rats exposed head-only to up to 84 mg/m^3 APFO dusts for up to 2 weeks, examinations of the sternebrae were unremarkable (Kennedy et al. 1986). Similarly, no gross or microscopic alterations were reported in the sternum from rats following dietary exposure to 100–110 mg/kg/day APFO for 90 days (Griffith and Long 1980) or in the femur, sternum, or thigh skeletal muscle from Cynomolgus monkeys dosed with up to 20 mg/kg/day APFO administered via a capsule for 26 weeks (Butenhoff et al. 2002). *In utero* exposure to 0.3 mg/kg/day PFOA resulted in morphometrical alterations in the femur (increases in the periosteal area) and decreases in bone mineral density in the tibia

of 13- or 17-month-old mice (Koskela et al. 2016). No alterations in biomechanical properties were found.

PFOS

Epidemiological Studies. Several epidemiological studies have evaluated the potential of PFOS to induce skeletal damage. In the participants of the C8 Health Study, a decreased risk of osteoarthritis was found in participants with serum PFOS levels in the 2nd, 3rd, and 4th quartiles (Innes et al. 2011). In contrast, Uhl et al. (2013) found an increased risk of osteoarthritis in NHANES participants with serum levels of >20.97 ng/mL. When categorized by sex and age, the osteoarthritis risk was approximately 5 times higher in women aged 20–49 years with serum PFOS levels in the 4th quartile. Another study of NHANES participants (Khalil et al. 2016) did not find an increased risk of osteoporosis in women. However, the study did find an inverse association between serum PFOS and femur neck bone mineral density, but no associations with total femur or lumbar spine bone mineral density. No associations between serum PFOS levels and measures of bone mineral density were observed in a study of obese children (Khalil et al. 2018).

Laboratory Animal Studies. Treatment of monkeys with up to 0.75 mg/kg/day PFOS (potassium salt) administered via a capsule for 26 weeks had no significant effect on the gross or microscopic appearance of the femur, sternum, or thigh skeletal muscle (Seacat et al. 2002). Similar observations were made in rats treated with up to 1.04 mg/kg/day PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Epidemiological Studies. A study of NHANES participants found an increase in the risk of osteoporosis among women that was associated with serum PFHxS levels (Khalil et al. 2016). An inverse association between serum PFHxS (fourth quartile) and total femur bone mineral density was also found in women. There were no associations between serum PFHxS and femur neck or lumbar spine bone mineral density (Khalil et al. 2016). In contrast, no association between serum PFHxS levels and bone mineral density were observed in obese children (Khalil et al. 2018).

Laboratory Animal Studies. No histological alterations were observed in bone or muscle of mice administered up to 3 mg/kg/day prior to mating and during mating, gestation, and lactation periods (Chang et al. 2018).

PFNA

Epidemiological Studies. Khalil et al. (2016) found an increase in the risk of osteoporosis in women NHANES participants that was associated with serum PFNA levels. Increasing serum PFNA levels did not result in alterations in bone mineral density of the lumbar spine or femur neck, but was inversely associated with total femur bone mineral density in women with serum PFNA levels in the fourth quartile. A study of 48 obese children found an inverse association between serum PFNA levels and bone mineral density; however, the association was no longer significant after adjusting for multiple testing (Khalil et al. 2018).

PFBS

Laboratory Animal Studies. Treatment of rats with up to 900 mg/kg/day PFBS by gavage for 28 days (3M 2001) or 90 days (Lieder et al. 2009a) did not induce morphological alterations in skeletal muscle.

PFBA

Laboratory Animal Studies. PFBA administered to rats by gavage in doses of up to 184 mg/kg/day for 5 days did not induce morphological alterations in skeletal muscle (3M 2007a). Administration of 150 mg/kg/day PFBA for 28 days or 30 mg/kg/day for 90 days did not induce gross or microscopic alterations in bone (femur and sternum) or skeletal muscle (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFHxA

Laboratory Animal Studies. An intermediate-duration gavage study did not find histological alterations in the bone or muscle of rats administered up to 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b).

2.9 HEPATIC

Overview. Epidemiological studies on perfluoroalkyls have examined three potential hepatic outcomes: liver disease, alterations in serum enzyme and bilirubin levels, and alterations in serum lipid levels. Summaries of the epidemiological studies examining these outcomes are presented in Tables 2-10, 2-11, and 2-12, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 7. There are limited epidemiological data on potential associations between serum perfluoroalkyls and risk of liver disease. Occupational exposure and community studies did not find increased risk of liver disease associated with PFOA or PFOS. As assessed by serum enzyme and bilirubin levels, the epidemiological studies provide suggestive evidence of liver damage. Increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) levels and decreases in serum bilirubin levels have been reported in occupational, community, and/or general population studies. These increases in serum enzyme levels, particularly ALT, are associated with increasing levels of PFOA, PFOS, and PFHxS; it is noted that there is considerable variability across studies and not all of the studies adjusted for potential confounders. No consistent results were found for PFNA. The results of available epidemiological studies suggest associations between increases in serum lipids, particularly total cholesterol and LDL cholesterol, and serum PFOA, PFOS, PFNA, and PFDA. For PFHxS, PFUnA, PFHpA, PFBS, PFBA, and PFDoDA, there are too few studies or the results are too inconsistent to determine if they also would affect serum lipid levels at environmental exposure levels. No epidemiological studies examining hepatic endpoints were identified for PFHxA or FOSA.

Numerous animal studies have evaluated the hepatotoxicity of perfluoroalkyls following inhalation, oral, and dermal exposure; summaries of these studies are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and the NOAEL and LOAEL values are graphically presented in Figures 2-6, 2-7, 2-8, 2-9, and 2-10. No laboratory animal studies were identified for PFHpA.

The results of these studies provide strong evidence that the liver is a sensitive target of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFBS, PFBA, PFDoDA, and PFHxA toxicity. Observed effects in rodents include increases in liver weight; hepatocellular hypertrophy, hyperplasia, and necrosis; and decreases in serum cholesterol and triglyceride levels. As discussed in greater detail in Section 2.20, these effects are believed to be initiated by PPARα; however, studies in PPARα-null mice suggest that other mechanisms are also involved. Increases in liver weight have also been observed in monkey studies for PFOA and PFOS; these studies have also found alterations in serum lipid levels and hepatocellular hypertrophy (PFOS only).

Table 2-10. Summary of Liver Disease in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Liver problems (self-reported)	SPR 1.01 (0.64–1.59)
Darrow et al. 2016 Community (C8) (n=28,831)	Estimated cumulative 16.5 ng/mL (median 2005/2006)	Liver disease	HR 0.97 (0.92–1.03), no lag per ln increase in PFOA HR 0.98 (0.93–1.04), 10-year lag
		Enlarged liver, fatty liver, or cirrhosis	HR 0.97 (0.91–1.04), no lag per ln increase in PFOA HR 1.00 (0.94–1.07), 10-year lag
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative	Non-hepatitis liver disease risk	NS (p=0.86), no lag NS (p=0.40), 10-year lag
PFOS			
Alexander et al. 2003 Occupational (n=2,083)	NR	Liver cirrhosis deaths	SMR 0.81 (0.10–2.94)
Grice et al. 2007 Occupational (n=1,400)	1,300–1,970 ng/mL (high potential workers)	Liver disease	OR 1.21 (0.56–2.60)
		Cholelithiasis	OR 0.91 (0.57–1.46)
		Cholecystitis	OR 1.15 (0.65–2.06)

Table 2-10. Summary of Liver Disease in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen et al. 2004a Occupational (n=652 exposed, n=659 for non-exposed)	NR	Cholelithiasis or acute cholecystitis	**RRE_pC 8.6 (1.1–>100)*** **RRE_pC 25 (2.1–>100)*, workers with ≥10 years high exposure potential**
		Liver disease	RRE_pC 1.2 (0.2–8.6)
		Biliary duct disorders	RRE_pC 1.6 (0.8–2.9) **RRE_pC 2.6 (1.2–5.5)*, workers with ≥10 years high exposure potential**

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 7 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; NR = not reported; NS = not significant; OR = odds ratio; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; RRE_pC = risk ratio episode of care; SMR = standardized mortality ratio; SPR = standard prevalence ratio

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa et al. 2009 Occupational (n=37 current workers; n=16 former workers; n=107 non-exposed workers)	12,930 ng/mL (mean PFOA current workers) 6,810 ng/mL (mean former workers)	AST	NS (p>0.05) (34 current workers)
		ALT	NS (p>0.05) (34 current workers) **Association (p<0.01)* (56 current, former, non-exposed workers)**
		GGT	NS (p>0.05) (34 current workers) **Association (p<0.01)* (56 current, former, non-exposed workers)**
		Total bilirubin	**Inverse association (p<0.01)* (56 current, former, non-exposed workers)**
Gilliland 1992; Gilliland and Mandel 1996 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	ALT	NS (p=0.32)
		AST	NS (p=0.80)
		GGT	NS (p=0.81)
Olsen et al. 2000 Occupational (n=111, 80, and 74 in 1993, 1995, and 1997)	5,000, 6,400, and 6,400 ng/mL (mean PFOA in 1993, 1995, and 1997) Workers divided into three groups: 0–<1,000, 1,000–<10,000, and ≥10,000 ng/mL	ALT	NS (p=0.82, 0.30, 0.73) differences between exposure groups for each measurement period
		AST	NS (p=0.33, 0.45, 0.83) differences between exposure groups for each measurement period
		GGT	NS (p=0.24, 0.41, 0.78) differences between exposure groups for each measurement period
		Total bilirubin	NS (p=0.48, 0.11, 0.58) differences between exposure groups for each measurement period
		Direct bilirubin	NS (p=0.82, 0.05, 0.74) differences between exposure groups for each measurement period

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen and Zobel 2007 Occupational (n=552)	2170 ng/mL (mean 8th PFOA decile) 12,150 ng/mL (mean 10th PFOA decile)	GGT Elevated GGT	**Association (p=0.05)*** OR 1.0 (0.3–2.9), 10th decile
		Total bilirubin	**Inverse association (p=0.001)***
		ALT Elevated ALT	NS (p=0.06) OR 1.2 (0.5–3.4), 10th decile
		AST	NS (p=0.55)
Sakr et al. 2007a Occupational (n=454)	1,130 ng/mL (mean PFOA)	Total bilirubin	**Association (p=0.006)***
		AST	**Association (p=0.009)***
		ALT	NS (p>0.05)
		GGT	NS (p>0.05)
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	GGT	**Association (p=0.016)***
		AST	NS (p=0.317)
		ALT	NS (p=0.124)
		Bilirubin	NS (p=0.590)
Wang et al. 2012 Occupational (n=55)	2,157.74 ng/mL (mean PFOA)	AST	**Association (p=0.02)***
		ALT	NS (p=0.38)
Darrow et al. 2016 Community (C8) (n=28,831)	Estimated cumulative 16.5 ng/mL (median PFOA in 2005/2006)	ALT	**Association (p<0.0001 for trend)*, estimated cumulative levels** **Association (p<0.0001 for trend)*, 2005/2006 levels**
		GGT	NS (p=0.1021), cumulative levels NS (p=0.1552), 2005/2006 levels
		Bilirubin	**Inverse association (p=0.0029 for trend)*, estimated cumulative levels** **Inverse association (p=0.0036 for trend)*, 2005/2006 levels**

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	ALT Abnormal ALT	NS (p>0.05) NS (p>0.05)
		AST Abnormal AST	NS (p>0.05) **Inverse association (p=0.03)***
		GGT Abnormal GGT	NS (p>0.05) NS (p>0.05)
Gallo et al. 2012 Community (C8) (n=46,452)	NR	ALT Abnormal ALT	**Correlation (p<0.001)*** **OR 1.19 (1.03–1.37)*, 3rd decile**
		GGT Abnormal GGT	**Correlation (p<0.001)*** NS (p=0.213 for trend)
		Direct bilirubin Abnormal bilirubin	NS (p>0.05) NS (p=0.496 for trend)
Wang et al. 2012 Community (n=132)	378.30 ng/mL (mean PFOA)	ALT	NS (p=0.05)
		AST	NS (p=0.22)
Gleason et al. 2015 General population (NHANES) (n=4,333)	3.7 ng/mL (median PFOA)	ALT	**Association (p<0.001)***
		Elevated ALT	**Association (p=0.007 for trend)***
		AST	**Association (p<0.01)***
		Elevated AST	NS (p=0.058 for trend).
		GGT	**Association (p<0.01)***
		Elevated GGT	**Association (p=0.042 for trend)***
		Total bilirubin	**Association (p<0.01)***
		Elevated bilirubin	**Association (p<0.001 for trend)***
Lin et al. 2010 General population (NHANES) (n=2,216)	5.05 and 4.06 ng/mL (geometric mean PFOA in males and females)	ALT	**Association (p=0.005)***
		GGT	**Association (p=0.019)***
		Total bilirubin	NS (p=0.645)
Yamaguchi et al. 2013 General population (n=608)	2.1 ng/mL (mean PFOA)	ALT	**Association (p=0.02)***
		AST	**Association (p=0.001)***
		GGT	**Association (p=0.03)***

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Grice et al. 2007 Occupational (n=1,400)	1,300–1,970 ng/mL (high potential workers)	Cholelithiasis	OR 0.91 (0.57–1.46)
		Cholecystitis	OR 1.15 (0.65–2.06)
Olsen et al. 1999 Occupational (n=178 in 1995; n=149 in 1997)	2,440 and 1,930 ng/mL (mean PFOS in 1995 in Decatur and Antwerp) 1,960 and 1,480 ng/mL (mean PFOS in 1997 in Decatur and Antwerp)	AST	NS (p=0.14 for trend), 1995 NS (p=0.67 for trend), 1997
		ALT	NS (p=0.38 for trend), 1995 NS (p=0.46 for trend), 1997
		GGT	NS (p=0.71 for trend), 1995 NS (p=0.34 for trend), 1997
Olsen et al. 2003a Occupational (n=518)	2460 ng/mL (median 4th PFOS quartile)	AST	NS (p>0.05), no adjustments
		ALT Risk of abnormal ALT	**Higher levels (p<0.05)*, males only with no adjustments** OR 2.1 (0.6–7.3)
		GGT Risk of abnormal GGT	**Difference (p<0.05)*, females only with no adjustments** OR 2.0 (0.7–5.8)
Gallo et al. 2012 Community (C8) (n=46,452)	NR	ALT Abnormal ALT	**Correlation (p<0.001)*** **OR 1.19 (1.04–1.37)*, 5th decile**
		GGT Abnormal GGT	NS (p>0.05) **Association (p=0.047 for trend)***
		Direct bilirubin Abnormal bilirubin	**Correlation (p<0.001)*** **Association (p=0.015 for trend)***

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gleason et al. 2015 General population (NHANES) (n=4,333)	11.3 ng/mL (median PFOS)	ALT	NS (p>0.01)
		Elevated ALT	NS (p=0.370 for trend)
		AST	NS (p>0.01)
		Elevated AST	NS (p=0.438 for trend)
		GGT	NS (p>0.01)
		Elevated GGT	NS (p=0.654 for trend)
		Total bilirubin	NS (p>0.01)
		Elevated bilirubin	**Association (p=0.028 for trend)***
Lin et al. 2010 General population (NHANES) (n=2,216)	27.39 and 22.20 ng/mL (geometric mean PFOS in males and females)	ALT	NS (p=0.066)
		GGT	NS (p=0.808)
		Total bilirubin	NS (p=0.223)
Yamaguchi et al. 2013 General population (n=608)	5.8 ng/mL (mean PFOS)	ALT	**Association (p=0.03)***
		AST	**Association (p=0.01)***
		GGT	**Association (p=0.03)***
PFHxS			
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.8 ng/mL (median PFHxS)	ALT	**Association (p<0.01)***
		Elevated ALT	NS (p=0.484 for trend)
		AST	**Association (p<0.001)***
		Elevated AST	NS (p=0.230 for trend)
		GGT	NS (p>0.01)
		Elevated GGT	NS (p=0.415 for trend)
		Total bilirubin	**Association (p<0.01)***
		Elevated bilirubin	**Association (p=0.041 for trend)***
Lin et al. 2010 General population (NHANES) (n=2,216)	2.29 and 1.72 ng/mL (geometric mean PFHxS in males and females)	ALT	NS (p=0.691)
		GGT	NS (p=0.898)
		Total bilirubin	NS (p=0.063)

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	ALT	NS, longitudinal analysis
		AST	NS, longitudinal analysis
		GGT	NS, longitudinal analysis
		Bilirubin	NS, longitudinal analysis
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.4 ng/mL (median PFNA)	ALT	**Association (p<0.001)***
		Elevated ALT	NS (p=0.042 for trend)
		AST	NS (p>0.01)
		Elevated AST	NS (p=0.516 for trend)
		GGT	**Association (p<0.01)***
		Elevated GGT	NS (p=0.126 for trend)
		Total bilirubin	NS (p>0.01)
		Elevated bilirubin	NS (p=0.614 for trend)
Lin et al. 2010 General population (NHANES) (n=2,216)	0.89 and 0.72 ng/mL (geometric mean PFNA in males and females)	ALT	NS (p=0.131)
		GGT	NS (p=0.857)
		Total bilirubin	NS (p=0.053)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 7 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; OR = odds ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa 2004 Occupational (n=35)	NR	Total cholesterol	**Association (p=0.03)***
		Non-HDL cholesterol	**Association (p=0.03)***
		HDL cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		Total triglycerides	NS (p>0.05)
Costa et al. 2009 Occupational (n=37 current workers; n=16 former workers; n=107 non-exposed workers)	12,930 ng/mL (mean PFOA in current workers) 6,810 ng/mL (mean PFOA in former workers)	Total cholesterol	**Association (p=0.005)* (current workers)** **Association (p<0.05)* (56 current, former, non-exposed workers)**
		HDL cholesterol	NS (p>0.05) (34 current workers)
		Triglycerides	NS (p>0.05)
Gilliland 1992; Gilliland and Mandel 1996 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	Total cholesterol	NS (p=0.62)
		Total LDL	NS (p=0.87)
		Total HDL	NS (p=0.66)
Olsen et al. 2000 Occupational (n=111, 80, and 74 in 1993, 1995, and 1997)	5,000, 6,400, and 6,400 ng/mL (mean PFOA in 1993, 1995, and 1997) Workers divided into three groups: 0–<1,000, 1,000–<10,000, and ≥10,000 ng/mL	Total cholesterol	NS (p=0.45, 0.48, 0.08) differences between exposure groups for each measurement period
		LDL cholesterol	NS (p=0.84, 0.96, 0.11) differences between exposure groups for each measurement period
		HDL cholesterol	NS (p=0.32, 0.70, 0.40) differences between exposure groups for each measurement period
		Triglycerides	NS (p=0.77, 0.07, 0.13) differences between exposure groups for each measurement period

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen and Zobel 2007 Occupational (n=552)	2,170 ng/mL (mean of 8th PFOA decile) 12,150 ng/mL (mean of 10th PFOA decile)	Total cholesterol	NS (p=0.20)
		Elevated total cholesterol	OR 1.1 (0.5–2.6), 10th decile
		LDL cholesterol	NS (p=0.81)
		Elevated LDL cholesterol	OR 1.2 (0.5–2.8), 10th decile
		HDL cholesterol	**Association (p=0.01)***
		Decreased HDL cholesterol	OR 1.8 (0.7–4.8), 10th decile
		Triglycerides	**Association (p=0.0001)***
		Elevated triglycerides	OR 1.8 (0.8–4.4), 10th decile
Sakr et al. 2007a Occupational (n=454)	1,130 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.011)***
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
		Total bilirubin	**Association (p=0.006)***
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.002)***
		LDL cholesterol	**Association (p=0.008)***
		VLDL cholesterol	**Association (p=0.031)***
		HDL cholesterol	NS (p=0.680)
		Triglycerides	NS (p=0.384)
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA	Elevated cholesterol	NS (p=0.56), no lag NS (p=0.62), 10-year lag
Wang et al. 2012 Occupational (n=55)	2,157.74 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.36)
		LDL cholesterol	NS (p=0.43)
		HDL cholesterol	**Inverse association (p=0.01)***
		Triglycerides	NS (p=0.37)
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	Total cholesterol Abnormal cholesterol	NS (p>0.05) NS (p>0.05)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fitz-Simon et al. 2013 Community (C8) (n=560 adults)	140.1 and 68.2 ng/mL (mean PFOA at first and second examinations)	Total cholesterol	**-1.65% (-0.32 to -2.97)*, 50% decrease in PFOA**
		LDL cholesterol	**-3.58% (-1.47 to -5.66)*, 50% decrease in PFOA**
		HDL cholesterol	-1.33% (0.21 to -2.85), 50% decrease in PFOA
		Triglycerides	0.78% (5.34 to -3.58), 50% decrease in PFOA
Frisbee et al. 2010 Community (C8) (n=12,476 children and adolescents)	77.7 ng/mL (mean PFOA in children) 61.8 ng/mL (mean PFOA in adolescents)	Total cholesterol	**Association (p<0.001)*, children 5th quintile** **Association (p<0.001)*, adolescents 5th quintile**
		Abnormal cholesterol	OR 1.1 (1.0–1.3), 2nd quintile
		LDL cholesterol	**Association (p=0.001)*, children 5th quintile** **Association (p=0.004)*, adolescents 5th quintile**
		Abnormal LDL levels	OR 1.2 (1.0–1.5), 2nd quintile
		HDL cholesterol	NS (p=0.88), children 5th quintile NS (p=0.20), adolescents 5th quintile
		Triglycerides	NS (p=0.1), children 5th quintile NS (p=0.1), adolescents 5th quintile
Steenland et al. 2009b Community (C8) (n=46,294)	80.3 ng/mL (mean PFOA) 13.2–26.5 ng/mL (2nd PFOA quartile)	Total cholesterol	**Association (p<0.001 for trend)***
		Abnormal cholesterol	**OR 1.21 (1.12–1.31)*, 2nd quartile**
		LDL cholesterol	**Association (p<0.05 for trend)***
		HDL cholesterol	NS (p>0.05)
		Triglycerides	**Association (p<0.05 for trend)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2012 Community (n=132)	378.30 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.85)
		LDL cholesterol	NS (p=0.97)
		Triglycerides	NS (p=0.73)
		HDL cholesterol	NS (p=0.39)
Winquist and Steenland 2014a Community (C8) (n=28,541)	142–<234 ng/mL (estimated 2nd quintile for cumulative PFOA)	Hypercholesterolemia	**HR 1.24 (1.15–1.33)*, 2nd quintile for estimated cumulative exposure**
Eriksen et al. 2013 General population (n=753)	7.1 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.01)***
Fisher et al. 2013 General population (n=2,368)	2.46 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.22)
		High cholesterol levels	OR 1.5 (0.86–2.62), 4th quartile
		Non HDL cholesterol	NS (p=0.13)
		LDL cholesterol	NS (p=0.63)
		HDL cholesterol	NS (p=0.96)
Fu et al. 2014a General population (n=133)	1.43 ng/mL (median PFOA)	Total cholesterol	**Association (p=0.015)***
		Elevated cholesterol	OR 0.55 (0.09–3.31)
		LDL cholesterol	**Association (p=0.022)***
		Elevated LDL	OR 0.71 (0.14–3.49)
		HDL cholesterol	NS (p=0.260)
		Elevated HDL	OR 0.67 (0.13–3.51)
		Triglycerides	NS (p=0.298)
		Elevated triglyceride	OR 1.97 (0.59–6.55)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Geiger et al. 2014b General population (NHANES) (n=815 12–18-year-old adolescents)	4.2 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.0170 for trend)***
		Elevated cholesterol	**OR 1.44 (1.11–1.88, p=0.0253 for trend)*, log transformed PFOA**
		LDL cholesterol	**Association (p=0.0027 for trend)***
		Elevated LDL	NS (p=0.0539 for trend)
		HDL cholesterol	NS (p=0.1769 for trend)
		Decreased HDL	NS (p=0.1493 for trend)
		Triglycerides	NS (p=0.9943 for trend)
		Elevated triglycerides	NS (p=0.5975 for trend)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	1.88 ng/mL (median serum PFOA)	Total cholesterol	β -2.256 (-11.490–6.978, p=0.630)
		LDL cholesterol	β 3.899 (-4.810–12.608, p=0.377)
		Triglycerides	β 0.020 (-0.134–0.175, p=0.796)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group)	Total cholesterol	**β 0.09 mg/dL (0.04–0.14, p<0.001)***
		LDL cholesterol	**β 0.11 mg/dL (0.03–0.19, p=0.006)***
		HDL cholesterol	β 0.04 mg/dL (-0.04–0.12, p=0.34)
		Triglycerides	**β 0.14 mg/dL (0.02 to 0.27, p=0.03)***
Liu et al. 2018b General population (NHANES, n=1,871 adults)	1.86 ng/mL (geometric mean serum PFOA)	Total cholesterol	Association (p<0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	Association (p<0.01)
		Triglycerides	NS (p>0.05)
Maisonet et al. 2015a General population (n=111 for 7-year-old and n=88 for 15-year-old girls	1.1–3.1, 3.2–4.4, and 4.5–16.4 ng/mL (maternal PFOA for 1st, 2nd, and 3rd tertiles)	Total cholesterol in 7-year-olds	**Association (β 13.75, 0.05–27.45)*, 1st tertile** NS, 2nd and 3rd tertiles
		Total cholesterol in 15-year-olds	**Association (β 17.19, 0.405–33.93)*, 1st tertile** NS, 2nd and 3rd tertiles
		LDL cholesterol in 7-year-olds	**Association (β 14.01, 3.26–24.76)*, 1st tertile** NS, 2nd and 3rd tertiles

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		LDL cholesterol in 15-year-olds	**Association (β 14.261, 0.25–28.26)*, 1st tertile** NS, 2nd and 3rd tertiles
		HDL cholesterol	NS (β -0.40, -1.82–1.01), 3rd tertile 7-year-olds NS (β -0.520, -2.10–1.06), 3rd tertile 15-year-olds
		Triglycerides	NS (β -0.020, -0.068–0.029), 3rd tertile, 7-year-olds NS (β -0.013, -0.051–0.025), 3rd tertile, 15-year-olds
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	2.32 ng/mL (maternal geometric mean PFOA)	Total cholesterol	β -0.02 (-0.10–0.15)
		LDL cholesterol	β 0.03 (-0.12–0.21)
		HDL cholesterol	β -0.04 (-0.15–0.08)
		Triglycerides	β -0.01 (-0.17–0.16)
Nelson et al. 2010 General population (NHANES) (n=860)	4.6 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.07)
		LDL cholesterol	NS (p=0.84)
		Non-HDL cholesterol	**Association (p=0.05)* β 1.38 (0.12–2.65), per ng/mL increase in PFOA**
		HDL cholesterol	NS (p=0.34)
Skuladottir et al. 2015 General population (n=854 pregnant women)	4.1 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.01 for trend)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014a General population (n=854 pregnant women)	2.25 ng/mL (50th PFOA percentile)	Total cholesterol	NS (β 2.58; -4.32–9.47), per ln-unit increase in PFOA
		LDL cholesterol	NS (β 0.35 -3.97–8.48), per ln-unit increase in PFOA
		HDL cholesterol	**Association (β 3.42 0.56–6.28)*, 4th quartile**
		Triglycerides	NS (β 0.00 (-0.07–0.06), per ln-unit increase in PFOA
Timmermann et al. 2014 General population (n=499 children, 8–10 years old)	9.3 ng/mL (median PFOA)	Triglycerides	NS (p=0.91), normal weight children **Association (p=0.002)*, obese children**
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	1.90 ng/mL (median serum PFOA)	HDL cholesterol	β 0.15 (-0.17–0.46)
		Triglycerides	**β 2.3 (0.77–8.38)***
Zeng et al. 2015 General population (n=225 adolescents, 12–15 years old)	1.1 and 0.92 ng/mL (mean PFOA in boys and girls)	Total cholesterol	**Association (p=0.001)***
		LDL cholesterol	**Association (p=0.002)***
		HDL cholesterol	NS (p=0.06)
		Triglycerides	**Association (p<0.001)***
PFOS			
Olsen et al. 1999 Occupational (n=178 in 1995; n=149 in 1997)	2,440 and 1,930 ng/mL (mean PFOS in 1995 in Decatur and Antwerp) 1,960 and 1,480 ng/mL (mean in 1997 in Decatur and Antwerp)	Total cholesterol	NS (p=0.96 for trend), 1995 **Association (p=0.006 for trend)*, 1997**
		LDL cholesterol	NS (p=0.87 for trend), 1995 **Association (p=0.01 for trend)*, 1997**
		HDL cholesterol	**Inverse association (p=0.04 for trend)*, 1995** NS (p=0.34) 1997
		Triglycerides	NS (p=0.35 for trend), 1995 NS (p=0.67 for trend), 1997

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen et al. 2003a Occupational (n=518)	2,460 ng/mL (median 4th PFOS quartile)	Total cholesterol	NS (p>0.05), no adjustments **Association (p=0.04)*, with adjustments**
		HDL cholesterol	NS (p>0.05), no adjustments
		Triglycerides	**Higher levels (p<0.05)*, males only with no adjustments** **Association (p=0.01)*, with adjustments**
Frisbee et al. 2010 Community (C8) (n=12,476 children and adolescents)	23.6 ng/mL (mean PFOS in children) 21.9 ng/mL (mean PFOS in adolescents)	Total cholesterol	**Association (p<0.001)*, children 5th quintile** **Association (p<0.001)*, adolescents 5th quintile**
		Abnormal cholesterol	**OR 1.3 (1.1–1.4)*, 2nd quintile**
		LDL cholesterol	**Association (p=0.002)*, children 5th quintile** **Association (p<0.001)*, adolescents 5th quintile**
		Abnormal LDL levels	**OR 1.2 (1.0–1.5)*, 2nd quintile**
		HDL cholesterol	**Association (p=0.007)*, children 5th quintile** **Association (p=0.001)*, adolescents 5th quintile**
		Triglycerides	NS (p=0.1), children 5th quintile NS (p=0.1), adolescents 5th quintile
Steenland et al. 2009b Community (C8) (n=46,294)	22.4 ng/mL (mean PFOS) 13.3–19.5 ng/mL (2nd quartile)	Total cholesterol	**Association (p<0.001 for trend)***
		Abnormal cholesterol	**OR 1.14 (1.05–1.23)*, 2nd quartile**
		LDL cholesterol	**Association (p<0.05 for trend)***
		HDL cholesterol	NS (p>0.05)
		Triglycerides	**Association (p<0.05 for trend)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Château-Degat et al. 2010 General population (n=723)	25.7 ng/mL (mean PFOS)	Total cholesterol	NS (p=0.086)
		LDL cholesterol	NS (p=0.242)
		HDL cholesterol	**Association (p<0.001)*, men** **Association (p=0.001)*, women**
		Triglycerides	**NS (p=0.162), men** **Inverse association (p=0.040)*, women**
Eriksen et al. 2013 General population (n=753)	36.1 ng/mL (mean PFOS)	Total cholesterol	**Association (p=0.02)***
Fisher et al. 2013 General population (n=2,368)	8.04 ng/mL (mean PFOS)	Total cholesterol	NS (p=0.35)
		High cholesterol levels	OR 1.36 (0.87–2.12), 4th quartile
		Non HDL cholesterol	NS (p=0.14)
		LDL cholesterol	NS (p=0.42)
		HDL cholesterol	NS (p=0.33)
Fu et al. 2014a General population (n=133)	1.47 ng/mL (median PFOS)	Total cholesterol	NS (p=0.287)
		Elevated cholesterol	OR 2.27 (0.47–10.92)
		LDL cholesterol	NS (p=0.357)
		Elevated LDL	OR 2.27 (0.50–10.37)
		HDL cholesterol	NS (p=0.260)
		Elevated HDL	OR 0.29 (0.06–1.50)
		Triglycerides	NS (p=0.711)
		Elevated triglycerides	OR 1.26 (0.41–3.90)
Geiger et al. 2014b General population (NHANES) (n=815 12–18-year-old adolescents)	17.7 ng/mL (mean PFOS)	Total cholesterol	NS (p=0.0512 for trend)
		Elevated cholesterol	**OR 1.35 (1.11–1.64, p=0.0183 for trend)*, log transformed PFOS**
		LDL cholesterol	**Association (p=0.0081 for trend)***
		Elevated LDL	**OR 1.48 (1.15–1.90, p=0.0178 for trend)*, log transformed PFOS**

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		HDL cholesterol	NS (p=0.9703)
		Decreased HDL	NS (p=0.9873 for trend)
		Triglycerides	NS (p=0.1104 for trend)
		Elevated triglycerides	NS (p=0.2418 for trend)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	5.68 ng/mL (median serum PFOS)	Total cholesterol	β -0.450 (-10.667–9.768, p=0.931)
		LDL cholesterol	β 2.507 (-6.879–11.893, p=0.598)
		Triglycerides	β -0.020 (-0.186–0.146, p=0.809)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group)	Total cholesterol	**β 0.08 mg/dL (0.05–0.12, p<0.001)***
		LDL cholesterol	**β 0.10 mg/dL (0.05–0.16, p<0.001)***
		HDL cholesterol	**β 0.06 mg/dL (0.003–0.13, p=0.04)***
		Triglycerides	β 0.04 mg/dL (0.05–0.13, p=0.36)
Liu et al. 2018b General population (NHANES, n=1,871 adults)	5.28 ng/mL (geometric mean serum PFOS)	Total cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
Maisonet et al. 2015b General population (n=111 for 7-year-old and n=88 for 15-year-old girls)	23.5–94.5 ng/mL (3rd tertile maternal PFOS)	Total cholesterol	NS (β -0.10, -0.73–0.54), 7-year-olds **Association (β -0.77, -1.40 to -0.13)*, 15-year-olds**
		LDL cholesterol	NS (β 0.02, -0.48–0.53), 7-year-olds **Association (β -0.54, -1.08 to -0.003)*, 15-year-olds**
		HDL cholesterol	NS (β -0.04, -0.33–0.25), 7-year-olds NS (β -0.18, -0.47–0.12), 15-year-olds
		Triglycerides	NS (β -0.004, -0.015–0.006), 7-year-olds NS (β -0.004, -0.011–0.004), 15-year-olds

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	5.80 ng/mL (maternal geometric mean PFOS)	Total cholesterol	β -0.02 (-0.10–0.15)
		LDL cholesterol	β 0.02 (-0.12–0.15)
		HDL cholesterol	β -0.03 (-0.14–0.09),
		Triglycerides	β 0.05 (-0.06–0.17)
Nelson et al. 2010 General population (NHANES) (n=860)	25.3 ng/mL (mean PFOS)	Total cholesterol	**Association (p=0.01)***
		LDL cholesterol	NS (p=0.27)
		Non-HDL cholesterol	**Association (p=0.02)***
		HDL cholesterol	NS (p=0.78)
Skuladottir et al. 2015 General population (n=854 pregnant women)	22.3 ng/mL (mean PFOS)	Total cholesterol	**Association (p=0.01 for trend)***
Starling et al. 2014a General population (n=854 pregnant women)	13.03 ng/mL (50th PFOS percentile)	Total cholesterol	**Association (p<0.05)***
		LDL cholesterol	NS (β 6.48, -0.07–13.03), per ln-unit increase in PFOS
		HDL cholesterol	**Association (β 4.39, 2.37–6.42)*, per ln-unit increase in PFOS**
		Triglycerides	NS (β -0.02, -0.09–0.04), per ln-unit increase in PFOS
Timmermann et al. 2014 General population (n=499 children, 8–10 years old)	41.5 ng/mL (median PFOS)	Triglycerides	NS (p=0.78), normal weight children **Association (p=0.002)*, obese children**
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	3.00 ng/mL (median serum PFOS)	HDL cholesterol	β 0.02 (-0.17–0.2)
		Triglycerides	β 0.3 (-0.63–1.22)
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	32.4 and 34.2 ng/mL (mean PFOS in boys and girls)	Total cholesterol	**Association (p<0.001)***
		LDL cholesterol	**Association (p<0.001)***
		HDL cholesterol	NS (p=0.72)
		Triglycerides	**Association (p=0.05)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Fisher et al. 2013 General population (n=2,368)	2.18 ng/mL (mean PFHxS)	Total cholesterol	**Association (p=0.005)***
		High cholesterol levels	**OR 1.27 (1.11–1.45)*, 4th quartile**
		Non HDL cholesterol	**Association (p=0.002)***
		LDL cholesterol	**Association (p=0.02)***
		HDL cholesterol	NS (p=0.67)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.793 ng/mL (median serum PFHxS)	Total cholesterol	β 0.989 (-9.526–11.503, p=0.853)
		LDL cholesterol	β -4.222 (-13.979–5.534, p=0.393)
		Triglycerides	β 0.081 (-0.092–0.253, p=0.355)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group)	Total cholesterol	**β 0.04 mg/dL (0.04–0.06, p=0.01)***
		LDL cholesterol	**β 0.05 mg/dL (0.01–0.09, p=0.02)***
		HDL cholesterol	β 0.03 mg/dL (-0.02–0.07, p=0.26)
		Triglycerides	β 0.04 mg/dL (-0.02–0.11, p=0.20)
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	0.61 ng/mL (maternal geometric mean PFHxS)	Total cholesterol	β 0.02 (-0.09–0.12)
		LDL cholesterol	β -0.01 (-0.12–0.09)
		HDL cholesterol	β -0.01 (-0.11 to 0.10)
		Triglycerides	**β 0.11 (0.01–0.21)***
Nelson et al. 2010 General population (NHANES) (n=860)	2.6 ng/mL (mean PFHxS)	Total cholesterol	NS (p=0.07)
		LDL cholesterol	NS (p=0.10)
		Non-HDL cholesterol	**Association (p=0.04)***
		HDL cholesterol	NS (p=0.11)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014a General population (n=854 pregnant women)	0.60 ng/mL (50th PFHxS percentile)	Total cholesterol	NS (β 3.00, -1.75–7.76), per ln-unit increase in PFHxS
		LDL cholesterol	NS (β 1.92, -2.50–6.33), per ln-unit increase in PFHxS
		HDL cholesterol	**Association (β 1.46; 0.19–2.73)*, per ln-unit increase in PFHxS**
		Triglycerides	NS (β -0.01, -0.05–0.03), per ln-unit increase in PFHxS
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	3.80 ng/mL (median serum PFHxS)	HDL cholesterol	**β 0.22 (0 to 0.43, p<0.05)***
		Triglycerides	**β 1.18 (0.12–2.25, p<0.05)***
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	2.1 and 2.1 ng/mL (mean PFHxS in boys and girls)	Total cholesterol	NS (p=0.23)
		LDL cholesterol	NS (p=0.17)
		HDL cholesterol	NS (p=0.54)
		Triglycerides	NS (p=0.15)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	Total cholesterol	NS, longitudinal analysis
		Triglycerides	NS, longitudinal analysis
Fu et al. 2014a General population (n=133)	0.37 ng/mL (median PFNA)	Total cholesterol	**Association (p=0.002)***
		Elevated cholesterol	OR 1.03 (0.24–4.46)
		LDL cholesterol	**Association (p=0.004)**
		Elevated LDL	OR 2.51 (0.59–10.74)
		HDL cholesterol	NS (p=0.191)
		Lowered HDL	OR 1.06 (0.20–5.57)
		Triglycerides	NS (p=0.460)
		Elevated triglycerides	OR 0.80 (0.26–2.49)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.938 ng/mL (median serum PFNA)	Total cholesterol	β -1.624 (-10.218–6.970, p=0.709)
		LDL cholesterol	β 2.304 (-6.558–11.167, p=0.607)
		Triglycerides	β 0.065 (-0.092–0.221, p=0.820)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group)	Total cholesterol	**β 0.05 mg/dL (0.01–0.09, p=0.01)***
		LDL cholesterol	**β 0.07 mg/dL (0.01–0.14, p=0.01)***
		HDL cholesterol	β 0.05 mg/dL (0.02–0.12, p=0.13)
		Triglycerides	β -0.07 mg/dL (0.11–0.01, p=0.89)
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	0.66 ng/mL (maternal geometric mean PFNA)	Total cholesterol	β-0.00 (-0.11–0.12)
		LDL cholesterol	β 0.01 (-0.10–0.12)
		HDL cholesterol	β -0.03 (-0.14–0.08),
		Triglycerides	β 0.03 (-0.07–0.14)
Nelson et al. 2010 General population (NHANES) (n=860)	1.3 ng/mL (mean PFNA)	Total cholesterol	**Association (p=0.04)***
		LDL cholesterol	NS (p=0.08)
		Non-HDL cholesterol	**Association (p=0.04)***
		HDL cholesterol	NS (p=0.31)
Starling et al. 2014a General population (n=854 pregnant women)	0.39 ng/mL (50th PFNA percentile)	Total cholesterol	NS (β 0.01, -5.98–6.00), per ln-unit increase in PFNA
		LDL cholesterol	NS (β -2.15, -7.31–3.02), per ln-unit increase in PFNA
		HDL cholesterol	**Association (β 2.84; 0.97–4.71)*, per ln-unit increase in PFNA**
		Triglycerides	NS (β -0.02, -0.07–0.03), per ln-unit increase in PFNA
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.50 ng/mL (median serum PFNA)	HDL cholesterol	**β 0.3 (0.05–0.56)***
		Triglycerides	**β 1.54 (0.27–2.8)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	0.8 and 0.9 ng/mL (mean PFNA in boys and girls)	Total cholesterol	**Association (p=0.04)***
		LDL cholesterol	**Association (p=0.05)***
		HDL cholesterol	NS (p=0.37)
		Triglycerides	**Association (p=0.007)***
PFDA			
Fu et al. 2014a General population (n=133)	0.19 ng/mL (median PFDA)	Total cholesterol	**Association (p=0.048)***
		Elevated cholesterol	OR 3.84 (0.87–16.95)
		LDL cholesterol	NS (p=0.251)
		Elevated LDL	OR 2.17 (0.52–9.04)
		HDL cholesterol	**Association (p=0.007)***
		Elevated HDL	OR 2.21 (0.49–10.07)
		Triglycerides	NS (p=0.317)
		Elevated triglycerides	OR 0.51 (0.17–1.58)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.0592 ng/mL (median serum PFDA)	Total cholesterol	β -3.330 (-7.484–0.824, p=0.115)
		LDL cholesterol	β -1.858 (-5.694–1.979, p=0.339)
		Triglycerides	β -0.036 (-0.103–0.032, p=0.302)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group)	Total cholesterol	**β 0.04 mg/dL (0.02–0.06, p<0.001)***
		LDL cholesterol	**β 0.04 mg/dL (0.02–0.06, p=0.03)***
		HDL cholesterol	**β 0.05 mg/dL (0.02–0.09, p=0.003)***
		Triglycerides	β -0.01 mg/dL (-0.047–0.057,p=0.85)
Starling et al. 2014a General population (n=854 pregnant women)	0.09 ng/mL (50th PFDA percentile)	Total cholesterol	NS (β 1.84, -2.12–5.79), per ln-unit increase in PFDA
		LDL cholesterol	NS (β 0.19, -3.30–3.69), per ln-unit increase in PFDA
		HDL cholesterol	**Association (β 2.54, 1.22–3.87)*, per ln-unit increase in PFDA**
		Triglycerides	NS (β -0.03, -0.07–0.01), per ln-unit increase in PFDA

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.40 ng/mL (median serum PFDA)	HDL cholesterol	β 0.24 (0.04–0.52)
		Triglycerides	β 0.64 (-0.77–2.05)
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	1.0 and 1.0 ng/mL (mean PFDA in boys and girls)	Total cholesterol	NS (p=0.74)
		LDL cholesterol	NS (p=0.85)
		HDL cholesterol	NS (p=0.47)
		Triglycerides	NS (p=0.92)
PFUnA			
Fu et al. 2014a General population (n=133)	0.26 ng/mL (median PFUnA)	Total cholesterol	NS (p=0.184)
		Elevated cholesterol	OR 3.70 (0.76–18.03)
		LDL cholesterol	NS (p=0.270)
		Elevated LDL	OR 4.16 (0.96–18.00)
		HDL cholesterol	NS (p=0.279)
		Elevated HDL	OR 0.54 (0.11–2.57)
		Triglycerides	NS (p=0.755)
		Elevated triglycerides	OR 0.74 (0.25–2.21)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.652 ng/mL (median serum PFUnA)	Total cholesterol	**β 7.906 (2.681–13.131, p=0.003)***
		LDL cholesterol	**β 7.101 (2.448–11.754, p=0.003)***
		Triglycerides	β 0.043 (-0.042–0.129, p=0.317)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCHR group and comparison group)	Total cholesterol	β 0.02 mg/dL (0–0.04, p=0.06)
		LDL cholesterol	β 0.01 mg/dL (-0.02–0.04, p=0.49)
		HDL cholesterol	**β 0.04 mg/dL (0.01–0.07, p=0.01)***
		Triglycerides	β -0.04 mg/dL (-0.09–0.003, p=0.07)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014a General population (n=854 pregnant women)	0.22 ng/mL (50th PFUnA percentile)	Total cholesterol	NS (β 0.89, -3.28–5.06), per ln-unit increase in PFUnA
		LDL cholesterol	NS (β -2.36, -5.97–1.25), per ln-unit increase in PFUnA
		HDL cholesterol	**Association (β 4.05, 2.75–5.35)*, per ln-unit increase in PFUnA**
		Triglycerides	NS (β -0.04, -0.08–0.00), per ln-unit increase in PFUnA
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.30 ng/mL (median serum PFUnA)	HDL cholesterol	β 0.11 (-0.11–0.34)
		Triglycerides	β 0.61 (-0.48–1.7)
PFHpA			
Fu et al. 2014a General population (n=133)	0.04 ng/mL (median PFHpA)	Total cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.20 ng/mL (median serum PFHpA)	HDL cholesterol	β -0.33 (-0.77–0.11)
		Triglycerides	β -0.92 (-3.12–1.28)
PFBS			
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	0.5 and 0.4 ng/mL (mean PFBS in boys and girls)	Total cholesterol	**Association (p=0.04)***
		LDL cholesterol	NS (p=0.14)
		HDL cholesterol	NS (p=0.15)
		Triglycerides	NS (p=0.81)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFBA			
Fu et al. 2014a General population (n=133)	0.11 ng/mL (median PFBA)	Total cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
PFDoDA			
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	4.5 and 4.4 ng/mL (mean PFDoDA in boys and girls)	Total cholesterol	NS (p=0.37)
		LDL cholesterol	NS (p=0.44)
		HDL cholesterol	NS (p=0.68)
		Triglycerides	NS (p=0.40)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 7 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HDL = high density lipoprotein; LDL = low density lipoprotein; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; VLDL = very low-density lipoprotein; WTCHR = World Trade Center Health Registry

To address concern over the relevance of liver enlargement in rodents to human health risk, the European Society of Toxicologic Pathology (ESTP) convened an expert panel to define what constitutes an adverse hepatic effect and whether hepatic effects induced by nuclear hormone receptors such as PPARα, constitutive androstane receptor (CAR), or pregnane X receptor (PXR) are rodent-specific adaptive reactions; the findings of the panel are summarized by Hall et al. (2012). As discussed by Hall et al. (2012), criteria were established for determining whether increases in liver organ weight and liver cell hypertrophy observed in studies of rodents exposed to agents inducing enzyme induction can be considered adaptive responses and of little relevance to humans. According to the ESTP criteria, increases in liver weight without histological evidence, such as (1) degenerative or necrotic changes including hepatocyte necrosis, inflammation, and steatotic vascular degeneration; (2) biliary/oval cell proliferation, degeneration, fibrosis, and cholestasis; or (3) necrosis and degeneration of other resident cells within the liver, are not considered adverse or relevant for human risk assessment. In the absence of histological changes, increases in liver organ weight are not considered relevant for human risk assessment unless at least two of the following three parameters are present: (1) at least 2–3 times increase in ALT levels; (2) biologically significant change in other biomarkers of hepatobiliary damage (alkaline phosphatase, AST, GGT, etc.); or (3) biologically significant change in another clinical pathology marker indicating liver dysfunction (albumin, bilirubin, bile acids, coagulation factors, cholesterol, triglycerides, etc.). ATSDR has adopted the criteria from Hall et al. (2012) for determining the adversity of the liver effects reported in the rodent perfluoroalkyl studies. Doses associated with increases in liver weight and hepatocellular hypertrophy were not considered adverse effect levels unless hepatocellular degenerative or necrotic changes or evidence of biliary or other liver cell damage were also present. The lowest doses associated with the liver weight increases and hepatocellular hypertrophy are noted in the LSE tables even though the dose levels are considered NOAELs.

PFOA

Epidemiological Studies—Liver Disease. Three studies of highly exposed populations have examined possible associations between PFOA and increased risk of liver disease. In workers, no association between estimated cumulative serum PFOA levels and the risk of non-hepatitis liver disease was observed (Steenland et al. 2015). Similarly, two studies of residents living near the Washington Works PFOA facility reported no increases in liver disease. In a study by Anderson-Mahoney et al. (2008), no significant increases in self-reported liver problems were found in residents primarily served by the Lubeck Public Water Service District or Little Hocking Water District; the study did not measure serum PFOA levels. In a C8 Health Project study that included workers at the Washington Works facility,

estimated cumulative serum PFOA levels were not associated with any liver disease or enlarged liver, fatty liver, or cirrhosis (Darrow et al. 2016).

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. The possible association between PFOA exposure and hepatic enzymes has been examined in seven occupational exposure studies that have found inconsistent results. A small study of Italian perfluoroalkyl workers did not find associations between serum PFOA and ALT, AST, or GGT activities when only current workers were examined (Costa et al. 2009). In analysis of all workers (current, former, and non-exposed workers), associations between serum PFOA levels and ALT and GGT activities were found; total bilirubin was also inversely associated with serum PFOA. Another small study of workers at a fluorochemical facility in China found an association between serum PFOA and AST activity, but not ALT activity (Wang et al. 2012). Gilliland and Mandel (1996; data also reported in Gilliland 1992) did not find associations between serum fluorine levels (used as a surrogate for serum PFOA) and ALT, AST, or GGT levels in workers. In a follow-up study of this facility, there were no differences between AST, ALT, GGT, or total bilirubin levels between workers in three exposure groups (Olsen et al. 2000); the mean serum PFOA levels in this study ranged from 5,000 to 6,400 ng/mL at three time points and the serum PFOA levels in the lowest exposure group ranged from 0 to <1,000 ng/mL. Increases in GGT and decreases in total bilirubin levels associated with increases in serum PFOA were observed in a study of workers exposed to high levels of PFOA and PFOS (Olsen and Zobel 2007); ALT activity was not affected. In a cross-sectional study of active workers at a PFOA facility, a modest but statistically significant positive association between serum PFOA and GGT activity was found (Sakr et al. 2007b). No associations were found for bilirubin levels or ALT and AST activities.

The possible associations between serum PFOA and serum enzyme and bilirubin levels were examined in two longitudinal occupational exposure studies. Sakr et al. (2007a) examined the relationship between serum PFOA and liver enzymes in a longitudinal study of 454 workers who had two or more measurements of serum PFOA from 1979 until the study was conducted. The average length of employment among workers with multiple PFOA measurements was 11 years, and, on average, 10.8 years elapsed between their first and last serum PFOA measurement. The means of the first and last PFOA measurement were 1,040 and 1,160 ng/mL, respectively. After adjustment for potential confounders, serum PFOA was associated with AST activity, but not ALT, GGT, or total bilirubin. The second study included 179 workers involved in the demolition of 3M perfluoroalkyl manufacturing facilities examined over a mean period of 164 days (Olsen et al. 2012). In workers with prior exposure to

PFOA who had a decrease in serum PFOA levels during the study period, there was a significant increase in ALT levels. An increase in serum PFOA levels did not significantly alter AST or total bilirubin levels.

Community and general population exposure studies have also examined possible associations between serum PFOA levels and alterations in serum hepatic enzyme and bilirubin levels. As with the occupational exposure studies, several studies of populations living near PFOA facilities have found inconsistent results. Darrow et al. (2016) found associations between ALT and bilirubin (inverse association) and estimated cumulative and 2005/2006 serum PFOA levels in participants of the C8 Health Project (6.5% of the participants also worked at the facility); there were no associations with GGT activity. Gallo et al. (2012) also reported a significant correlation between serum PFOA levels and ALT activity in C8 Health Project participants. Unlike the Darrow et al. (2016) study, a significant correlation between serum PFOA levels and GGT activity, but no correlation with direct bilirubin levels, was found. An earlier study of residents in the same area, as well as a study of residents near a facility in China, did not find associations between serum PFOA and ALT, AST, or GGT (Emmett et al. 2006b; Wang et al. 2012).

More consistent results were found in three general population studies. In studies utilizing data from NHANES, Gleason et al. (2015) and Lin et al. (2010) reported associations between serum PFOA levels and ALT, AST, and GGT activities; total bilirubin was also found to be associated with serum PFOA in the Gleason et al. (2015) study, but not in the Lin et al. (2010) study. A general population study conducted in Japan (Yamaguchi et al. 2013) also found associations between serum PFOA levels and AST, ALT, and GGT activities.

Although a number of epidemiological studies have found associations between serum PFOA and serum hepatic enzyme and bilirubin levels, many of the investigators noted that liver biomarker levels were typically within the normal range. Four studies examining the risk of having biomarker levels outside of the normal range provide useful information for evaluating the health impact of the enzyme level alterations. For ALT, Gallo et al. (2012) and Gleason et al. (2015) found increased risks of abnormal levels in C8 and NHANES participants, respectively. In contrast, Olsen and Zobel (2007) and Emmett et al. (2006b) did not find increased risks of abnormal ALT levels in workers and C8 participants, respectively. No alterations in the risk of abnormal AST levels associated with elevated serum PFOA levels were observed in NHANES participants (Gleason et al. 2015). Emmett et al. (2006b) found a decrease in the risk of abnormal AST levels with increasing serum PFOA levels in community members. Associations between the risk of elevated GGT and serum PFOA were found in the study conducted by

Gleason et al. (2015), but not in the Olsen and Zobel (2007), Gallo et al. (2012), or Emmett et al. (2006b) studies. Similarly, Gleason et al. (2015) reported an association between serum PFOA and the risk of elevated bilirubin levels, whereas Gallo et al. (2012) did not find this association in the higher exposed population.

One limitation to the interpretation of the serum hepatic enzyme data is confounding factors that should be considered in analyses; these include age, body mass index (BMI), serum lipid levels (triglycerides and total cholesterol), alcohol consumption, smoking, physical activity, and glucose levels (Deb et al. 2018; Kim et al. 2008). Although many of the studies accounted for age, BMI, smoking, and alcohol consumption, none of the studies adjusted for all of these potential confounders.

Epidemiological Studies—Serum Lipids. Occupational, community, and general population studies have examined the possible associations between serum PFOA levels and serum lipid levels; the results of these studies are presented in Table 2-12. Summaries of the changes in serum total cholesterol and LDL cholesterol levels, as well as the risk associated with elevated serum cholesterol and LDL cholesterol levels, are presented in Figures 2-11, 2-12, 2-13, and 2-14.

A study of workers at a manufacturing facility in Italy found higher total cholesterol and non-high-density lipoprotein (HDL)-cholesterol levels (non-HDL cholesterol was estimated by subtracting HDL cholesterol from total cholesterol) in the PFOA-exposed workers, as compared to levels in workers who were not exposed to PFOA (Costa 2004). A second study at this facility (Costa et al. 2009) also found an association between serum PFOA levels and total cholesterol levels, but no association with HDL cholesterol levels. No associations were found for HDL cholesterol or triglyceride levels. In another small study of workers at a fluorochemical facility in China (Wang et al. 2012), no associations between serum PFOA and total cholesterol, LDL cholesterol, or triglyceride levels were observed; the study did find an inverse association between serum PFOA and HDL cholesterol levels.

Several studies have examined workers at 3M facilities in Cottage Grove, Minnesota, Decatur, Alabama, and/or Antwerp, Belgium; workers at these facilities were also exposed to high levels of PFOS. Gilliland and Mandel (1996; data also reported in Gilliland 1992) examined workers at the Cottage Grove facility in 1990 and found no associations between serum fluorine levels (used as a surrogate for PFOA) and total cholesterol, LDL cholesterol, or HDL cholesterol. In a follow-up to this study, Olsen et al. (2000) examined workers in 1993, 1995, and 1997; only 17 workers were examined at all three time periods,

Figure 2-11. Serum Total Cholesterol Levels Relative to Serum PFOA Levels
(Presented as percent change in cholesterol levels)

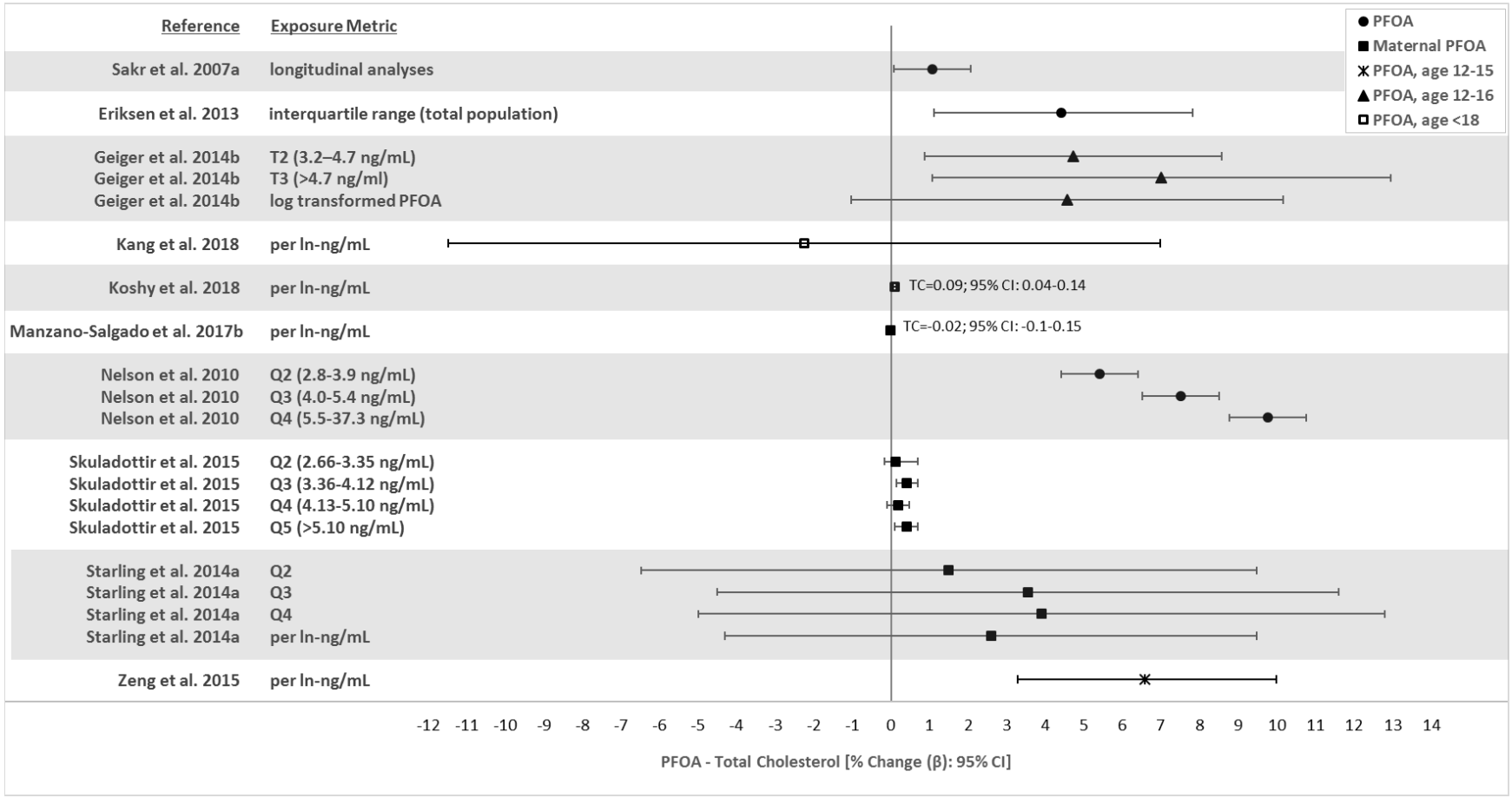


Figure 2-12. Risk of Abnormal Cholesterol Levels Relative to PFOA Levels (Presented as Adjusted Ratios)

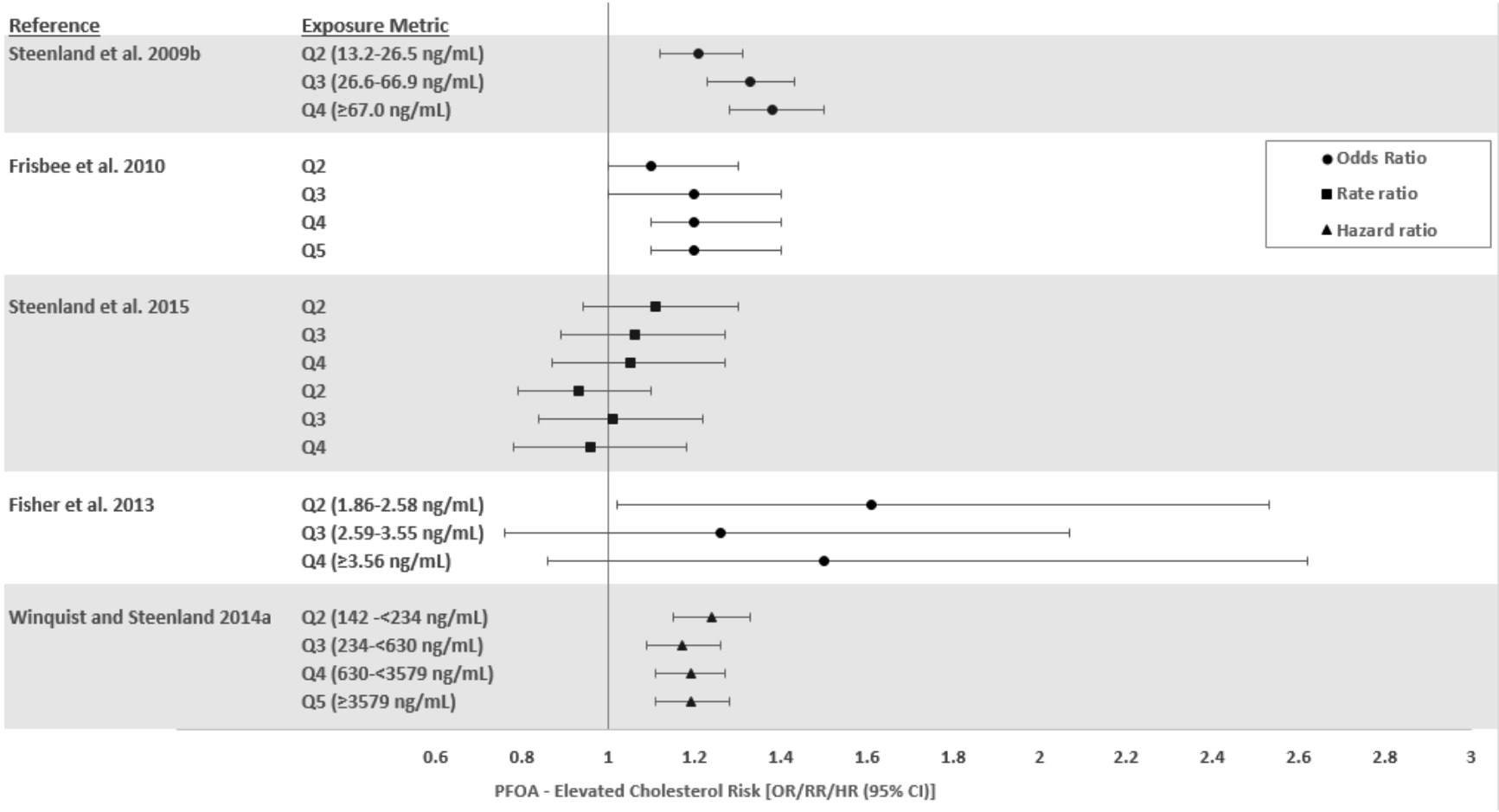


Figure 2-13. Serum LDL Cholesterol Levels Relative to Serum PFOA Levels
(Presented as percent change in LDL cholesterol levels)

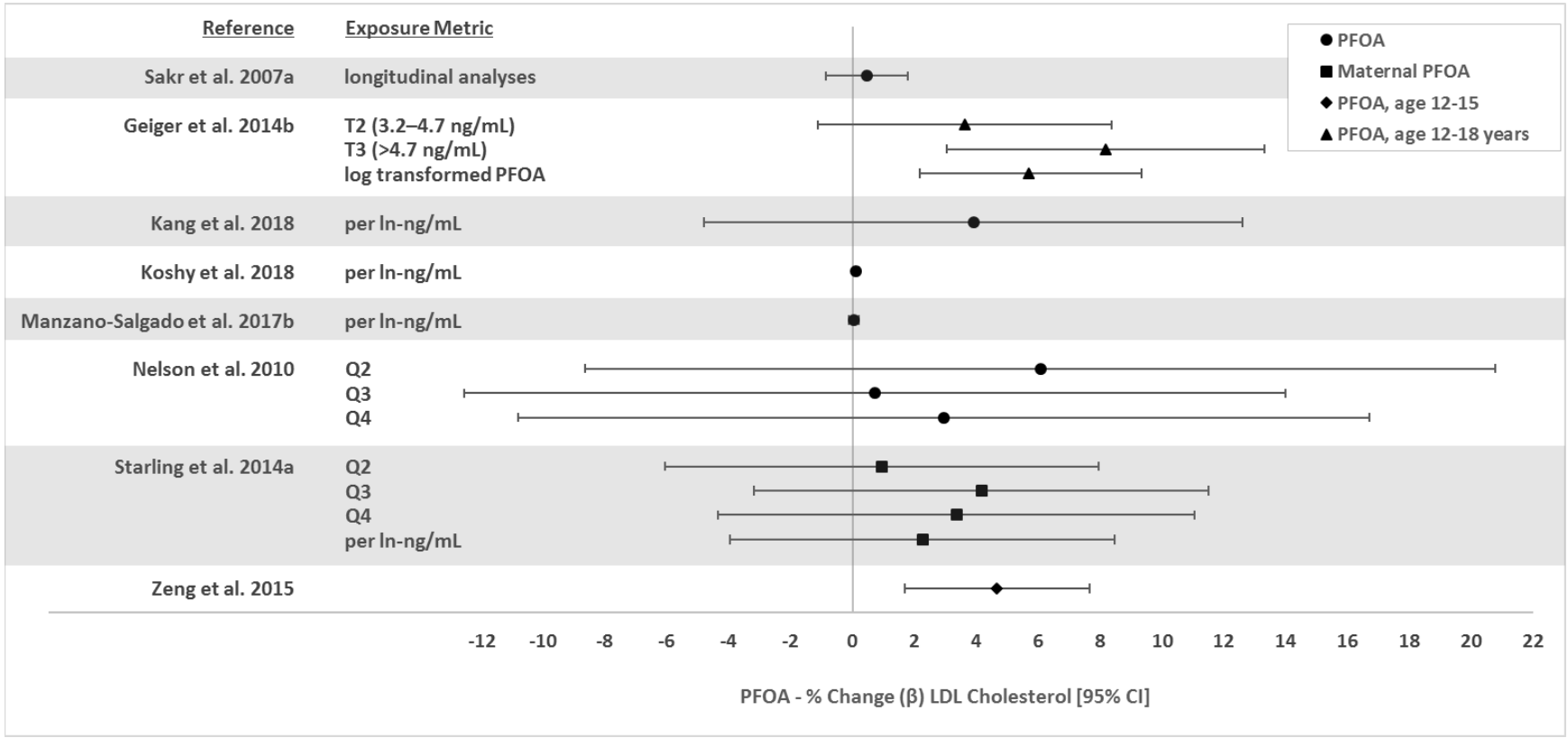
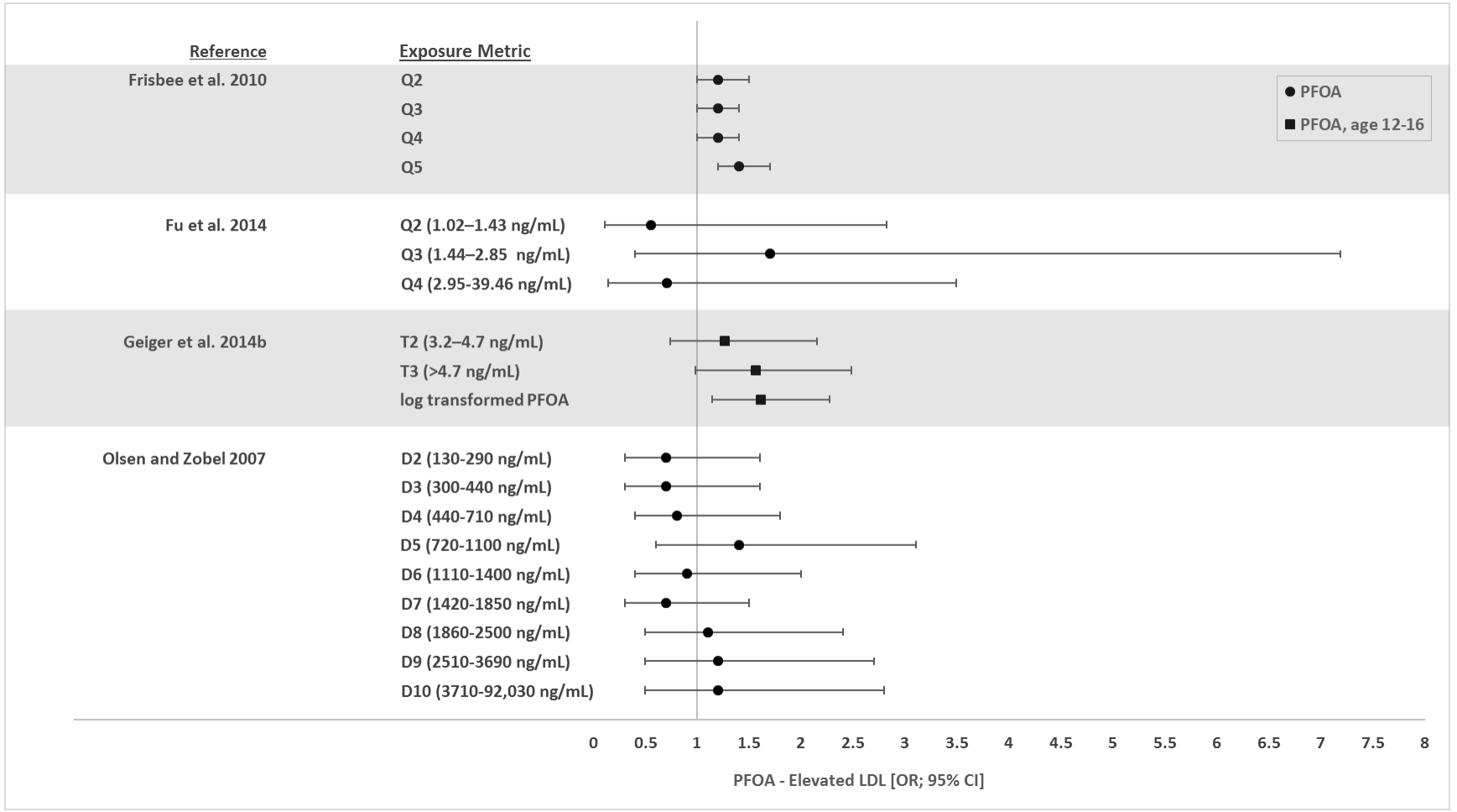


Figure 2-14. Risk of Abnormal LDL Cholesterol Levels Relative to PFOA Levels (Presented as Adjusted Ratios)



21 workers were examined in 1995 and 1997, and 68 workers were examined in 1993 and 1995. The study did not adjust for the use of cholesterol-lowering medication. When workers were categorized by blood PFOA levels (0–<1,000, 1,000–<10,000, and >10,000 ng/mL), no significant differences in serum cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels were found at any of the monitoring periods. A study in workers at the three 3M facilities, most of whom were not taking cholesterol-lowering medications, did not find associations between serum PFOA levels and total cholesterol or LDL cholesterol levels; however, serum PFOA levels were associated with elevated triglyceride levels and inversely associated with HDL cholesterol levels (Olsen and Zobel 2007). The study did not find increases in the risk of elevated total cholesterol (≥200 mg/dL), elevated LDL cholesterol (≥130 mg/dL), elevated triglyceride (≥150 mg/dL), or decreased HDL cholesterol (≤40 mg/dL) levels in workers with serum PFOA levels in the highest deciles. In addition to these cross-sectional studies, two longitudinal studies were conducted at these facilities. Using data for 174 workers with medical surveillance data in 2000 and 1997 and/or 1995, Olsen et al. (2003a) found that serum PFOA was a significant predictor of cholesterol and triglyceride levels, which was primarily due to 21 workers at the Antwerp facility (mean serum level 8,400 ng/mL) whose serum PFOA levels increased over time. In a longitudinal study, Olsen et al. (2012) examined workers (none of the subjects reported using cholesterol-lowering medication) involved in the demolition of 3M perfluoroalkyl manufacturing facilities; serum PFOA and lipid levels were measured prior to the demolition and after demolition (mean time interval of 164 days). The mean baseline serum PFOA levels were 881 ng/mL in 14 3M workers with prior PFOA or PFOS exposure and 28.9 ng/mL in the remaining 165 workers. Among the 119 workers whose serum PFOA/PFOS levels (mean increase 50.9 ng/mL) increased during the observation period, there was a significant increase in HDL cholesterol levels, but no change in total cholesterol or non-HDL cholesterol levels. No significant alterations in serum lipid levels were observed in the 55 workers whose serum PFOA/PFOS levels decreased during the observation period. In workers whose baseline levels of PFOA and PFOS were <15 and <50 ng/mL, respectively, there were no significant differences between pre- and post-exposure serum lipid levels.

Investigators have also examined workers at the DuPont Washington Works facility in West Virginia. In a cross-sectional study, Sakr et al. (2007b) found associations between serum PFOA levels and total cholesterol, LDL cholesterol, and very-low-density lipoprotein (VLDL) cholesterol levels in all subjects and in a subset of subjects not taking cholesterol-lowering medication. The study did not find any association between serum PFOA and HDL cholesterol or triglyceride levels. In a second study, Steenland et al. (2015) did not find an association between estimated serum PFOA levels and the occurrence of elevated cholesterol levels that required medication. In a longitudinal study of workers

who had at least two serum PFOA measurements between 1979 and 2004, Sakr et al. (2007a) found a positive association between serum PFOA and total cholesterol levels; no associations with triglycerides, LDL cholesterol, or HDL cholesterol were found. Total cholesterol levels increased 1.06 mg/dL for each 1,000 ng/mL increase in serum PFOA.

Several studies have been conducted of residents living near the Washington Works facility. A study by Emmett et al. (2006b) of adults and children living in a community serviced by the Little Hocking Water Authority did not find an association between serum PFOA levels and total cholesterol levels; the study included an adjustment for the use of cholesterol-lowering medication. Four larger-scale studies of participants in the C8 Science Panel studies found associations between serum PFOA levels and serum lipid levels (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009b; Winquist and Steenland 2014a). Positive associations between serum PFOA levels and total cholesterol and LDL cholesterol were found in a study of over 12,000 children and adolescents, with mean serum PFOA levels of 32.6 ng/mL in children (aged 1.0–11.9 years) and 26.3 ng/mL in adolescents (aged 12.0–17.9 years) (Frisbee et al. 2010). Serum PFOA was also positively associated with triglyceride levels. Additionally, there was an increased risk of elevated cholesterol (≥170 mg/dL) in subjects with serum PFOA levels in the 4th or 5th quintiles. Increased odds of high LDL cholesterol (≥110 mg/dL) were also observed for the 5th PFOA quintile (OR 1.4, 95% CI 1.2–1.7). The investigators noted that the dose-response relationship between serum PFOA and serum lipids was nonlinear, with greater increases in lipids observed at the lower serum PFOA levels. Similar findings were reported in a study of >46,000 adults with a median serum PFOA level of 26.6 ng/mL; the study excluded subjects who reported taking cholesterol-lowering medication (Steenland et al. 2009b). Associations were found between serum PFOA levels and total cholesterol, LDL cholesterol, and non-HDL cholesterol; a positive association between serum PFOA and triglycerides was also found. No associations between serum PFOA levels and HDL cholesterol levels were found. Increased risks of having high total cholesterol (≥240 mg/dL) were found in subjects with serum PFOA levels in the 2nd, 3rd, and 4th quartiles. The investigators noted that the odds of high total cholesterol from the 1st to the 5th quartile were approximately 40% for PFOA, which may be important given that the Framingham study found that the risk of coronary heart disease was about 1.8 times higher in subjects with total cholesterol levels >240 mg/dL as compared to subjects with levels <200 mg/dL. Steenland et al. (2009b) also found an association between serum PFOA levels and total cholesterol levels in a study of 10,746 adults taking cholesterol-lowering medication. Using both groups of subjects (taking or not taking cholesterol-lowering medication), the investigators analyzed whether taking cholesterol-lowering medication was associated with lower serum PFOA levels, which may be indicative of reverse causality. Although serum PFOA levels were significantly lower in subjects taking cholesterol-lowering

medication, the difference between the groups was low (4%). Using estimated cumulative serum PFOA levels as the exposure metric, Winquist and Steenland (2014a) found increased risks of hypercholesterolemia at estimated cumulative exposure levels ≥142 ng/mL. In a longitudinal study by Fitz-Simon et al. (2013), adults participating in the C8 Health Project and not taking cholesterol-lowering medication were examined twice, with an average of 4.4 years between examinations. Mean serum PFOA levels were 74.8 ng/mL at the first examination and 30.8 ng/mL at the second examination. In subjects whose serum PFOA levels halved between examinations, there was a 3.6% decrease in LDL cholesterol levels and 1.7% decrease in total cholesterol levels. However, there were very small changes in LDL cholesterol and total cholesterol levels in subjects whose serum PFOA levels decreased by >64% and there were slight increases in LDL cholesterol and total cholesterol levels in subjects whose serum PFOA levels fell by <50%. Changes in PFOA levels were not associated with changes in HDL cholesterol or triglyceride levels. Similarly, Wang et al. (2012) found no associations between serum PFOA levels and total cholesterol, HDL cholesterol, LDL cholesterol, or triglycerides in a study of adults living near a PFOA manufacturing facility in China; the mean serum PFOA level was 378.30 ng/mL and did not include an adjustment for the use of cholesterol-lowering medication.

General population studies were conducted in the United States, Canada, Denmark, Norway, Spain, Japan, Korea, China, and Taiwan; these studies have examined possible associations between serum PFOA levels and serum lipid levels in children, adolescents, pregnant women, and adults. In a study of 8–10-year-old children (median serum PFOA of 9.3 ng/mL), Timmermann et al. (2014) found an association between serum PFOA and triglyceride levels among obese children; this association was not found among normal weight children. In a study of adolescents (12–18 years of age) participating in NHANES (mean serum PFOA level of 4.2 ng/mL), Geiger et al. (2014b) found associations between serum PFOA and total cholesterol and LDL cholesterol levels; no associations were found for HDL cholesterol or triglycerides. The study also found increased risks of elevated total cholesterol levels (>170 mg/dL) associated with serum PFOA levels. No alterations in the risk of elevated LDL cholesterol or triglycerides or decreased HDL cholesterol were found. Associations between serum total cholesterol, LDL cholesterol, and triglycerides have also been observed in a study of Taiwanese adolescents (12–15 years of age, median PFOA level of 9.3 ng/mL) (Zeng et al. 2015); no association was found for HDL cholesterol. A fourth study found associations between maternal PFOA levels and total cholesterol and LDL cholesterol in 7- and 15-year-old girls, but no associations for girls whose maternal PFOA levels were in the 2nd or 3rd tertiles (Maisonet et al. 2015b). No associations were found for HDL cholesterol or triglyceride levels. A study of children enrolled in the World Trade Center Health Registry found associations between serum PFOA levels and elevated serum cholesterol, LDL cholesterol, and

triglyceride levels, but no association with HDL cholesterol (Koshy et al. 2017). Another study of children aged 3–18 years found no associations between serum PFOA levels and total cholesterol, LDL cholesterol, or triglycerides (Kang et al. 2018). Manzano-Salgado et al. (2017b) found no association between maternal serum PFOA levels and serum lipid levels in 4-year-old children.

Studies in adults have found mixed results for serum lipids. Using NHANES data for adults not taking cholesterol-lowering medication (mean serum PFOA level of 4.6 ng/mL), Nelson et al. (2010) found an association between serum PFOA levels and non-HDL cholesterol levels; no associations were found for total cholesterol, LDL cholesterol, or HDL cholesterol. Another study of NHANES participants that statistically adjusted for use of cholesterol-lowering medication found no associations between serum PFOA and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels (Liu et al. 2018b). Associations between serum PFOA levels and total cholesterol levels were also found in a study of Danish adults not taking cholesterol-lowering medication (mean serum PFOA level of 7.1 ng/mL) (Eriksen et al. 2013). A study in Chinese adults (median PFOA level of 1.43 ng/mL) also found associations between serum PFOA and total cholesterol and LDL cholesterol, with no associations for HDL cholesterol or triglycerides (Fu et al. 2014a). This study did not find increased risks of elevated total cholesterol, LDL cholesterol, or triglycerides or decreased HDL cholesterol associated with serum PFOA. A second study of Chinese men found an association between serum PFOA and triglyceride levels, but no association with HDL cholesterol levels (Yang et al. 2018). A study of pregnant women in Denmark also found an association between serum PFOA (mean serum PFOA level of 4.1 ng/mL at gestation week 30) and total cholesterol levels (Skuladottir et al. 2015). No associations between serum PFOA levels and total cholesterol, LDL cholesterol, or non-HDL cholesterol levels were found in Canadian adults not taking cholesterol-lowering medication with a geometric mean serum PFOA level of 2.46 ng/mL (Fisher et al. 2013). In a second study of pregnant women (median PFOA level of 2.25 ng/mL at gestation week 18), no associations between plasma PFOA and total cholesterol, LDL cholesterol, or triglycerides were found (Starling et al. 2014a). The study did find an association between plasma PFOA and HDL cholesterol.

A number of epidemiological studies have reported associations between serum PFOA levels and serum lipid levels; the most consistently found alteration was for increased serum total cholesterol levels. Associations between serum PFOA and serum cholesterol levels have been observed in occupational (Costa 2004; Costa et al. 2009; Sakr et al. 2007a, 2007b), community (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009b; Winquist and Steenland 2014a), and general population (Eriksen et al. 2013; Fu et al. 2014a; Geiger et al. 2014b; Skuladottir et al. 2015; Zeng et al. 2015) studies, whereas

other investigators have not found associations in worker populations (Gilliland and Mandel 1996; Olsen et al. 2000; Olsen and Zobel 2007; Steenland et al. 2015; Wang et al. 2012), community populations (Emmett et al. 2006b; Wang et al. 2012), or general populations (Fisher et al. 2013; Nelson et al. 2010; Starling et al. 2014a). Longitudinal studies conducted in workers and highly exposed residents strengthen the interpretation of this association between serum PFOA and serum lipid levels. Serum PFOA levels were found to be a significant predictor of serum cholesterol levels in workers examined at least twice in a ≥5-year period (Olsen et al. 2003a; Sakr et al. 2007a). Similarly, a study of highly-exposed residents examined twice with approximately 4 years between examinations found that there was a 1.7% decrease in serum total cholesterol levels in subjects whose serum PFOA levels decreased by 50% between examinations (Fitz-Simon et al. 2013). As noted in Steenland et al. (2010a), there is considerable variation in the strength of the association between PFOA and serum cholesterol, with the greatest changes in serum cholesterol occurring at lower PFOA levels. The change in cholesterol levels per ng/mL change in serum PFOA ranged from 0.0007, calculated from data from the Olsen et al. (2000) occupational exposure study, to 2.0 calculated from data from the Nelson et al. (2010) general population study; the mean serum PFOA levels in these studies were ~22,000 and 4 ng/mL respectively. In a clinical trial, administration of APFO to patients with advanced solid tumors at doses of 50–1,200 mg weekly for 6 weeks resulted in decreases in serum cholesterol levels; the marked decreases in serum cholesterol levels were observed at serum PFOA concentrations of 175,000–230,000 ng/mL (Convertino et al. 2018). These results are similar to those observed in laboratory animals, suggesting that the dose-response curve may be biphasic. Steenland et al. (2010a) and Frisbee et al. (2010) suggested that this may be due to a steep dose-response curve at low PFOA levels, which flattens out at higher PFOA levels and may be indicative of saturation. A similar pattern was also observed in the risks of elevated cholesterol per increases in serum PFOA levels (Figure 2-14). Several investigators have explored whether PFOA and cholesterol could be jointly affected or whether the associations were due to reverse causality (i.e., increased cholesterol resulted in increased serum PFOA levels). Butenhoff et al. (2012c) explored the issues of whether PFOA distributes into serum lipoprotein fractions, and whether increases in serum lipoproteins would result in increases in serum PFOA. They concluded that there was limited distribution to plasma lipoproteins, and did not consider it a non-causal factor. The Steenland et al. (2009b) study found slightly lower serum PFOA levels (4%) among individuals taking cholesterol medication, as compared to those not taking medication and noted that this was primarily a function of the large sample size. This finding does not support reverse causality.

Laboratory Animal Studies. Information from inhalation studies in animals is limited. Head-only exposure of male rats to 810 mg/m^3 APFO dusts for 4 hours caused liver enlargement, but

microscopically, the liver tissue appeared normal (Kennedy et al. 1986). Exposure head-only of male rats to 0, 1, 7.6, or 84 mg/m^3 APFO dusts 6 hours/day, 5 days/week for 2 weeks resulted in significant increases in absolute and relative liver weight at 7.6 and 84 mg/m^3 on exposure day 10; in rats from the 84 mg/m^3 group, absolute and relative liver weights were still significantly increased 28 days after exposure ceased (Kennedy et al. 1986). The activities of serum enzymes markers of liver function were unremarkable except for alkaline phosphatase, which was significantly increased in the 7.6 and 84 mg/m^3 groups immediately after exposure on day 10 and remained elevated in the 84 mg/m^3 group on day 14 of recovery. Histopathological changes were restricted to the 7.6 and 84 mg/m^3 groups and consisted of panlobular and centrilobular hepatocellular hypertrophy and necrosis. Panlobular hepatocellular hypertrophy was seen only after the 10th exposure, but was limited to the centrilobular hepatocytes 14 or 28 days after exposure terminated, and was absent 42 days following cessation of exposure. Inhalation exposure of pregnant rats to 25 mg/m^3 APFO dusts 6 hours/day during GDs 6–15 induced an 18% increase in absolute liver weight (Staples et al. 1984); no significant effect was reported in rats exposed to ≤10 mg/m^3.

Nose-only exposure of male CD rats to 67 mg/m^3 ammonium perfluorononanoate dusts for 4 hours induced significant increases (28–37%) in absolute and relative liver weight, assessed 5 and 12 days after exposure (Kinney et al. 1989). Histopathological examinations were not conducted in this study.

The liver is the main target organ for perfluoroalkyls in animals following short- or long-term oral exposures. The hepatic response to exposure to many perfluoroalkyls, particularly in rodents, is initiated by the activation of the nuclear hormone receptor, PPARα, which triggers a characteristic sequence of morphological and biochemical events characterized by liver hypertrophy and alteration of a wide range of enzymes, particularly those involved in lipid metabolism. It appears that PFOA can also damage the liver via a method independent of PPARα resulting in increases in liver weight, hepatocellular hypertrophy, microvesicular steatosis, and cholangiopathy (Abbott et al. 2007; Das et al. 2017; Minata et al. 2010; Wolf et al. 2008a; Yang e al. 2002b).

The most sensitive liver effect observed in rats and mice after acute oral exposure to PFOA is an increase in liver weight (Cook et al. 1992; Das et al. 2017; Eldasher et al. 2013; Haughom and Spydevold 1992; Ikeda et al. 1985; Iwai and Yamashita 2006; Kawashima et al. 1995; Kennedy 1987; Liu et al. 1996; Loveless et al. 2006; Pastoor et al. 1987; Permadi et al. 1992, 1993; Qazi et al. 2012; White et al. 2009; Wolf et al. 2007, 2008a; Xie et al. 2003; Yahia et al. 2010; Yang et al. 2001, 2002b). In rats orally administered 50 mg/kg/day PFOA for 1, 3, or 7 days, a 10% increase in liver weight was observed after

the first dose; however, the relative liver weight was not significantly different from controls (Pastoor et al. 1987). After 3 days of exposure, the relative liver weight was significantly higher (36%) than controls. Similarly, in mice, exposure to 390 mg/kg/day PFOA in the diet resulted in a significant increase in liver weight after 5 days of exposure, but not after 2 days of exposure (Permadi et al. 1992). The lowest LOAELs for increased relative liver weight in rats were 4.7 mg/kg/day in a 7-day study (Kawashima et al. 1995) and 2 mg/kg/day in a 14-day study (Liu et al. 1996); these studies also identified NOAELs of 2.4 and 0.2 mg/kg/day, respectively. In mice, the lowest LOAEL for increases in liver weight was 1 mg/kg/day PFOA administered in the diet for 10 days (Yang et al. 2001) or administered via gavage for 7 days (Eldasher et al. 2013; Wolf et al. 2008a). Pastoor et al. (1987) noted that oral administration of 50 mg/kg/day PFOA to rats for 7 days resulted in a 2-fold increase in absolute and relative liver weight, but no significant change in total deoxyribonucleic acid (DNA), indicating that the hepatomegaly represented hypertrophy rather than hyperplasia. Few acute-duration studies included histological examinations of the liver. Centrilobular and midzonal hypertrophy was observed in mice administered 1 or 3 mg/kg/day PFOA via gavage for 7 days; panlobular hypertrophy with cytoplasmic vacuolation was observed at 10 mg/kg/day (Wolf et al. 2008a). Qazi et al. (2010a) reported hepatocellular hypertrophy in mice exposed to 3.5 mg/kg/day PFOA in the diet for 10 days. Elcombe et al. (2010) reported hepatocellular hypertrophy in rats orally exposed to 18 mg/kg/day for 7 days, but not after 1 day of exposure. Increases in steatosis and triglyceride levels were observed in the livers of mice administered 10 mg/kg/day for 7 days (Das et al. 2017). A related liver effect was the finding of reduced serum cholesterol and triacylglycerol levels in rats administered 16 mg/kg/day PFOA in the diet for 7 days (Haughom and Spydevold 1992) and decreases in serum cholesterol and triglyceride levels in rats administered 18 mg/kg/day PFOA via gavage for 7 days (Elcombe et al. 2010).

Similar to the acute-duration studies, intermediate-duration oral exposure to PFOA resulted in increases in absolute and relative liver weights in rats (Biegel et al. 2001; Butenhoff et al. 2004b; Griffith and Long 1980; Perkins et al. 2004) and mice (Abbott et al. 2007; Ahmed and Abd Ellah 2012; Albrecht et al. 2013; Griffith and Long 1980; Kennedy 1987; Lau et al. 2006; Son et al. 2008; Wolf et al. 2007; Yang et al. 2009). The lowest dose resulting in increases in liver weight in rats was 0.96 mg/kg/day, observed following gavage administration of APFO for 28 days (Loveless et al. 2008); the lowest dose in mice was 0.5 mg/kg/day, observed in two 28-day studies using APFO (Kennedy 1987; Son et al. 2008). No significant alterations in liver weight were observed in rats administered 0.29 mg/kg/day for 28 days (Loveless et al. 2008) or in mice exposed to 0.2 mg/kg/day for 21 days (Kennedy 1987). Hepatocellular hypertrophy was the predominant histopathological alteration in rats (Cui et al. 2009; Griffith and Long 1980; Loveless et al. 2008; Perkins et al. 2004) and mice (Albrecht et al. 2013; Filgo et al. 2015a; Griffith

and Long 1980; Loveless et al. 2008; Tan et al. 2013); the severity of the hypertrophy was dose-related (Filgo et al. 2015a; Loveless et al. 2008). At higher doses, focal necrosis was observed (LOAEL of 29 mg/kg/day in rats and 0.96 mg/kg/day in mice exposed for 28 days) (Loveless et al. 2008). Fatty changes were observed in rats administered 20 mg/kg/day for 28 days (Cui et al. 2009) and mice administered 9.6 mg/kg/day (Loveless et al. 2008). No significant alterations in liver weight or histopathology were observed in rats allowed to recover for 8 weeks following a 13-week exposure to 0.6–6.5 mg/kg/day (Perkins et al. 2004). Intermediate-duration exposure to PFOA also resulted in decreases in serum HDL cholesterol levels in rats and mice administered ≥0.29 or 0.96 mg/kg/day, respectively, for 28 days (Loveless et al. 2008). Serum cholesterol levels were decreased in rats administered 0.29 or 0.96 mg/kg/day (no changes were observed at higher doses) and in mice administered 9.6 or 29 mg/kg/day (Loveless et al. 2008). Similarly, serum triglyceride levels were decreased in rats administered 0.29–9.6 mg/kg/day and in mice administered 9.6 or 29 mg/kg/day (Loveless et al. 2008). In a study of mice fed a western-type diet, increases in plasma cholesterol levels were observed after 6 weeks of dietary exposure to 0.55 mg/kg/day in BALB/c or C57BL/6 mice (Rebholz et al. 2016). The results of this study suggest that diet (fat intake and/or cholesterol levels) may influence the response to PFOA and may account for some of the differences observed in humans and rats fed a standard diet, which is typically low in fat.

Chronic exposure of rats to PFOA resulted in hepatocellular hypertrophy, hepatocellular necrosis, and portal mononuclear cell infiltration after a 1-year exposure to a LOAEL of 15 mg/kg/day in the diet (3M 1983; Butenhoff et al. 2012c). A 2-year exposure to 15 mg/kg/day resulted in hepatocellular hypertrophy, cystoid degeneration, and portal mononuclear cell infiltration (3M 1983; Butenhoff et al. 2012c). The study also found significant increases in ALT and AST levels in male rats exposed to 1.5 mg/kg/day. A second chronic exposure study found significant increases in relative liver weight in rats exposed to 13.6 mg/kg/day in the diet for 2 years; no non-neoplastic lesions were noted in the liver (Biegel et al. 2001).

Studies in monkeys suggest that longer-term exposure may also result in liver effects. Significant increases in absolute and relative liver weight were observed in Cynomolgus monkeys exposed to 20/30 mg/kg/day administered via capsules for 26 weeks (Butenhoff et al. 2002). A significant increase in absolute, but not relative, liver weight was also observed in monkeys administered 3 or 10 mg/kg/day. However, no histological alterations were observed in the livers at the doses tested. Similarly, no histological alterations were observed in the livers of Cynomolgus monkeys administered 2 or 20 mg/kg/day via capsules for 30 days (Thomford 2001) or Rhesus monkeys administered 3 or

10 mg/kg/day via gavage for 90 days (Griffith and Long 1980). Significant increases in serum triglyceride levels were observed in the 10 and 20/30 mg/kg/day groups; the increases were statistically significant at only some of the time points (Butenhoff et al. 2002). At 10 mg/kg/day, increases in serum triglyceride levels at 4, 10, and 14 weeks of exposure were significantly higher than pre-treatment levels. Increases in cholesterol levels were only observed in the 20/30 mg/kg/day group after 13 weeks of exposure, but not after 26 weeks. No alterations in serum cholesterol or triglyceride levels were observed in the Thomford (2001) study.

Several studies have examined PPARα-null mice to assess whether PFOA-induced liver effects can also occur via a mechanism independent of PPARα-receptor activation. Similar to wild-type mice, exposure to PFOA resulted in significant increases in liver weight (Abbott et al. 2007; Das et al. 2017; Minata et al. 2010; Wolf et al. 2008a; Yang e al. 2002b). Abbott et al. (2007) found that the effect level was slightly higher in PPARα-null mice than wild-type mice (3 versus 1 mg/kg/day) following oral exposure on GDs 1–17 (liver weights measured at weaning). Wolf et al. (2008a) and Minata et al. (2010) reported the same effect level (1 or 5 mg/kg/day, respectively) in PPARα-null mice and wild-type mice administered PFOA via gavage for 7 days or 4 weeks. Wolf et al. (2008a) found dose-related increases in hepatocellular cytoplasmic vacuoles at ≥1 mg/kg/day and suggested that the increase in liver weight was due to the accumulation of PFOA in the hepatocytes rather than a toxic response. Hepatocyte proliferation was also observed at 10 mg/kg/day. Unlike the Wolf et al. (2008a) study, the Minata et al. (2010) 4-week study reported hepatocellular hypertrophy and microvesicular steatosis in the PPARα-null mice (no incidence data were provided and it is unclear at what dose levels these effects were found); cytoplasmic vacuolation was also reported in the hepatocytes. Filgo et al. (2015a) also reported hepatocellular hypertrophy in PPARα-null mice; the LOAEL was 3 mg/kg/day, which was higher than the LOAEL of 0.3 mg/kg/day found in wild-type mice. Minata et al. (2010) also reported cholangiopathy in both the wild-type and PPARα-null mice, but noted that the effect was more intensive in the PPARα-null mice. No significant alterations in steatosis or triglyceride accumulation were observed in PPARα-null mice administered 10 mg/kg/day for 7 days, but were observed in wild-type mice (Das et al. 2017). Additionally, significant decreases in serum total cholesterol levels at 5.2 and 10.2 mg/kg/day and increases at 20.7 mg/kg/day were observed in the PPARα-null mice; significant decreases in total cholesterol were observed in the wild-type mice at 10.2 and 20.7 mg/kg/day doses. Serum triglyceride levels were increased in both strains at 5.2 and 10.2 mg/kg/day doses and in the PPARα-null mice at 20.7 mg/kg/day (Minata et al. 2010).

Intermittent application of 20, 200, or 2,000 mg/kg APFO to the skin of rats for 2 weeks resulted in the presence of one or more foci of coagulative necrosis in the livers from all treated groups (Kennedy 1985). The Kupffer cells within the foci of hepatocellular necrosis contained large vesicular nuclei and were markedly increased in number. At 2,000 mg/kg/day, these changes were seen in three out of five rats killed on the 10th day of exposure, in three out of five rats killed on recovery day 14, and in one out of five rats killed on recovery day 42. This lesion occurred in two out of five rats from the 20 mg/kg/day dose group killed on day 10 of exposure. Serum ALT activity appeared elevated at termination of exposure in a dose-related manner, but without achieving statistical significance. A similar trend was seen for AST activity, but achieving statistical significance in the high-dose group. The blood concentrations of organofluorine on the 10th day of exposure were 10.2, 52.4, 79.2, and 117.8 µg/mL in the control, low-, mid-, and high-dose groups, respectively. A study in mice reported that application of 6.25 mg/kg/day PFOA on the dorsal surface of each ear for 4 days resulted in a 52% increase in absolute liver weight (Fairley et al. 2007); no significant effect occurred after application of 2.5 mg/kg/day.

Summary. Epidemiological studies examining the hepatotoxicity of PFOA have examined three outcomes—risk of liver disease, evidence of hepatocellular damage (as measured by alterations in serum hepatic enzymes and bilirubin levels), and alterations in serum lipid levels (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides)—among workers, residents living near a PFOA manufacturing facility with high levels of drinking water contamination, and the general population. Exposure to PFOA does not appear to be associated with increased risks of liver disease in workers or highly exposed community members. The epidemiological studies have found associations between serum PFOA levels and increases in serum ALT, AST, and GGT enzyme levels and decreases in serum bilirubin levels. However, the results have not been consistently found, and serum enzyme levels were typically within the normal range. Four studies examined the risk of serum enzyme levels outside of the normal range; the results were mixed for the risk of elevated ALT, with two studies finding an increased risk and two studies finding no association. A number of occupational, community, and general population studies have found associations between serum PFOA levels and serum total cholesterol levels; several studies have also found no associations. Studies examining the change in cholesterol levels per change in serum PFOA levels have found greater increases in serum cholesterol levels associated with serum PFOA levels at the lower range of PFOA levels and the dose-response curve suggests a biphasic relationship. Positive associations have also been observed for LDL cholesterol, although associations have not been consistently found. In general, no consistent associations were found between serum PFOA and HDL cholesterol or triglyceride levels.

Studies in laboratory animals have found strong associations between PFOA exposure and hepatotoxicity. Liver effects have been observed in rats exposed to airborne APFO dusts; in rats, mice, and monkeys following oral exposure for acute-, intermediate-, or chronic-durations; and in rats following dermal exposure. The observed effects typically include increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol and triglyceride levels. Other effects that have been observed include hyperplasia, necrosis, and fatty degeneration. Available evidence suggests that the increased liver weight, hypertrophy, and serum lipid alterations are likely due to PPARα initiation and therefore, may not be relevant to humans. However, other mechanisms of liver toxicity are also involved, as evidenced by liver effects observed in PPARα-null mice (Das et al. 2017; Minata et al. 2010; Wolf et al. 2008a). In contrast to the results observed in epidemiological studies, a clinical trial study in humans with advanced solid tumors exposed to very large doses of PFOA (Convertino et al. 2018) and human exposure to other PPARα agonists, such as fibrates (Staels et al. 1998), suggest that hypolipidemic effects, similar to those observed in rodents, may occur in humans exposed to PFOA, although humans may not be as sensitive as rodents.

PFOS

Epidemiological Studies—Liver Disease. Several studies have examined the possible association between PFOS exposure and liver diseases. No increases in deaths from cirrhosis of the liver were found in workers at the 3M facility in Decatur, Alabama (Alexander et al. 2003). Another study of workers at this facility found no significant alterations in the episodes of care for all liver disorders or all biliary duct disorders (Olsen et al. 2004a). However, among workers with at least 10 years of high potential exposure to PFOS, there were significant increases in episodes of care for cholelithiasis or acute cholecystitis and for all biliary tract disorders. A third study of workers at a PFOS facility in Decatur, Alabama did not find increases in cholelithiasis, cholecystitis, or liver disease (including cirrhosis and hepatitis) (Grice et al. 2007).

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. A series of studies conducted by Olsen and associates evaluated liver function (as assessed by serum liver enzymes) in workers at several 3M facilities involved in PFOS production. Using health data collected in 1995 and 1997, Olsen et al. (1999) did not find associations between serum PFOS and serum ALT, AST, or GGT enzymes at PFOS levels <6,000 ng/mL; a positive association with total bilirubin levels was found. No conclusions were drawn from the few workers with serum PFOS ≥6,000 ng/mL due to their small number (seven in 1995 and five in 1997 data). Similarly, no association of ALT, AST, or GGT and serum PFOA levels

were observed in groups of workers at these facilities examined in 1993 (111 subjects), 1995 (80 subjects), and/or 1997 (74 subjects) (Olsen et al. 2000). A subsequent evaluation of workers from the same plants, but that included women and a longitudinal analysis of the workers, reported that, after adjusting for potential confounding factors, there were no substantial changes in hepatic parameters (Olsen et al. 2003a). GGT levels in females and ALT levels in males with PFOS levels in the 4th quartile were significantly elevated in comparisons between individuals with serum PFOS levels in the 4th quartile to those with levels in the 1st quartile; however, there were no statistical adjustments for potential confounders. In contrast to these findings in workers, Gallo et al. (2012) reported significant increases in the risks of elevated ALT, GGT, and bilirubin levels in a study of C8 participants. Conflicting results have been found in general populations studies. Studies using the NHANES data set (Gleason et al. 2015; Lin et al. 2010) did not find associations between serum PFOS and ALT, AST, GGT, or total bilirubin levels. No increases in the risk of elevated levels of ALT, AST, or GGT were found (Gleason et al. 2015), although there was an increased risk of elevated total bilirubin levels. In a study of adults in Japan (Yamaguchi et al. 2013), significant correlations between serum PFOS and ALT, AST, and GGT levels were found.

Epidemiological Studies—Serum Lipids. Occupational, community, and general population studies have examined possible associations between serum PFOS levels and serum lipids; these data are summarized in Table 2-12. A graphical presentation of differences in total cholesterol and LDL cholesterol levels relative to serum PFOS levels and the risks of elevated total cholesterol and LDL cholesterol are presented in Figures 2-15, 2-16, 2-17, and 2-18.

In the Olsen occupational studies, significantly higher serum total cholesterol levels were found in workers with serum PFOS levels between 3,000 and 6,000 ng/mL (Olsen et al. 1999, 2003a). However, the studies found mixed results for associations between serum PFOS and other serum lipids, with one study finding an association with LDL cholesterol (Olsen et al. 1999) and the other finding an association with triglycerides (Olsen et al. 2003a). Longitudinal analysis was conducted using data for 174 workers with medical surveillance data in 2000 and 1997 and/or 1995 (Olsen et al. 2003a). No significant differences in serum PFOS levels were observed across the three time periods, and serum PFOS levels were not a significant predictor of cholesterol or triglyceride levels.

Two large-scale studies of participants in the C8 Science Panel studies found associations between serum PFOS levels and serum lipid levels (Frisbee et al. 2010; Steenland et al. 2009b). Associations between serum PFOS levels and total cholesterol, LDL cholesterol, and HDL cholesterol were found in a study of

Figure 2-15. Serum Total Cholesterol Levels Relative to Serum PFOS Levels
(Presented as percent change in cholesterol levels)

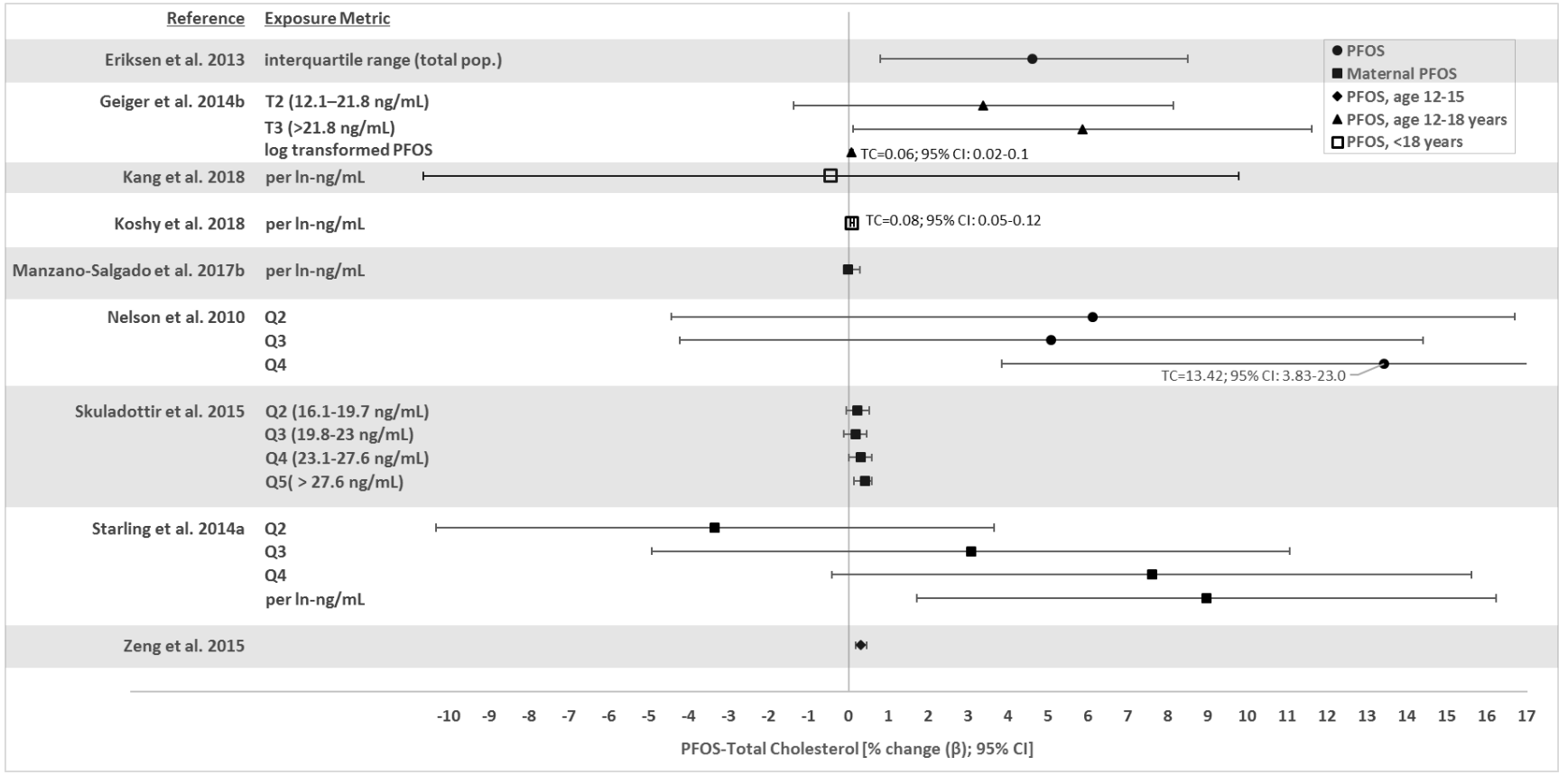


Figure 2-16. Risk of Abnormal Cholesterol Levels Relative to PFOS Levels (Presented as Adjusted Ratios)

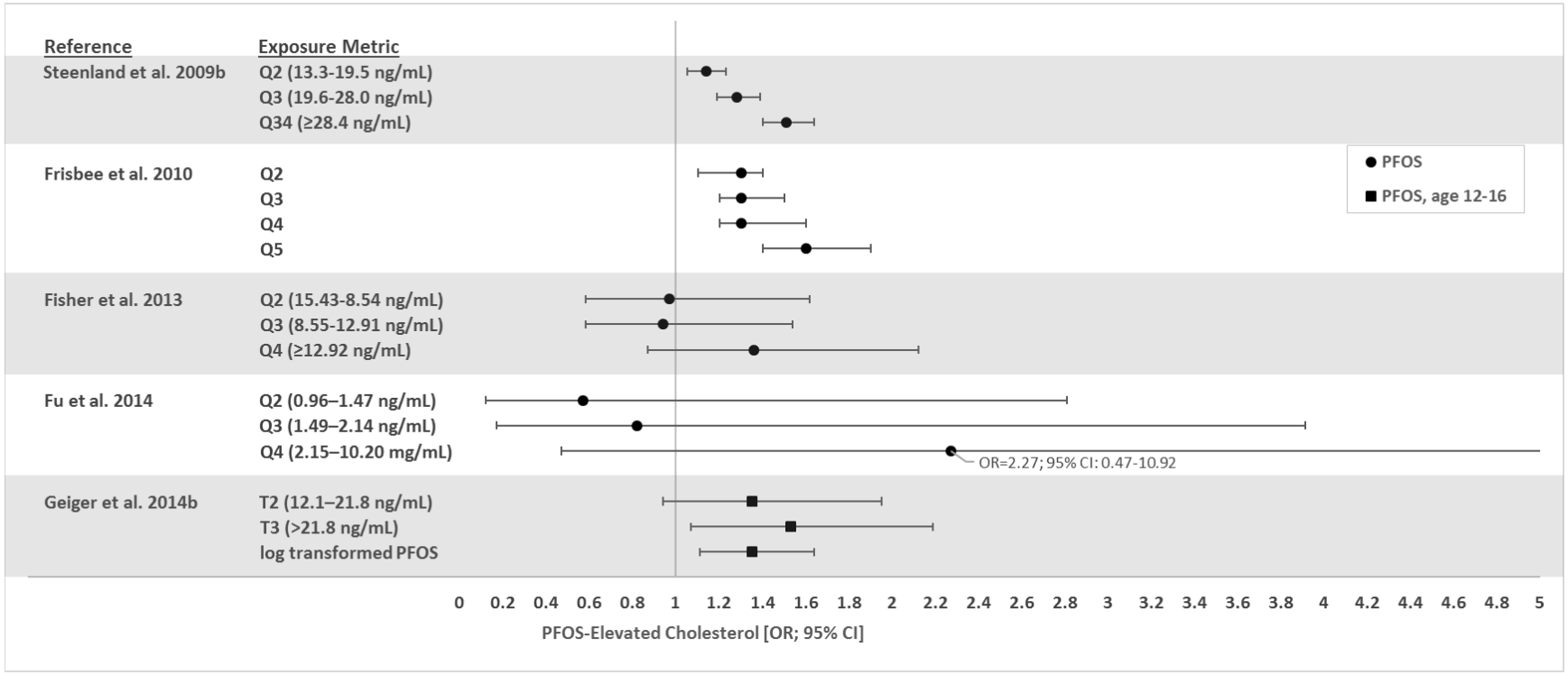


Figure 2-17. Serum LDL Cholesterol Levels Relative to Serum PFOS Levels
(Presented as percent change in LDL cholesterol levels)

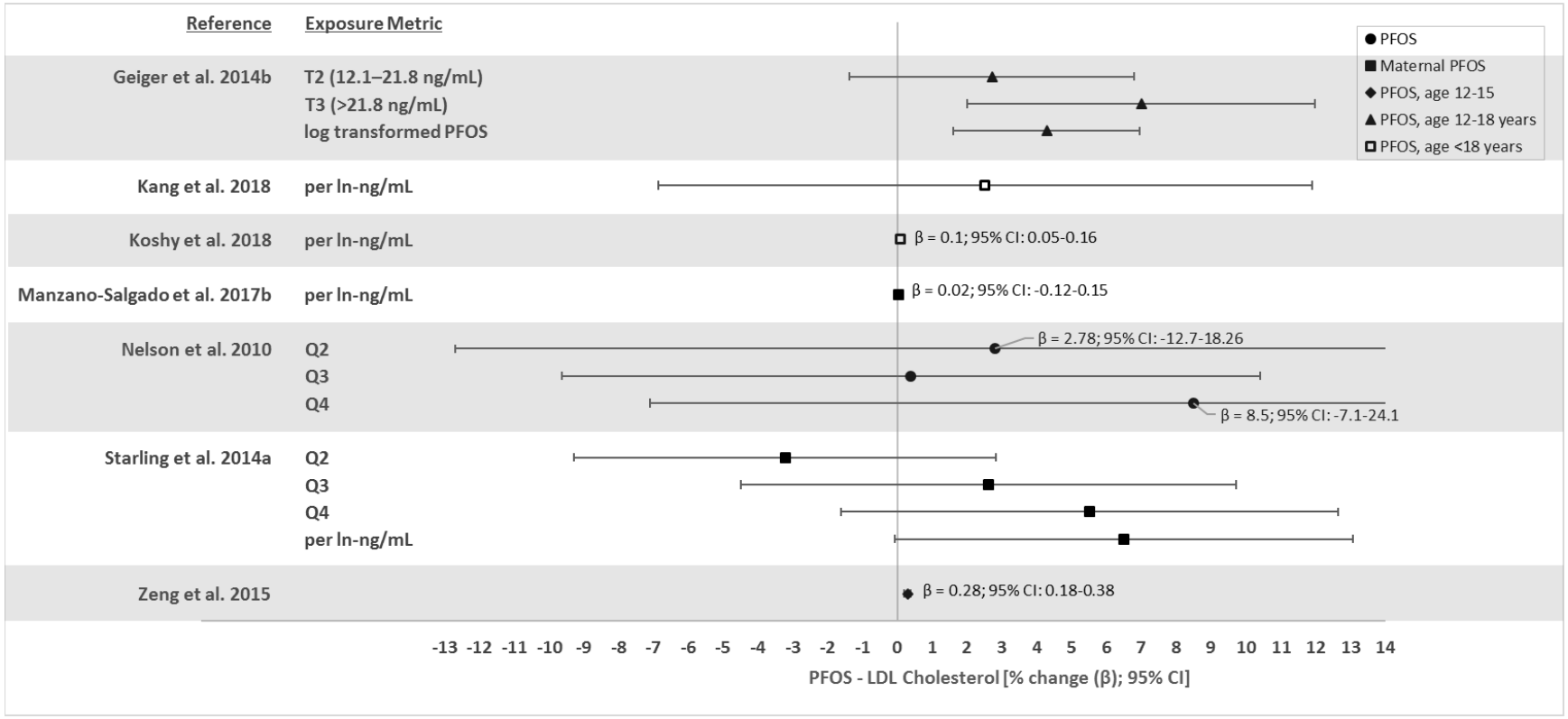
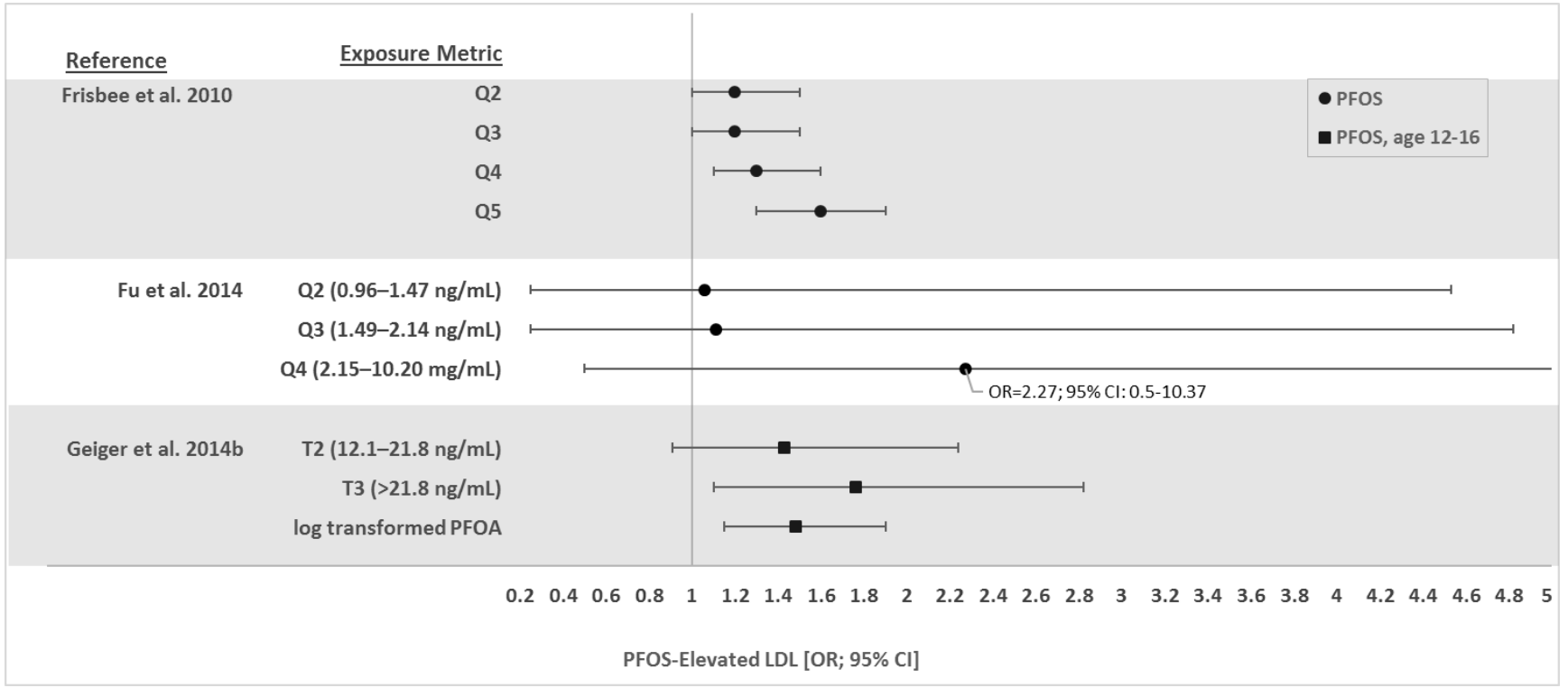


Figure 2-18. Risk of Abnormal LDL Cholesterol Levels Relative to PFOS Levels (Presented as Adjusted Ratios)



over 12,000 children and adolescents; the mean serum PFOS levels were 20.7 ng/mL in children (aged 1.0–11.9 years) and 19.3 ng/mL in adolescents (aged 12.0–17.9 years) (Frisbee et al. 2010). Similar findings were reported in a study of adults with a median serum PFOS level of 19.6 ng/mL; the study excluded subjects who reported taking cholesterol-lowering medication (Steenland et al. 2009b).

Associations were found between serum PFOS and total cholesterol, LDL cholesterol, and triglyceride levels, but not with HDL cholesterol. Participants with serum PFOS levels in the 2nd, 3rd, and 4th quartiles also had elevated risks of high cholesterol levels. Steenland et al. (2009b) noted that the odds of high cholesterol from the 1st to the 5th quintile was approximately 50% for PFOS, which may be important given that the Framingham study found that the risk of coronary heart disease was about 1.8 times higher in subjects with total cholesterol levels >240 mg/dL as compared to subjects with levels <200 mg/dL.

Steenland et al. (2009b) also examined over 10,000 participants who were taking cholesterol-lowering medication; an association between serum PFOS and total cholesterol levels was found in this group. Using both groups of subjects (taking or not taking cholesterol-lowering medication), the investigators analyzed whether taking cholesterol medication was associated with lower serum PFOA or PFOS levels, which may be indicative of reverse causality; no differences in serum PFOS levels were found between the two groups.

General population studies were conducted in the United States, Canada, and several European and Asian countries; these studies have found mixed results for associations between serum PFOS levels and serum lipids. Some studies have found associations between serum PFOS levels and serum total cholesterol (Nelson et al. 2010; Skuladottir et al. 2015; Starling et al. 2014a) and HDL cholesterol (Châteu-Degat et al. 2010); inverse associations between serum PFOS and HDL cholesterol (Starling et al. 2014a) and triglycerides (Châteu-Degat et al. 2010) were also found. However, other studies in adults have not found associations between serum PFOS and total cholesterol (Châteu-Degat et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Fu et al. 2014a; Liu et al. 2018b), non-HDL cholesterol (Fisher et al. 2013), LDL cholesterol (Châteu-Degat et al. 2010; Fisher et al. 2013; Fu et al. 2014a; Liu et al. 2018b; Starling et al. 2014a), HDL cholesterol (Fisher et al. 2013; Fu et al. 2014a; Liu et al. 2018b; Yang et al. 2018), or triglycerides (Fu et al. 2014a; Starling et al. 2014a; Liu et al. 2018b; Yang et al. 2018). Additionally, two studies did not find increased risks of elevated cholesterol levels (Fisher et al. 2013; Fu et al. 2014a). Several of these studies controlled for use of cholesterol-lowering medication (Châteu-Degat et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Nelson et al. 2010; Liu et al. 2018b). Overall, studies of children and adolescents have found associations for serum lipid levels. Geiger et al. (2014b) found increases in

the risk of elevated cholesterol and LDL cholesterol in children and adolescents aged 12–18 years; an association between serum PFOS and LDL cholesterol levels was also found. Zeng et al. (2015) found associations between serum PFOS and serum total cholesterol, LDL cholesterol, and triglyceride levels in children aged 12–15 years. Koshy et al. (2017) found an association between serum PFOS levels and serum total cholesterol, LDL cholesterol, and HDL cholesterol in children enrolled in the World Trade Center Health Registry. Timmermann et al. (2014) also found an association between serum PFOS and triglycerides only in obese Danish children (8–10 years of age), but not in normal weight children. In contrast, Maisonet et al. (2015b) found an inverse association between maternal serum PFOS and total cholesterol and LDL cholesterol in 15-year-old girls; no association was found when the girls were 7 years of age. Kang et al. (2018) did not find an association between serum PFOS and cholesterol, LDL cholesterol, or triglyceride levels in children aged 3–18 years, and Manzano-Salgado et al. (2017b) did not find associations between maternal serum PFOS and cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels in 4-year-old children.

Laboratory Animal Studies. Unpublished data summarized by OECD (2002) indicate that inhalation exposure of rats to lethal concentrations (1,890–45,970 mg/m^3) of PFOS dusts for 1 hour resulted in varying discoloration of the liver.

Consistent with the results for PFOA, acute-duration oral exposure of rats to PFOS resulted in increases in liver weight (Elcombe et al. 2012b; Era et al. 2009; Haughom and Spydevold 1992), hepatocellular hypertrophy (Elcombe et al. 2012b), and decreases in serum cholesterol and/or triglyceride levels (Elcombe et al. 2012a, 2012b; Haughom and Spydevold 1992). The lowest adverse effect level for increased liver weight, hypertrophy, and decreased serum cholesterol was 1.79 mg/kg/day in rats exposed to PFOS in the diet for 7 days (Elcombe et al. 2012b); however, a similar study by this group did not find significant alterations in liver weight or ALT, AST, or serum cholesterol levels after 7 days of exposure to 1.72 mg/kg/day (Elcombe et al. 2012a). Likewise, in mice, increases in liver weight (Fuentes et al. 2006; Qazi et al. 2009b, 2010a; Wan et al. 2011), hepatocellular hypertrophy (Qazi et al. 2010a), and decreases in serum cholesterol levels (Qazi et al. 2010a) were observed following acute exposure to PFOS. The lowest LOAEL for liver weight was 3 mg/kg/day in mice administered PFOS via gavage on GDs 6–18 (Fuentes et al. 2006); no effects were observed at 1.5 mg/kg/day. The only acute-duration mouse study that included histopathological examination of the liver and measurement of serum cholesterol levels identified a LOAEL of 8.5 mg/kg/day in mice exposed to PFOS in the diet for 10 days (Qazi et al. 2010a).

Intermediate-duration exposure to PFOS resulted in increased liver weight in rats (Cui et al. 2009; Curran et al. 2008; Elcombe et al. 2012a; Seacat et al. 2003; Thibodeaux et al. 2003) and mice (Bijland et al. 2011; Thibodeaux et al. 2003; Wan et al. 2011, 2014b; Xing et al. 2016; Yahia et al. 2008), hepatocellular hypertrophy in rats (Cui et al. 2009; Curran et al. 2008; Elcombe et al. 2012a; Seacat et al. 2003), decreased serum cholesterol levels in rats (Curran et al. 2008; Elcombe et al. 2012a; Luebker et al. 2005b; Seacat et al. 2003), decreased total cholesterol, triglyceride, non-HDL cholesterol, and HDL cholesterol levels in mice (Bijland et al. 2011), and increased serum AST and GGT levels in mice (Xing et al. 2016). A mouse study (Bijland et al. 2011) also showed dramatic decreases in the hepatic production of VLDL and HDL (Bijland et al. 2011). Another mouse study (Lee et al. 2015b) did not find increases in hepatic lipid levels in dams, although there were alterations in fetal livers. Only one of the intermediate-duration mouse studies included histopathological examination of the liver. Xing et al. (2016) reported cytoplasmic vacuolization, focal necrosis, and hepatocellular hypertrophy in mice exposed to PFOS via gavage for 30 days; however, the study did not report incidence; the lowest dose tested was 2.5 mg/kg/day. The lowest adverse effect level for liver effects in rats was 0.14 mg/kg/day for a significant increase in relative liver weight in female rats, but not male rats, exposed to PFOS in the diet for 28 days (Curran et al. 2008). This study also found significant decreases in serum cholesterol levels and increases in absolute and relative liver weights in males and females at 2.98 mg/kg/day and hepatocellular hypertrophy at 5.89 mg/kg/day. Seacat et al. (2003) reported increases in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol levels in rats following a 14-week dietary exposure to 1.33 mg/kg/day; however, no significant alterations in liver weight or liver histopathology were observed in rats exposed to 1.77 mg/kg/day PFOS in the diet for 4 weeks (Seacat et al. 2003). In contrast, Elcombe et al. (2012a) reported increases in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol in rats exposed to 1.54 mg/kg/day PFOS in the diet for 28 days.

Data on the chronic toxicity of PFOS to the liver in rodents are limited to a study in rats (Butenhoff et al. 2012b; Thomford 2002b). Hepatotoxicity characterized by centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, and centrilobular hepatocytic vacuolation was noted in rats exposed to PFOS in the diet for 2 years. Among rats sacrificed at the end of the study, significant increases in the incidence of centrilobular hepatocellular hypertrophy were observed in male and female rats exposed to ≥0.25 mg/kg/day (Thomford 2002b). When animals sacrificed at interim periods (14 or 52 weeks) and unscheduled deaths were included with animals sacrificed at exposure termination, the incidence of centrilobular hepatocellular hypertrophy was also increased in males exposed to 0.1 mg/kg/day. At ≥0.1 mg/kg/day, significant increases in the incidences of eosinophilic clear cell altered foci and cystic hepatocellular degeneration were observed in male rats. An increase in cystic degeneration was observed

in male rats exposed to ≥0.025 mg/kg/day. However, this was mainly due to a high incidence in unscheduled deaths; among animals sacrificed at exposure termination, the incidence was only increased in males exposed to 1.04 mg/kg/day. An increased incidence of single cell necrosis was observed in males and females at 1.04 mg/kg/day (all groups combined). Observations made in a group of rats exposed to 1.17 mg/kg/day PFOS for 52 weeks and allowed to continue on the control diet for an additional year showed that hepatotoxicity was not a persistent response, as hepatotoxicity was generally absent at the end of the recovery period. At termination, electron microscopy showed mild to moderate smooth endoplasmic reticulum hyperplasia and minimal to mild hepatocellular hypertrophy primarily in rats dosed with 1.5 mg/kg/day PFOS, the highest dose tested.

In a study of Cynomolgus monkeys administered via gavage three doses of PFOS over 315 days, decreases in HDL cholesterol levels were found; the investigators noted that the levels were still within the normal variation (Chang et al. 2017). No alterations in other serum clinical chemistry parameters were found. Treatment of Cynomolgus monkeys with up to 2 mg/kg/day PFOS administered via a capsule for 4 weeks did not induce gross or microscopic morphological alterations in the liver and did not increase cell proliferation (Thomford 2002a). In a 26-week study in Cynomolgus monkeys, exposure to 0.75 mg/kg/day PFOS, administered via a capsule resulted in increased absolute liver weight after 183 days of treatment (Seacat et al. 2002). Significant decreases in serum total cholesterol were also observed at 0.75 mg/kg/day after 91, 153, and 182 days of exposure. On day 182, total cholesterol decreased to 35 and 53% of predosing values in males and females, respectively. The HDL cholesterol levels were significantly lower in males at 0.03 and 0.75 mg/kg/day on days 153 and 182 and in females at 0.15 and 0.75 mg/kg/day on days 153 and 182; the lack of pre-treatment HDL cholesterol measurements precludes within-group comparisons. Serum bilirubin was significantly lower in males at 0.75 mg/kg/day on days 91, 153, and 182. Light microscopy of liver sections showed centrilobular vacuolation, hypertrophy, and mild bile stasis in some monkeys exposed to 0.75 mg/kg/day. Electron microscopy showed lipid-droplet accumulation in some males and females exposed to 0.75 mg/kg/day. Increased glycogen content was also noted at this dose level. No histological alterations were observed in the livers of monkeys exposed to 0.75 mg/kg/day for 26 weeks and allowed to recover for 7 months or 1 year. Similarly, serum cholesterol returned to pretreatment levels 36 days post exposure and HDL cholesterol levels returned to pretreatment levels after 61 days of recovery.

Summary. Epidemiological studies have examined the possible associations between PFOS exposure and liver disease in workers and hepatocellular damage and alterations in serum lipid levels in workers and the general population. The available occupational exposure studies or general population studies do not

consistently suggest an association between PFOS exposure and increases in the risk of liver disease or biliary tract disorders. A small number of occupational exposure studies have not found associations between serum PFOS levels and increases in ALT, AST, or GGT levels. Overall, the epidemiological studies suggest an association between serum PFOS levels and increases in serum total cholesterol levels and possibly serum LDL cholesterol levels. Studies of workers at a PFOS manufacturing facility found elevated serum total cholesterol levels in workers with high serum PFOS levels; however, a longitudinal analysis at the same facility did not find that serum PFOS was a significant predictor of cholesterol levels. Studies of residents living in an area with very high PFOA water levels found increases in serum total cholesterol levels associated with elevated serum PFOS levels in children, adolescents, and adults. Mixed results have been found for associations between serum PFOS and increases in serum total cholesterol levels in general population studies. Associations have been found between serum PFOS levels and serum LDL-cholesterol levels among non-occupational populations.

In laboratory animals, oral exposure to PFOS results in increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipid levels. A small number of studies also reported focal necrosis and centrilobular hepatocytic vacuolization. The proposed mechanism of action for the increased liver weight, hepatocellular hypertrophy, and decreased serum lipid levels involves PPARα receptor activation. Due to species differences for this mechanism, these effects observed in rodents are not considered relevant to humans. The applicability of the hepatic hypertrophy and serum lipid alterations observed in rodent studies to humans has been questioned due to species differences in the presumed mechanism of action for these effects in rodents.

PFHxS

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. Lin et al. (2010) did not find associations between serum ALT and GGT levels with serum PFHxS levels in a general population study using the NHANES data set.

Epidemiological Studies—Serum Lipids. Eight studies have evaluated the potential association between serum PFHxS levels and serum lipids in the general population. A study utilizing the NHANES data set for adults not taking cholesterol-lowering medication reported an association between serum PFHxS and non-HDL cholesterol, but no associations with total cholesterol, LDL cholesterol, or HDL cholesterol (Nelson et al. 2010). In a study of Canadian adults not taking cholesterol-lowering medication with a geometric mean serum PFHxS level of 2.16 ng/mL, associations were found for total cholesterol, LDL

cholesterol, and non-HDL cholesterol (Fisher et al. 2013). The study also found increased odds of having a high cholesterol level with increasing PFHxS levels. Associations between serum PFHxS levels and HDL cholesterol and triglyceride levels were found in a study of Chinese men (Yang et al. 2018). In pregnant women in Norway with median serum PFHxS levels of 0.60 ng/mL, serum PFHxS levels were associated with serum HDL cholesterol, but not with total cholesterol, LDL cholesterol, or triglycerides (Starling et al. 2014a). No associations between serum PFHxS and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels were found in a study of Taiwanese children aged 12–15 years (mean serum PFHxS of 2.1 ng/mL) (Zeng et al. 2015) or Korean children aged 3–18 years (mean serum PFHxS of 0.793 ng/mL) (Kang et al. 2018). A study of Spanish children aged 4 years found an association between maternal serum PFHxS and triglyceride levels, but not with cholesterol, LDL cholesterol, or HDL cholesterol (Manzano-Salgado et al. 2017b). A fourth study in children reported associations between serum PFHxS levels and serum cholesterol and LDL cholesterol, but not HDL cholesterol or triglycerides, in World Trade Center Health Registry enrollees (Koshy et al. 2017).

Laboratory Animal Studies. Acute-duration gavage administration of PFHxS resulted in increases in liver weight, steatosis, and increases in hepatic triglyceride levels in mice; increases in liver weight and steatosis were also observed in similarly exposed PPARα-null mice (Das et al. 2017). An intermediate-duration study with PFHxS in rats reported that gavage doses of ≥3 mg/kg/day induced a significant increase in absolute and relative liver weight in males (Butenhoff et al. 2009a). Light microscopy revealed minimal to moderate enlargement of centrilobular hepatocytes. Clinical chemistry tests showed a significant decrease in serum cholesterol at ≥0.3 mg/kg/day and decreased serum triglycerides at 10 mg/kg/day. None of these alterations were observed in female rats. Centrilobular hepatocellular hypertrophy was observed in mice administered ≥0.3 mg/kg/day PFHxS for 42–60 days (Chang et al. 2018); at 3 mg/kg/day single cell necrosis and microvascular fatty changes were also observed. In male mice, dietary exposure to PFHxS in a western-type diet resulted in >50% decreases in plasma triglyceride, total cholesterol, non-HDL cholesterol, and HDL cholesterol levels and approximately 75% decreases in the hepatic production of VLDL (Bijland et al. 2011). Increases in liver weight and hepatic triglyceride levels were also observed.

PFNA

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. A health evaluation of workers at a U.S. polymer production facility using PFNA did not find alterations in ALT, AST, GGT, or bilirubin levels related to increases in exposure intensity score in a longitudinal analysis (Mundt et al.

2007). Associations between serum PFNA and ALT and GGT levels were observed in a NHANES data study (Gleason et al. 2015); however, another study (Lin et al. 2010) utilizing the NHANES data did not find associations between serum PFNA and these enzymes. Neither study found associations for AST or total bilirubin.

Epidemiological Studies—Serum Lipids. Longitudinal analysis of serum lipid levels in the occupational exposure study (Mundt et al. 2007) did not find significant differences in serum total cholesterol or triglycerides over time. In general population studies, associations have been observed between serum PFNA levels and total cholesterol levels in adults (Fu et al. 2014a; Nelson et al. 2010) and children (Koshy et al. 2017; Zeng et al. 2015). No associations with cholesterol were found in a study in pregnant women (Starling et al. 2014a) or studies in children (Kang et al. 2018; Manzano-Salgado et al. 2017b). Several studies have also found associations with LDL cholesterol (Fu et al. 2014a; Koshy et al. 2017; Zeng et al. 2015) or non-HDL cholesterol (Nelson et al. 2010), but others did not find associations for LDL cholesterol (Nelson et al. 2010; Kang et al. 2018; Starling et al. 2014a). Most studies did not find an association between serum PFNA and HDL cholesterol (Fu et al. 2014a; Nelson et al. 2010; Koshy et al. 2017; Manzano-Salgado et al. 2017b; Zeng et al. 2015) or triglycerides (Fu et al. 2014a; Kang et al. 2018; Koshy et al. 2017; Manzano-Salgado et al. 2017b;Starling et al. 2014a). Exceptions were the Starling et al. (2014a) study of pregnant women, which found a positive association for HDL cholesterol, Yang et al. (2018) study of men, which found associations for HDL cholesterol and triglycerides, and Zeng et al. (2015), which found an association with triglycerides in children. Fu et al. (2014a) did not find increased risks of elevated cholesterol, LDL cholesterol, or triglyceride levels or lowered HDL cholesterol levels in adults.

Laboratory Animal Studies. Ten studies have evaluated the hepatic toxicity of PFNA. The observed effects are consistent with effects observed for other perfluoroalkyls. Alterations in serum lipid levels consisted of decreases in serum HDL cholesterol levels in rats administered via gavage ≥1 mg/kg/day PFNA for 14 days (Fang et al. 2012a), decreases in serum triglyceride and cholesterol levels in mice receiving gavage doses of ≥1 mg/kg/day PFNA (Wang et al. 2015a), and decreases in serum cholesterol levels in mice administered 0.5 mg/kg/day PFNA (Singh and Singh 2018). Increases in liver weight were observed in rats nose-only exposed to ≥67 mg/m^3 PFNA (Kinney et al. 1989), in mice administered via gavage 10 mg/kg/day PFNA for 7 days (Das et al. 2017), in mice exposed to 0.5 mg/kg/day PFNA in the diet for 14 days (Kennedy 1987), in mice administered ≥0.2 mg/kg/day PFNA via gavage for 14 days (Wang et al. 2015a), and in the offspring of mice administered via gavage ≥0.83 mg/kg/day PFNA on GDs 1–17 or 1–18 (Das et al. 2015; Wolf et al. 2010). The increases in liver weight, hepatocellular

hypertrophy, and deceases in serum lipid levels are considered adaptive and not relevant to humans (Hall et al. 2012). Hepatocellular vacuolation was observed in mice administered via gavage 5 mg/kg/day PFNA for 14 days (Fang et al. 2012b). In PPARα-null mice, increases in liver weight were observed in non-pregnant mice administered via gavage ≥1.5 mg/kg/day PFNA for 18 days, but were not found in pregnant animals (Wolf et al. 2010). Das et al. (2017) found increases in liver weight, steatosis, and increases in liver triglyceride levels in PPARα-null mice administered 10 mg/kg/day PFNA for 10 days.

PFDA

Epidemiological Studies—Serum Lipids. Six general population studies have evaluated the potential relationships between serum PFDA and serum lipids and reported inconsistent results. Fu et al. (2014a) found an association between serum PFDA and total cholesterol in adults and Koshy et al. (2017) found associations between serum PFDA and total cholesterol in children. A study of men did not find associations between serum PFDA and HDL cholesterol or triglycerides (Yang et al. 2018). Studies in pregnant women (Starling et al. 2014a) and other studies in children (Kang et al. 2018; Zeng et al. 2015) did not find associations. Fu et al. (2014a), Starling et al. (2014a), and Koshy et al. (2017) found positive associations with HDL cholesterol; this was not found in the Zeng et al. (2015) study. Koshy et al. (2017) also found an association with LDL cholesterol. The other studies did not find associations between serum PFDA and LDL cholesterol (Fu et al. 2014a; Starling et al. 2014a; Zeng et al. 2015), and none found association with triglycerides (Fu et al. 2014a; Kang et al. 2018; Koshy et al. 2017; Starling et al. 2014a; Zeng et al. 2015). Only the Fu et al. (2014a) study looked for alterations in the risk of elevated cholesterol, LDL cholesterol, or triglyceride levels or decreased HDL cholesterol levels, but the study did not find significant increases in the risk.

Laboratory Animal Studies. Hepatic effects observed in laboratory animals exposed to PFDA include alterations in liver weight and morphology. Increases in liver weight have been observed in mice following a single gavage dose of PFDA; the alterations were observed 2 days after exposure to 40 mg/kg/day (Brewster and Birnbaum 1989) or 30 days after exposure to ≥20 mg/kg/day (Harris et al. 1989). Repeated dietary exposure to 2.4 mg/kg/day PFDA for 1 week (Kawashima et al. 1995) or 78 mg/kg/day for 10 days (Permadi et al. 1992, 1993) also resulted in increases in liver weight. Oral doses ≥9.5 mg/kg/day also resulted in increases in hepatic cholesterol levels in rats (Kawashima et al. 1995) and hepatic lipids in mice (Brewster and Birnbaum 1989). These acute doses were also associated with hepatocellular hypertrophy and evidence of peroxisome proliferation. Thirty days after a single gavage dose of ≥20 mg/kg/day PFDA, effects included periportal to panlobular hepatocellular

hypertrophy characterized by swollen hepatocytes with abundant granular eosinophilic cytoplasm and enlarged and hyperchromatic nuclei (Harris et al. 1989).

In intermediate-duration exposure studies, an increased incidence of minimal single cell hepatocellular necrosis were observed in rats administered 0.5 mg/kg/day PFDA for 28 days (Frawley et al. 2018).

PFUnA

Epidemiological Studies—Serum Lipids. Of the five studies evaluating potential associations between serum PFUnA and serum lipids, only a study by Kang et al. (2018) in children found an association between serum PFUnA and total cholesterol and LDL cholesterol. The other studies did not find associations between serum PFUnA and total cholesterol or LDL cholesterol (Fu et al. 2014a; Koshy et al. 2107; Starling et al. 2014a) or with HDL cholesterol or triglycerides (Yang et al. 2018). None of the studies found associations with triglyceride levels. Starling et al. (2014a) and Koshy et al. (2017) found associations of serum PFUnA levels with HDL cholesterol levels; Fu et al. (2014a) did not find an association for this parameter. No alterations in the risk of abnormal serum lipid levels were found in the adults examined by Fu et al. (2014a).

Laboratory Animal Studies. Only one animal study was identified that examined the liver following oral exposure to PFUnA. In an intermediate-duration study of rats administered PFUnA via gavage, increases in relative liver weight were observed in males at 0.3 mg/kg/day and in females at 1.0 mg/kg/day, and mild to moderate centrilobular hepatocellular hypertrophy was observed in males and females at 1.0 mg/kg/day (Takahashi et al. 2014).

PFHpA

Epidemiological Studies—Serum Lipids. Epidemiological data on PFHpA are limited to a study in adults conducted by Fu et al. (2014a), which found no associations between serum PFHpA and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels and a study in men conducted by Yang et al. (2018), which found no associations between serum PFHpA and HDL cholesterol or triglycerides.

PFBS

Epidemiological Studies—Serum Lipids. In the only epidemiological study examining serum lipids and possible associations with serum PFBS, Zeng et al. (2015) found an association with total cholesterol levels in children. No associations were found between serum PFBS and LDL cholesterol, HDL cholesterol, or triglycerides.

Laboratory Animal Studies. Treatment of male rats with 900 mg/kg/day PFBS by gavage for 28 days induced a significant increase in absolute and relative liver weight (25–30%) relative to controls, which was no longer detected following a 14-day recovery period (3M 2001). Clinical chemistry tests of liver function were unremarkable and there were no chemical-related microscopic alterations. No alterations in liver weight, serum chemistry parameters (ALT, AST, cholesterol), or liver morphology were observed in rats administered gavage doses as high as 600 mg/kg/day PFBS for 90 days (Lieder et al. 2009a). Significant increases in liver weight were observed at 300 and 1,000 mg/kg/day in a 2-generation study (Lieder et al. 2009b); the alterations were only observed in male rats. An increase in hepatocellular hypertrophy was also observed in the male P0 and F1 rats administered via gavage 1,000 mg/kg/day. Dietary exposure to mice resulted in decreases in plasma triglyceride levels and hepatic cholesterol levels, but no alterations in liver weight or plasma cholesterol, HDL cholesterol, or non-HDL cholesterol (Bijland et al. 2011).

PFBA

Epidemiological Studies—Serum Lipids. Only one epidemiological study examined hepatic outcomes; this study (Fu et al. 2014a) did not find any associations between serum PFBA levels and total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides in adults.

Laboratory Animal Studies. Treatment of rats with up to 184 mg/kg/day PFBA by gavage for 5 days did not affect liver weight, nor did it cause gross or microscopic morphological alterations in the liver (3M 2007a). In addition, clinical chemistry tests did not indicate altered liver function. Similarly, administration of approximately 20 mg/kg/day PFBA in the diet to male rats for 2 weeks did not significantly affect relative liver weight, but the same dose of PFOA induced a 45% increase in liver weight (Ikeda et al. 1985). Dietary administration of doses of approximately 78 mg/kg/day PFBA to male mice for 10 days induced a 63% increase in absolute liver weight (Permadi et al. 1992, 1993).

PFBA intermediate-duration studies have consistently found increases in liver weight and histological alterations. Dosing rats with PFBA by gavage for 28 days resulted in significant increases in absolute and relative liver weight and decreases in serum cholesterol at ≥30 mg/kg/day and hepatocellular hypertrophy at 150 mg/kg/day (Butenhoff et al. 2012a; van Otterdijk 2007a). Administration of 150 mg/kg/day PFBA induced hepatocyte hypertrophy. These liver effects were no longer detected after a 21-day recovery period. In a similar 90-day study, administration of 30 mg/kg/day PFBA resulted in increased absolute liver weight and panlobular hepatocyte hypertrophy (Butenhoff et al. 2012a; van Otterdijk 2007b); no liver effects were observed at 6 mg/kg/day. None of the liver alterations were observed after a 21-day recovery period.

PFDoDA

Epidemiological Studies—Serum Lipids. A general population study of adolescents (Zeng et al. 2015) did not find any associations between serum PFDoDA and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels.

Laboratory Animal Studies. Dosing of male Sprague-Dawley rats with 10 mg/kg/day PFDoDA by gavage for 14 days induced a 35% increase in total serum cholesterol; doses of 1 or 5 mg/kg/day had no significant effect (Shi et al. 2007). In a subsequent study, the same group of investigators reported that in rats dosed via gavage with 1 or 5 mg/kg/day PFDoDA, there was a trend for decreased serum triglycerides, but the differences with controls were not statistically significant (Zhang et al. 2008); at 10 mg/kg/day, serum triglyceride levels were significantly increased. Liver triglyceride and liver cholesterol levels were increased at ≥5 mg/kg/day. Absolute liver weight was significantly reduced in the 5 mg/kg/day group (19%) relative to controls, but this may have been due to a marked reduction in body weight (shown in Shi et al. [2007], but not in Zhang et al. [2008]).

In a 42-day PFDoDA gavage administration study, increases in relative liver weight were observed in males at ≥0.5 mg/kg/day and hepatocellular hypertrophy was observed at 2.5 mg/kg/day (Kato et al. 2015). The study also found decreases in serum cholesterol at 0.1 and 0.5 mg/kg/day, but not at 2.5 mg/kg/day. In pregnant females (most dying before the end of the study), single cell hepatocyte necrosis was observed at 2.5 mg/kg/day (Kato et al. 2015). Prebiliary infiltration of inflammatory cells (males), disposition of bilirubin (females), and hepatocellular hypertrophy (females) were observed in males and nonpregnant females administered 2.5 mg/kg/day PFDoDA for 42 days followed by a 42-day recovery period (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Increases in liver weight, decreases in serum cholesterol levels, and centrilobular hepatocellular hypertrophy have been observed in rats administered 315 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005) or ≥100 mg/kg/day NaPFHx for 90–93 days (Chengelis et al. 2009b; Loveless et al. 2009). In a chronic-duration study, gavage administration of 200 mg/kg/day for 2 years resulted in increases in the incidence of hepatocellular necrosis in female rats (Klaunig et al. 2015). At 100 mg/kg/day, decreases in triglyceride levels were observed in male rats.

FOSA

Laboratory Animal Studies. In the only study examining hepatic effects, Seacat and Luebker (2000) reported no alterations in liver weight in rats receiving a single gavage dose of 5 mg/kg FOSA.

2.10 RENAL

Overview. Epidemiological and laboratory animal studies have evaluated the potential of perfluoroalkyls to be renal toxicants. Human studies have evaluated the risk of kidney disease, alterations in renal function, damage to the kidney, and alterations in uric acid levels. The results of epidemiological studies evaluating kidney disease and renal function are summarized in Table 2-13; Table 2-14 contains the studies evaluating alterations in uric acid levels. More detailed descriptions of these studies can be found in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 8. Although there are a couple of studies finding associations between PFOA exposure and kidney disease, the results are not consistent across study populations. However, there is some indication that perfluoroalkyls may affect renal function. Decreases in estimated glomerular filtration rate and increases in uric acid levels associated with serum PFOA or PFOS have been reported in a number of epidemiological studies. However, these alterations may be due to reverse causality (i.e., increases in serum perfluoroalkyl levels could be due to a decrease in glomerular filtration and shared renal transporters for perfluoroalkyls and uric acid). Based on the small number of epidemiological studies or the inconsistency of the results, possible associations between other perfluoroalkyls (PFHxS, PFNA, PFDA, PFBS, PFDoDA, or PFHxA) and renal functions cannot be assessed. No studies were available for PFUnA, PFHpA, PFBA, or FOSA.

Table 2-13. Summary of Renal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa et al. 2009 Occupational (n =53)	12,930 ng/mL (mean PFOA in current workers)	Serum urea	NS (p>0.05)
		Serum creatinine	NS (p>0.05)
		Total proteins	NS (p>0.05)
		α1 globulins, α2 globulins, β globulins, or γ globulins	NS (p>0.05)
		α2 globulins	**Association (p<0.01), current, former and non-exposed workers.**
Lundin et al. 2009 Occupational (n=3,992)	NR	Nephritis and nephrosis deaths	SMR 5.2 (0.6–18.9)
Raleigh et al. 2014 Occupational (n=9,027)	NR	Chronic kidney disease deaths	HR 0.73 (0.21–2.48), 4th quartile
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA	Chronic kidney disease risk	NS (p=0.92), no lag NS (p=0.99), 10-year lag
Steenland and Woskie 2012 Occupational (n=1,084)	7,800 ng/mL-year (mean PFOA)	Chronic kidney disease deaths	**SMR 3.79 (1.03–9.71)*, 2nd quartile**
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Kidney disease (self-reported)	**SPR 2.26 (1.45–3.51)***
Dhingra et al. 2016b Community (C8) (n=28,541)	Estimated cumulative PFOA	Chronic kidney disease	NS (p=0.80 for trend), no lag NS (p=0.81 for trend), 5-year lag NS (p=0.88 for trend), 10-year lag NS (p=0.30 for trend), 20-year lag
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	Serum creatinine	NS (p>0.05)
		BUN	NS (p>0.05)
		Total serum proteins	NS (p>0.05)

Table 2-13. Summary of Renal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Watkins et al. 2013 Community (C8) (9,660 children)	28.3 ng/mL (median PFOA)	GFR	**Inverse association (p=0.02)***
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4.7 ng/mL (4th PFOA quartile)	GFR	**Inverse association (p<0.01)*, 4th quartile**
		Serum uric acid	**Association (p<0.01)***
Shankar et al. 2011a General population (NHANES) (n=4,587)	>5.9 ng/mL (4th PFOA quartile)	GFR	**Inverse association (p<0.001 for trend)***
		Chronic kidney disease	**OR 1.73 (1.04–2.88)*, 4th quartile**
PFOS			
Olsen et al. 1998a Occupational (n=178 in 1995; n=149 in 1997)	2,440 and 1,930 ng/mL (mean PFOS in 1995 in Decatur and Antwerp) 1,960 and 1,480 ng/mL (mean in 1997 in Decatur and Antwerp)	Serum creatinine	**Association (p<0.06)*, 1997 only**
		BUN	NS (p>0.1)
Watkins et al. 2013 Community (C8) (9,660 children)	20.0 ng/mL (median PFOS)	GFR	**Inverse association (p=0.0001)***
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	7.9–12.8 ng/mL (2nd PFOS quartile) ≥19.4 ng/mL (4th PFOS quartile)	GFR	**Inverse association (p<0.05)*, 2nd quartile**
Shankar et al. 2011a General population (NHANES) (n=4,587)	>29.5 ng/mL (4th PFOS quartile) 11.2–17.8 ng/mL (2nd PFOS quartile)	GFR	**Inverse association (p<0.001 for trend)***
		Chronic kidney disease	**OR 1.82 (1.02–3.27)*, 4th quartile**
PFHxS			
Watkins et al. 2013 Community (C8) (9,660 children)	5.2 ng/mL (median PFHxS)	GFR	**Inverse association (p=0.003)***

Table 2-13. Summary of Renal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4 ng/mL (4th PFHxS quartile)	GFR	NS (p>0.05)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	BUN Creatinine	Small, but not clinically significant
Watkins et al. 2013 Community (C8) (9,660 children)	1.5 ng/mL (median PFNA)	GFR	**Inverse association (p=0.002)***
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥1.5 ng/mL (4th PFNA quartile)	GFR	NS (p>0.05)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 8 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BUN = blood urea nitrogen; GFR = glomerular filtration rate; HR = hazard ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SMR = standardized mortality ratio

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa et al. 2009 Occupational (n=53)	12,930, ng/mL (mean PFOA in current workers)	Serum uric acid	**Association (p=0.039)***
Sakr et al. 2007b Occupational (n=1,025)	490 ng/mL (median PFOA)	Serum uric acid	**Association (reported by investigator)***
Steenland et al. 2010b Community (n=54,591)	11.5–20.6 ng/mL (2nd quintile PFOA)	Hyperuricemia risk	**OR 1.33 (1.24–1.43)* (2nd quintile)**
Gleason et al. 2015 General population (NHANES) (n=4,333)	3.7 ng/mL (median PFOA)	Serum uric acid	**Association (p<0.001)***
		Hyperuricemia risk	**Association (p<0.001)***
Geiger et al. 2013 General population (NHANES) (n=1,772 adolescents and adults)	4.3 ng/mL (mean PFOA), >5.4 ng/mL (4th PFOA quartile)	Serum uric acid	**Association (p=0.0001)***
		Hyperuricemia risk	**OR 1.62 (1.10–2.37)* (4th quartile)**
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4.7 ng/mL (4th PFOA quartile)	Serum uric acid	**Association (p<0.01)***
Qin et al. 2016 General population (n=225 adolescents)	0.5 ng/mL (median PFOA)	Serum uric acid	**Association (p<0.05)***
		Hyperuricemia risk	**OR 2.16 (1.29–3.61)* (full cohort)** **OR 2.76 (1.37–5.56)* (boys only)**
Shankar et al. 2011b General population (NHANES) (n=3,883 adults)	3.5–5.1 ng/mL (3rd PFOA quartile)	Serum uric acid	**Association (p<0.0001)***
		Hyperuricemia risk	**OR 1.90 (1.35–2.69)*, 3rd quartile**
PFOS			
Steenland et al. 2010b Community (n=54,591)	17.5–23,26 ng/mL (3rd PFOS quintile)	Hyperuricemia risk	**OR 1.11 (1.04–1.20)* (3rd quintile)**

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gleason et al. 2015 General population (NHANES, n=4,333)	11.3 ng/mL (median PFOS)	Serum uric acid	**Association (p<0.01)***
		Hyperuricemia risk	NS (p=0.502)
Geiger et al. 2013 General population (NHANES) (n=1,772 adolescents and adults)	18.4 ng/mL (mean PFOS), >25.5 ng/mL (4th PFOS quartile)	Serum uric acid	NS (p=0.0575)
		Hyperuricemia risk	**OR 1.65 (1.10–2.49)* (4th quartile)**
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥19.4 ng/mL (4th PFOS quartile)	Serum uric acid	**Association (p<0.05)***
Qin et al. 2016 General population (n=225 adolescents)	28.9 ng/mL (median PFOS)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.35 (0.95–1.93) (full cohort)
Shankar et al. 2011b General population (NHANES) (n=3,883 adults)	11.2–17.8 ng/mL (2nd PFOS quartile)	Serum uric acid	**Association (p=0.0018)***
		Hyperuricemia risk	**OR 1.46 (1.11–1.91)*, 2nd quartile**
PFHxS			
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.8 ng/mL (median PFHxS)	Serum uric acid	NS (p>0.01)
		Hyperuricemia risk	NS (p=0.110 for trend)
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4 ng/mL (4th PFHxS quartile)	Serum uric acid	NS (p>0.05)
Qin et al. 2016 General population (n=225 adolescents)	1.3 ng/mL (median PFHxS)	Serum uric acid	**Association (p<0.05)***
		Hyperuricemia risk	OR 1.39 (0.93–2.07)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	Serum uric acid	Small, but not clinically significant

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.4 ng/mL (median PFNA)	Serum uric acid	**Association (p<0.001)***
		Hyperuricemia risk	NS (p=0.42 for trend)
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥1.5 ng/mL (4th PFNA quartile)	Serum uric acid	NS (p>0.05)
Qin et al. 2016 General population (n=225 adolescents)	0.8 ng/mL (median PFNA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.28 (0.83–1.96)
PFDA			
Qin et al. 2016 General population (n=225 adolescents)	0.9 ng/mL (median PFDA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.26 (0.82–1.92)
PFBS			
Qin et al. 2016 General population (n=225 adolescents)	0.5 ng/mL (median PFBS)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.23 (0.86–1.75)
PFDoDA			
Qin et al. 2016 General population (n=225 adolescents)	2.7 ng/mL (median PFDoDA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 0.93 (0.65–1.34)

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxA			
Qin et al. 2016 General population (n=225 adolescents)	0.2 ng/mL (median PFHxA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.08 (0.77–1.61)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 8 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBS = perfluorobutane sulfonic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Laboratory animal studies have primarily evaluated kidney morphology; these studies are summarized in Tables 2-1, 2-3, 2-4, 2-5, and 2-6. The NOAEL and LOAEL values for these studies are illustrated in Figures 2-6, 2-8, 2-9, and 2-10. In general, the laboratory animal studies have not found evidence of impaired renal function or morphological damage following exposure to PFOA, PFOS, PFHxS, PFDA, PFUnA, PFBS, PFBA, PFDoDA, or PFHxA. No laboratory animal studies examining renal endpoints were available for PFNA, PFHpA, or FOSA.

PFOA

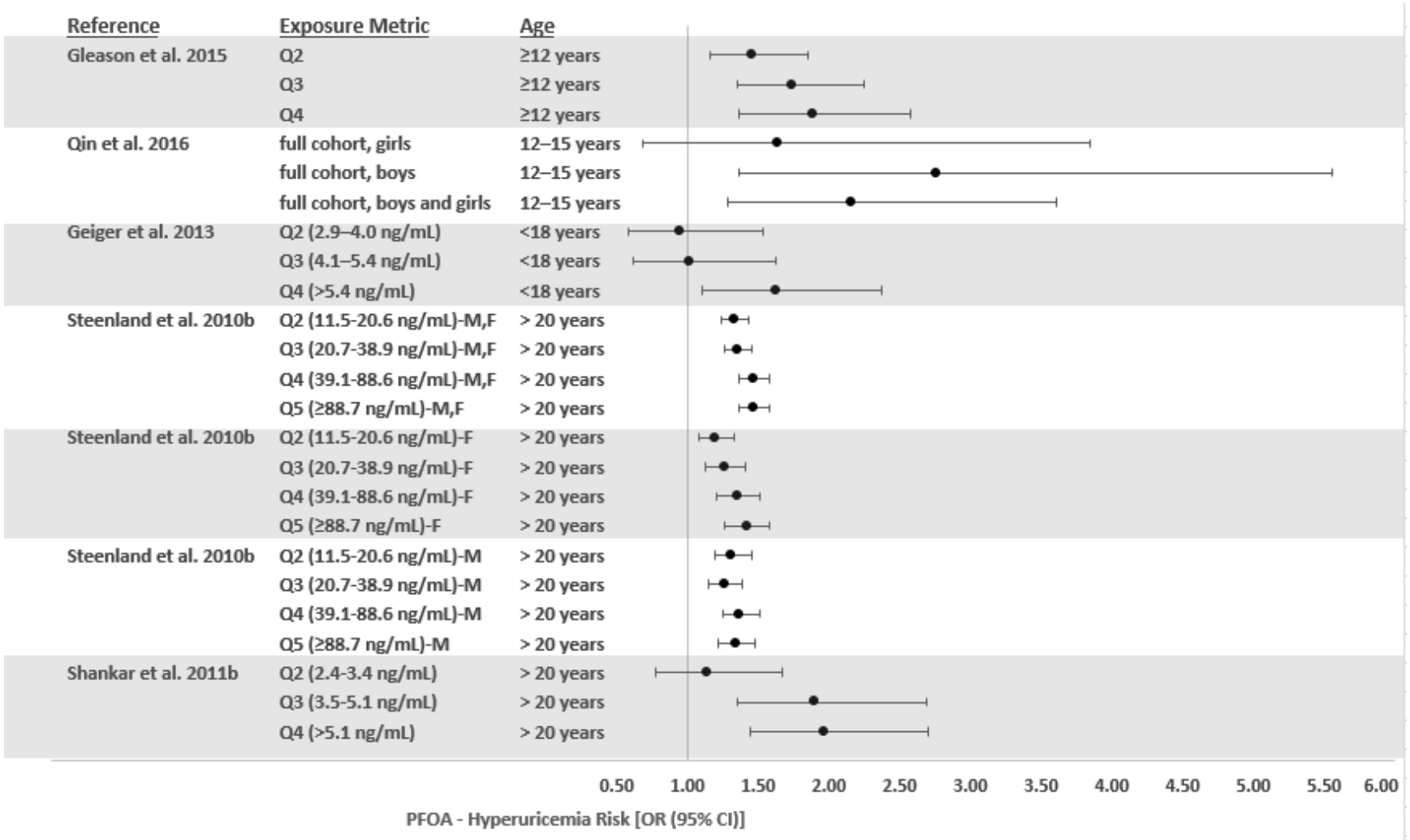
Epidemiological Studies—Kidney Disease. Several epidemiological studies have examined the possible association between PFOA exposure and increased risk of kidney disease. In a cohort mortality study of workers at the DuPont PFOA facility in West Virginia, Steenland and Woskie (2012) found an increase in deaths from chronic renal disease when compared to DuPont workers at other regional facilities. When estimated cumulative PFOA exposure was estimated based on the worker's job history and data from a biomonitoring survey conducted from 1979 to 2004, there was a significant positive trend for nonmalignant kidney disease when the workers were divided in estimated cumulative exposure quartiles. Two studies of workers at the 3M APFO facility in Cottage Grove, Minnesota did not find increases in deaths from chronic kidney disease (Raleigh et al. 2014) or nephritis and nephrosis (Lundin et al. 2009) as compared to mortality rates for the state of Minnesota. Similar results were found when chronic kidney disease deaths were compared to those in a cohort of workers in St. Paul Minnesota who worked at a non-APFO facility (Raleigh et al. 2014). An occupational exposure study (Steenland et al. 2015) and C8 community study (Dhingra et al. 2016b) found no associations between estimated cumulative PFOA exposure and the risk of chronic kidney disease. Another study of the community living near the Washington Works facility found a higher prevalence of self-reported kidney disease as compared to rates reported in NHANES (Anderson-Mahoney et al. 2008).

Epidemiological Studies—Biomarkers of Renal Function. Several biomarkers of renal function have been evaluated in epidemiological studies; these include BUN, serum creatinine, glomerular filtration rate, and uric acid levels (discussed in the following section). Kidney function, assessed by levels of BUN and serum creatinine, was not associated with exposure to PFOA in the occupational exposure studies by Olsen et al. (2003a) or Costa et al. (2009) or a community exposure study by Emmett et al. (2006b).

Three studies have found inverse associations between serum PFOA and glomerular filtration rate. Using the NHANES data for the 1999–2008 cycles, Shankar et al. (2011a) found an inverse association between serum PFOA levels and estimated glomerular filtration rate in adults. The likelihood of chronic kidney disease, defined as a glomerular filtration rate of <60 mL/minute/1.73 m^2, was significantly higher in adults with the highest serum PFOA (>5.9 ng/mL, OR 1.73, 95% CI 1.04–2.88) levels than in adults with serum PFOA levels in the lowest quartile. The study also investigated whether the association between serum PFOA levels and chronic kidney disease was due to reverse causality (i.e., decreased glomerular filtration leads to a decrease in perfluoroalkyl filtration) and found a stronger negative correlation between estimated glomerular filtration rate and serum PFOA levels in subjects without chronic kidney disease, suggesting that it was not due to reverse causality. In another study utilizing NHANES data, an inverse association was found in adolescents with serum PFOA levels in the 4th quartile (Kataria et al. 2015). Similarly, an inverse association between serum PFOA and glomerular filtration rate was found in children participating in the C8 Health Project (Watkins et al. 2013). Unlike Shankar et al. (2011a), Watkins et al. (2013) suggested that the association between serum perfluoroalkyl levels and estimated glomerular filtration rates may be a consequence of reverse causation because no associations were found between estimated serum PFOA levels 3 or 10 years prior to enrollment in the study or at the time of study enrollment and estimated glomerular filtration rates; predicted serum PFOA levels were based on environmental PFOA levels, self-reported residential history, and PBPK modeling.

Epidemiological Studies—Alterations in Uric Acid Levels. Associations between serum PFOA levels and serum uric acid levels have been found in several occupational, community, and general population studies. Costa et al. (2009) and Sakr et al. (2007b) reported associations between serum PFOA levels and serum uric acid levels in workers with high serum PFOA levels. In adult participants of the C8 Health Project, positive linear trends between serum uric acid levels and serum PFOA levels were found (Steenland et al. 2010b). When the subjects were categorized by PFOA levels, significantly increased risks of hyperuricemia (>6.0 mg/dL for women, >6.8 mg/dL for men) were observed for subjects with serum PFOA levels in the 2nd, 3rd, 4th, and 5th quintiles (≥11.5 ng/mL). Four studies utilizing NHANES data have found associations between serum PFOA and serum uric acid levels in adults (Gleason et al. 2015; Shankar et al. 2011b) and adolescents (Geiger et al. 2013; Kataria et al. 2015). A study in Taiwanese adolescents also found this association between PFOA and uric acid (Qin et al. 2016). Several studies have also found increases in the risk of hyperuricemia in a highly exposed population (Steenland et al. 2010b) and the general population (Gleason et al. 2015; Geiger et al. 2013; Qin et al. 2016; Shankar et al. 2011b). The ORs for the risk of hyperuricemia in these studies are summarized in Figure 2-19.

Figure 2-19. Risk of Hyperuricemia Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



Laboratory Animal Studies. No gross or microscopic alterations were observed in the kidneys from male rats following head-only inhalation exposure to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986). Significantly elevated absolute and relative kidney weight was reported in male rats dosed with ≥3 mg/kg/day PFOA by gavage in water for 70 days (Butenhoff et al. 2004b), but histological evaluation of the kidney was not conducted in this study. Rats that received much higher doses (100–110 mg/kg/day) of APFO for 90 days in the diet showed no significant morphological alterations in the kidneys, and BUN and the urinalysis were unremarkable (Griffith and Long 1980). Also, male mice dosed with up to 47 mg/kg/day APFO in the drinking water for 21 days showed no morphological alterations in the kidneys, and BUN and serum creatinine levels were not significantly affected (Son et al. 2008). Treatment of Cynomolgus monkeys with daily doses of up to 20 mg/kg/day APFO, administered via a capsule, for 26 weeks (Butenhoff et al. 2002) or Rhesus monkeys dosed with up to 10 mg/kg/day by gavage for 90 days (Griffith and Long 1980) did not cause morphological alterations in the kidneys, and blood chemistries and urinalyses provided no evidence of alterations in kidney function. In a 2-year dietary study in rats, relative kidney weight from males dosed with 15 mg/kg/day APFO was significantly elevated (14%) at the 1-year interim evaluation relative to controls, but gross and microscopic appearance (at 1 year and at termination), BUN, and urinalyses (several times during the study) were not significantly affected (3M 1983; Butenhoff et al. 2012c). No gross or microscopic alterations were seen in the kidneys from rats that received dermal applications of up to 2,000 mg/kg/day APFO to the shaven skin for 2 weeks (Kennedy 1985).

Summary. Epidemiological studies have examined possible associations between exposure to PFOA and increases in the risk of kidney disease and alterations in renal function. Mixed results for associations between serum PFOA and risks of kidney disease have been reported in occupational exposure studies and studies of highly exposed residents with more studies not finding associations. Several general population and community studies have found inverse associations between serum PFOA and glomerular filtration rate; however, there is suggestive evidence that this association may be due to reverse causation rather than a direct effect. Associations between serum PFOA levels and serum uric acid levels have been consistently observed in occupational, community, and general populations. Laboratory animal studies have not found evidence of alterations in renal function or histological alterations.

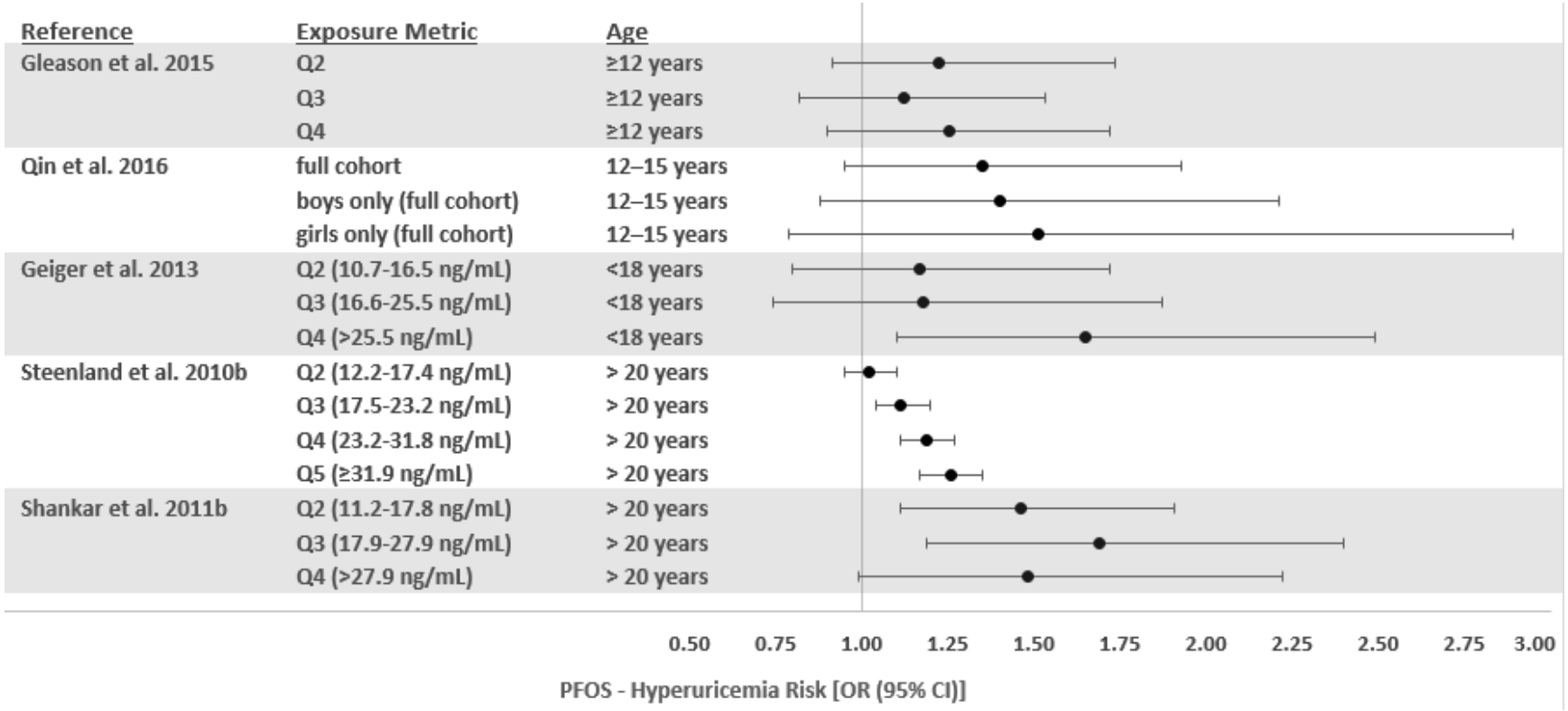
PFOS

Epidemiological Studies—Biomarkers of Renal Function. Three studies have found inverse associations between serum PFOS levels and glomerular filtration rate in adults (Shankar et al. 2011a), adolescents (Kataria et al. 2015), and children (Watkins et al. 2013). In the Watkins et al. (2013) study of C8 Health Project participants, a concentration-related linear trend between decreasing estimated glomerular filtration rates and increases in serum PFOS levels was observed in children and adolescents 1–<18 years old. In adolescents 12–19 years of age participating in NHANES, the estimated glomerular filtration rate was lower in participants with serum PFOA levels in the 2nd, 3rd, and 4th quartiles than those with levels in the 1st quartile (Kataria et al. 2015). In addition to the inverse association between serum PFOS and estimated glomerular filtration rate observed in adult NHANES participants, Shankar et al. (2011a) also found increased risks of chronic kidney disease (defined as a glomerular filtration rate of <60 mL/minute/1.73 m^2) in participants with serum PFOS levels in the 4th quartile.

Epidemiological Studies—Alterations in Uric Acid Levels. In a study of C8 Health Project participants, a linear trend between serum uric acid levels and serum PFOS levels was found (Steenland et al. 2010b). When the subjects were categorized by serum PFOS levels, increased risks of hyperuricemia (>6.0 mg/dL for women, >6.8 mg/dL for men) were observed for subjects with serum PFOS levels in the 3rd, 4th, and 5th quintiles. Similar findings were found in NHANES adult participants (Shankar et al. 2011b). A study of adolescent NHANES participants found associations between serum PFOS and serum uric acid levels (Kataria et al. 2015); a second study did not find an association (Geiger et al. 2013). The Geiger et al. (2013) study did find an increased risk of hyperuricemia for adolescents with serum PFOS levels in the 4th quartile. A study of Taiwanese adolescents did not find associations between serum PFOS and uric acid or an increased risk of hyperuricemia (Qin et al. 2016). The ORs for the risk of hyperuricemia in these studies are summarized in Figure 2-20.

Laboratory Animal Studies. No significant morphological alterations or clinical evidence of impaired kidney function was reported in male and female rats dosed with up to 1.77 mg/kg/day PFOS (potassium salt) (Seacat et al. 2003) or 5.89 mg/kg/day (Curran et al. 2008) for 4 weeks. Extending the treatment to 14 weeks resulted in an increase in BUN in male (23% increase) and female rats (41% increase), but histopathology of the kidneys and urinalyses were unremarkable (Seacat et al. 2003). The NOAEL values were 0.34 and 0.4 mg/kg/day in males and females, respectively. Gavage administration of three doses of PFOS to Cynomolgus monkeys over 315 days did not result in alterations in BUN or serum creatinine or

Figure 2-20. Risk of Hyperuricemia Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



total protein levels (Chang et al. 2017). Treatment of Cynomolgus monkeys with up to 0.75 mg/kg/day PFOS (potassium salt) administered via a capsule for 26 weeks did not cause morphological alterations in the kidneys, nor did it affect BUN, serum creatinine, or urinary parameters (Seacat et al. 2002). Similar results were reported in a 4-week study in monkeys dosed with up to 2 mg/kg/day PFOS (Thomford 2002a). A mild increase in BUN was reported in rats treated with approximately 0.25 or 1.04 mg/kg/day PFOS in the diet for 53 weeks in a 2-year study (Butenhoff et al. 2012b; Thomford 2002b). However, there were no significant gross or microscopic alterations in the kidneys at week 53 or at termination.

PFHxS

Epidemiological Studies—Biomarkers of Renal Function. A small number of epidemiological studies have evaluated biomarkers of renal function. In a study of C8 Health Project child participants (aged 1–<18 years), an inverse association between serum PFHxS and estimated glomerular filtration rate was observed (Watkins et al. 2013). A study of adolescent participants in NHANES did not find this association (Kataria et al. 2015). It is noted that the reported median PFHxS level in the Watkins et al. (2013) study (5.2 ng/mL) exceeded the lower end of the 4th quartile serum PFHxS level in the Kataria et al. (2015) study (≥4 ng/mL).

Epidemiological Studies—Alterations in Uric Acid Levels. In NHANES participants ≥12 years of age (Gleason et al. 2015) and adolescent NHANES participants (Kataria et al. 2015), no associations between serum PFHxS levels and serum uric acid levels or risk of hyperuricemia (Gleason et al. 2015) were found. A study of Taiwanese adolescents found an association between serum PFHxS levels and serum uric acid levels, but did not find increased risks of hyperuricemia (Qin et al. 2016).

Laboratory Animal Studies. Male rats treated by gavage with 10 mg/kg/day PFHxS for at least 42 days showed a significant increase in BUN levels, but there were no significant gross or microscopic alterations in the kidneys (Butenhoff et al. 2009a); the NOAEL was 3 mg/kg/day. No significant effect on BUN was reported in female rats. No histological alterations were observed in the kidneys of mice following intermediate-duration administration of ≤3 mg/kg/day PFHxS (Chang et al. 2018).

PFNA

Epidemiological Studies—Biomarkers of Renal Function. Two epidemiological studies have evaluated the possible associations between serum PFNA and alterations in renal function biomarkers. In a study of

children participating in the C8 Health Project, an inverse association between serum PFNA and estimated glomerular filtration rate was observed, but not in adolescents participating in NHANES (Watkins et al. 2013). Mundt et al. (2007) noted that there were small, but not clinically significant, alterations in BUN, creatinine, and serum uric acid levels in workers exposed to PFNA.

Epidemiological Studies—Alterations in Uric Acid Levels. Gleason et al. (2015) found an association between serum PFNA and serum uric acid levels in NHANES participants; this association was not found in studies of adolescents (Kataria et al. 2015; Qin et al. 2016). Studies by Gleason et al. (2015) and Qin et al. (2016) did not find increases in the risk of hyperuricemia associated with serum PFNA levels.

PFDA

Epidemiological Studies—Alterations in Uric Acid Levels. Epidemiological studies examining renal outcomes are limited to a study of Taiwanese adolescents that found no association between serum PFDA levels and serum uric acid levels and did not find increased risks of hyperuricemia (Qin et al. 2016).

Laboratory Animal Studies. Administration of a single dose of up to 80 mg/kg PFDA to female C57BL/6N mice by gavage did not induce gross or microscopic changes in the kidneys (Harris et al. 1989). However, 2 out of 10 mice that died following administration of a dose of 320 mg/kg showed mild acute necrosis of the proximal convoluted tubules. No histological alterations were observed in the kidneys of rats administered 0.5 mg/kg/day PFDA for 28 days or mice receiving weekly gavage doses of 5 mg/kg for 4 weeks (Frawley et al. 2018).

PFUnA

Laboratory Animal Studies. Treatment of male and female rats with 1.0 mg/kg/day PFUnA via gavage for 41–46 days resulted in significant increases in BUN levels (35–61% in males, 19–45% in females) and alkaline phosphatase activity (86–140% in males, 83% in females) and significant decreases in total protein (11% in males, 10–13% in females) and albumin (7% in males) levels (Takahashi et al. 2014); the NOAEL was 0.3 mg/kg/day.

PFBS

Epidemiological Studies—Alterations in Uric Acid Levels. Serum PFBS levels were not associated with serum uric acid levels or increases in the risk of hyperuricemia in a study of adolescents in Taiwan (Qin et al. 2016).

Laboratory Animal Studies. Treatment of female rats with 900 mg/kg/day PFBS by gavage for 28 days caused a significant increase (9–11%) in absolute and relative kidney weight, but caused no significant alterations in the microscopic appearance of the kidneys (3M 2001). The weight of the kidneys returned to control levels following a recovery period of approximately 14 days; the NOAEL for kidney weight effects was 900 mg/kg/day PFBS. In a 90-day rat study, PFBS did not result in alterations in kidney weights, but did result in hyperplasia of the medullary and papillary tubular and ductal epithelial cells in the inner medullary region at 600 mg/kg/day, but not at 200 mg/kg/day (Lieder et al. 2009a). Minimal to moderate papillary epithelial tubular/acinar hyperplasia was also observed in a 2-generation rat study at 300 mg/kg/day; the study identified a NOAEL of 100 mg/kg/day (Lieder et al. 2009b).

PFBA

Laboratory Animal Studies. No alterations in renal morphology or clinical indications of impaired renal function were reported in rats treated with PFBA in doses of up to 184 mg/kg/day for 5 days (3M 2007a), 150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or 30 mg/kg/day by gavage for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b).

PFDoDA

Epidemiological Studies—Alterations in Uric Acid Levels. In adolescents, no associations between serum PFDoDA levels and serum uric acid levels or the risk of hyperuricemia were observed (Qin et al. 2016).

Laboratory Animal Studies. No histopathological alterations were observed in rats administered up to 2.5 mg/kg/day PFDoDA for 42–47 days (Kato et al. 2015).

PFHxA

Epidemiological Studies—Alterations in Uric Acid Levels. In adolescents, no associations between serum PFHxA levels and serum uric acid levels or the risk of hyperuricemia were observed (Qin et al. 2016).

Laboratory Animal Studies. Renal papillary necrosis was determined to be one of the causes of death in rats administered 450 mg/kg/day PFHxA for 4 days (Kirkpatrick 2005). No increases in renal lesions were observed in surviving rats administered a TWA dose of 315 mg/kg/day for 32–44 days (Kirkpatrick 2005). No histological alterations were observed in the kidneys of rats administered up to 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b). In a 2-year gavage study, treatment of female rats with 200 mg/kg/day PFHxA resulted in mild renal tubular degeneration and mild to severe papillary necrosis (Klaunig et al. 2015); the NOAEL was 100 mg/kg/day. In addition, urinalysis revealed an increased mean urine volume and reduced specific gravity. There were no histological alternations in the kidneys of males.

2.11 DERMAL

Overview. No studies were located regarding dermal effects in humans. Studies in laboratory animals have not found dermal effects following head-only inhalation exposure to PFOA (see Table 2-1) or oral exposure to PFOA, PFOS, or PFBA (see Tables 2-3, 2-4, and 2-5). Dermal exposure to PFOA has resulted in skin damage (see Table 2-6).

PFOA

In an inhalation head-only exposure study, no histopathological alterations were observed in the abdominal skin of male rats exposed to ≤84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986).

No microscopic alterations were observed in the skin following oral exposure of rats to ≤100–110 mg/kg/day APFO via the diet for 90 days (Griffith and Long 1980) or monkeys exposed to up to 20 mg/kg/day PFOA or 0.75 mg/kg/day PFOS for 26 weeks (Butenhoff et al. 2002; Seacat et al. 2002).

Application of a single dose of 5,000 mg/kg of an aqueous paste of APFO to a clipped area of the skin of rats, and left in place covered for 24 hours produced mild skin irritation (Kennedy 1985); no irritation was

apparent with a dose of 3,000 mg/kg. In a 2-week dermal exposure study, skin irritation was observed in rats exposed to 200 mg/kg/day (Kennedy 1985). Acute necrotizing dermatitis was observed in two out of five rats exposed to 2,000 mg/kg/day; this lesion was observed after the 10th treatment. Application of 500 mg/kg APFO to the intact or abraded skin of young rabbits and left covered for 24 hours was non-irritating, as scored according to the Draize procedure immediately after removal of the cover and 48 hours later (Griffith and Long 1980).

PFOS

Administration of up to approximately 1.04 mg/kg/day PFOS to rats in the diet for 2 years did not induce morphological alterations in the skin (Butenhoff et al. 2012b; Thomford 2002b).

PFBA

There were no significant gross or microscopic alterations in the skin of rats receiving gavage doses of ≤150 mg/kg/day PFBA for 28 days or ≤30 mg/kg/day PFBA for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

2.12 OCULAR

Overview. No information was located regarding ocular effects in humans. Ocular irritation has been observed in laboratory animals following exposure to airborne APFO dust or instillation of PFOA into the eye (see Tables 2-1 and 2-6). However, ocular effects have not been found following oral exposure to PFOA, PFOS, PFBS, PFBA, or PFHxA (see Tables 2-3, 2-4, and 2-5).

PFOA

Rats exposed to 18,600 mg/m^3 APFO dusts for 1 hour exhibited a red material around the eyes and lacrimation during exposure (Griffith and Long 1980). Male rats exposed to ≥810 mg/m^3 APFO dusts for 4 hours showed corneal opacity and corrosion, which was confirmed by fluorescein staining (Kennedy et al. 1986). Examination of the eyes of male rats exposed intermittently to up to 84 mg/m^3 APFO for 2 weeks using a bright light and a slit-lamp biomicroscope on days 5 and 9 of exposure did not reveal any significant exposure-related alterations (Kennedy et al. 1986). Microscopic examination of the eyes from these rats at termination and following a recovery period of up to 42 days was unremarkable.

In oral exposure studies, examination of the eyes from rats exposed to approximately 100–110 mg/kg/day APFO in the diet for 90 days did not reveal any significant gross or microscopic alterations (Griffith and Long 1980). Similar results were reported in rats that received dietary doses up to 15 mg/kg/day APFO for 2 years (3M 1983; Butenhoff et al. 2012c) and in monkeys dosed with up to 20 mg/kg/day APFO for 26 weeks (Butenhoff et al. 2002).

No significant gross alterations were observed in the eyes of rats following repeated dermal exposure to APFO (Kennedy 1985). Microscopic examination of the eyes also did not reveal treatment-related changes. In a study in rabbits, 0.1 g APFO was instilled once in the conjunctival sac of the right eye and examinations were conducted after 1, 24, 48, and 72 hours and 5 and 7 days after the application (Griffith and Long 1980). APFO produced moderate irritation of the eye characterized by iridal and conjunctival effects. The effects were most pronounced 1 hour after instillation. The irritation was persistent, but by day 7, it had subsided. In a different experiment in which 0.1 g APFO was instilled for 5 or 30 seconds before washing with 200 mL of water, there was limited conjunctival irritation, but the effects were immediate and persistent.

PFOS

No gross or microscopic alterations were observed in the eyes from rats exposed to ≤1.77 mg/kg/day PFOS in the diet for 4 weeks or ≤1.56 mg/kg/day for 14 weeks (Seacat et al. 2003). Similar findings were reported in monkeys dosed daily with up to 2 mg/kg/day PFOS administered via a capsule for 4 weeks (Thomford 2002a) or up to 0.75 mg/kg/day PFOS administered via a capsule for 26 weeks (Seacat et al. 2002), and in rats dosed with up to 1.04 mg/kg/day in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b).

PFBS

No gross or microscopic alterations were observed in the eyes of rats administered ≤900 mg/kg/day PFBS via gavage for 28 days (3M 2001).

PFBA

Examination of the eyes of rats orally exposed to ≤150 mg/kg/day PFBA for 28 days or ≤30 mg/kg/day for 90 days did not reveal any significant alterations in the eyes (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFHxA

No ophthalmological alterations were observed in rats administered up to 500 mg/kg/day NaPFHx for 90–93 days (Chengelis et al. 2009b; Loveless et al. 2009).

2.13 ENDOCRINE

Overview. Epidemiological studies have examined a number of endocrine targets including thyroid gland and hormones, reproductive hormones, and insulin levels. A discussion of the thyroid effects is included in this section; the reproductive hormone effects are discussed in Section 2.16, Reproductive, and the insulin effects (as well as other effects associated with glucose metabolism and utilization) are discussed in Section 2.18, Other Noncancer. Summaries of results of epidemiological studies evaluating thyroid outcomes are presented in Table 2-15; more in-depth summaries of the studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 9. Although some associations between serum PFOA, PFOS, PFHxS, PFNA, PFDA, and PFUnA and thyroid stimulating hormone (TSH), triiodothyronine (T3), or thyroxine (T4) levels or thyroid disease have been found, the results are not consistent across studies and a larger number of studies have not found associations. A small number of studies have evaluated PFDoDA and most studies have not found consistent associations between serum perfluoroalkyl levels and thyroid hormone levels. No epidemiological studies examining endocrine health outcomes were identified for PFHpA, PFBS, PFBA, PFHxA, or FOSA.

Laboratory animal studies have primarily evaluated potential morphological alterations in endocrine tissues following oral exposure; these studies are summarized in Tables 2-3, 2-4, and 2-5. Some alterations in thyroid hormone levels have been observed in laboratory animals exposed to PFOA, PFOS, PFHxS, or PFDA. Histopathological alterations have been observed in the thyroid of some laboratory animal studies for PFHxS, PFBA, and PFHxA; the investigators noted that these effects were likely secondary to the hepatocellular hypertrophy, although the mechanism has not been established for these compounds. In general, the pituitary, parathyroid, thyroid, and adrenal glands do not appear to be

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gilliland 1992 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	TSH	**Association (p=0.004)***
Olsen et al. 1998b Occupational (n=111 in 1993 and n=80 in 1995)	10,000–<30,000 ng/mL (PFOA range)	TSH	NS (p=0.09 for trend), 1993 group Association (p=0.002), 1995 group
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	TSH	The investigators noted that the levels were within the reference range
		T4	
		T3	
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA	Thyroid disease risk	NS (p=0.98 for trend) no lag, males NS (p=0.55 for trend) 10-year lag, males NS (p=0.97 for trend) no lag, females NS (p=0.27 for trend) 10-year lag, females
Olsen and Zobel 2007 Occupational (n=552)	2,210 ng/mL (mean PFOA)	Free T4	**Association (p=0.01)***
		T4	NS (p=0.29)
		T3	**Association (p=0.05)***
		TSH	NS (p=0.08)
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Self-reported thyroid problems	**SPR 1.56 (1.22–1.98)***
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	TSH	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Knox et al. 2011a Community (n=50,113 adults ≥20 years of age)	52.6, 91.0, 98.6, and 124.3 ng/mL (mean PFOA in women ≤50 years, men ≤50 years, women >50 years, men >50 years, respectively)	T4	**Association (p≤0.0001)*, women ≤50 years** **Association (p<0.001), men and women >50 years**
		T3 uptake	**Inverse association (p=0.0001)* women ≤50 years** **Inverse association (p=0.005)*, women >50 years** **Inverse association (p=0.037)*, men >50 years**
Lopez-Espinosa et al. 2012 Community (n=10,725 children aged 1–17 years)	29.3 and 67.7–2,071 ng/mL (median and 4th quartile PFOA)	Thyroid disease	**OR 1.44 (1.02–2.03)*, per interquartile shift**
		Hypothyroidism	**OR 1.54 (1.00–2.37)*, per interquartile shift**
		Subclinical hypothyroidism	OR 0.98 (0.86–1.15), per interquartile shift
		Subclinical hyperthyroidism	OR 0.81 (0.58–1.15), per interquartile shift
		TSH	β -1.1 (-5.3–3.4), 4th quartile
		Total T4	β -0.1 (-1.7–1.4), 4th quartile
Winquist and Steenland 2014b Community (C8 and occupational) (n=28,541)	114.7–<202.2 ng/mL-year (2nd quintile estimated cumulative PFOA)	Functional thyroid disease	**HR 1.24 (1.02–1.51;p=0.031)* (women), retrospective analysis** HR 1.01 (0.94–1.07 per log linear increase in PFOA, p=0.853) (men), retrospective analysis NS (p=0.549) (women), prospective analysis NS (p=0.087) (men), prospective analysis

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Hyperthyroidism	NS (p=0.074) (women), retrospective analysis NS (p=0.858) (men), retrospective analysis NS (p=0.268) (women), prospective analysis NS (p=0.760) (men), prospective analysis
		Hypothyroidism	NS (p=0.076) (women), retrospective analysis NS (p=0.684) (men), retrospective analysis NS (p=0.247) (women), prospective analysis **HR 1.24 (1.03–1.49)* (men), prospective analysis**
Berg et al. 2017 General population (n=370 pregnant women)	1.53 ng/mL (median maternal serum PFOA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	1.33 ng/mL (geometric mean PFOA)	TSH	NS (p=0.871)
		Free T4	NS (p=0.896)
Chan et al. 2011 General population (n=94 women with hypothyroxinemia and 175 matched controls)	1.28 and 1.37 ng/mL (geometric mean PFOA in cases and controls)	Hypothyroxinemia risk	OR 0.94 (0.74–1.18)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Crawford et al. 2017 General population (n=99 30–44-year-old women)	2.79 ng/mL (geometric mean serum PFOA)	Total T4	NS (p=0.07)
		Free T4	NS (p=0.11)
		T3	**Association (β 6.05, p=0.03)***
		TSH	NS (p=0.37)
Dufour et al. 2018 General population (n=214 pregnant women)	0.80 ng/mL (cord blood mean PFOA); 0.44–0.68 ng/mL (2nd quartile cord blood PFOA)	Hypothyroidism	**OR 4.42 (1.23–21.14)*, 4th quartile**
		TSH in infants	NS (p=0.196)
Jain 2013 General population (NHANES) (n=1,525)	NR	Total T3	**Association (p=0.013)***
		TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	2.74 ng/mL (median PFOA)	TSH	NS (p=0.4055)
		T4	NS (p=0.2221)
Kang et al. 2018 General population (150 children, 3–18 years)	1.88 ng/mL (median serum PFOA)	Free T4	NS (p=0.075)
		TSH	NS (p=0.565)
Lewis et al. 2015 General population (NHANES) (n=1,682)	1.42–2.55 ng/mL (range of median PFOA for different age groups)	TSH	**Association (p<0.05)*, 12–20-year-old females**
		Free T4	**Association (p<0.05)*, 20–<40-year-old females**
		Total T4	NS (p>0.05)
		Free T3	**Association (p<0.05)*, 60–80-year-old females**
		Total T3	**Association (p<0.05)*, 60–80-year-old females**

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Melzer et al. 2010 General population (NHANES) (n=3,966)	9.47 and 10.39 ng/mL (4th PFOA quartile mean in women and men)	Thyroid disease risk	**OR 1.64 (1.09–2.46)*, females** OR 1.58 (0.74–3.39), males
Preston et al. 2018 General population (n=732 mothers and 480 infants)	5.6 ng/mL (maternal median serum PFOA)	Total T4	β 0.09 (-0.08–0.27)
		Free T4	**β -1.87 (-3.40 to -0.31)***
		TSH	β 0.28 (-9.26–10.8)
		Neonatal T4	**β -1.1 (-2.1 to -0.1)*, 4th quartile**
Raymer et al. 2012 General population (n=256)	10.4 ng/mL (mean PFOA)	TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.91 ng/mL (cord blood median PFOA)	Cord blood T4	NS (p=0.99)
		Cord blood T3	NS (p=0.99)
		Cord blood TSH	NS (p=0.24)
Shrestha et al. 2015 General population (n=87 with thyroid disease)	9.17 ng/mL (geometric mean PFOA)	TSH	NS (p=0.176)
		Free T4	NS (p=0.536)
		T4	NS (p=0.097)
		T3	NS (p=0.208)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	3.14 ng/mL (mean cord blood PFOA)	Cord blood T4	β -0.031 (-0.414–0.342)
		Cord blood T3	β 0.025 (-0.054–0.103)
		Cord blood TSH	β 0.059 (-0.136–0.254)
Wang et al. 2013a General population (n=903 pregnant women)	2.13 ng/mL (geometric mean PFOA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	2.39 ng/mL (median PFOA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Webster et al. 2016 General population (NHANES) (n=1,525)	4.2 ng/mL (geometric mean PFOA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	**Association (p<0.05)***
		Total T3	NS (p<0.05)
Wen et al. 2013 General population (NHANES) (n=1,181)	4.15 ng/mL (geometric mean PFOA)	TSH	NS (p=0.916), men NS (p=0.732), women
		Total T4	NS (p=1.0), men NS (p=0.705), women
		Total T3	NS (p=0.673), men **Association (p=0.035)*, women**
		Thyroglobulin	NS (p=0.226), men NS (p=0.341), women
		Subclinical hypothyroidism risk	OR1.29 (0.40–4.10), men **OR 7.42 (1.14–48.12, p<0.05), women**
		Subclinical hyperthyroidism risk	**OR 0.38 (0.16–0.95, p<0.05)*, men** OR 0.99 (0.13–7.59), women
Yang et al. 2016a General population (n=157 pregnant women)	1.95 ng/mL (mean PFOA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFOS			
Olsen et al. 1998a Occupational (n=327)	1,480–2,440 ng/mL (range of mean PFOS)	TSH	NS (p=0.95)
		Cortisol	NS (p=0.45)
Olsen et al. 2003a Occupational (n=518)	1,320 and 800 ng/mL (mean PFOS at the Decatur and Antwerp facilities, respectively)	T3	**Association (p=0.04)***

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Knox et al. 2011a Community (n=50,113 adults ≥20 years of age)	17.3, 24.8, 25.7, and 29.1 ng/mL (mean PFOA in women ≤50 years, men ≤50 years, women >50 years, men >50 years, respectively)	T4	**Association (p<0.0001)*, women ≤50 or >50 years** **Association (p=0.0001)*, men ≤50 or >50 years**
		T3 uptake	**Inverse association (p<0.0001)* women ≤50 years** **Inverse association (p=0.0001)*, women >50 years** **Inverse association (p=0.009)*, men ≤50 years** **Inverse association (p=0.0001)*, men >50 years**
Lopez-Espinosa et al. 2012 Community (n=10,725 children aged 1–17 years)	20.0 ng/mL (median PFOS)	Thyroid disease	OR 0.8 (0.62–1.08), per interquartile shift
		Hypothyroidism	OR 0.91 (0.63–1.31), per interquartile shift
		Subclinical hypothyroidism	OR 0.99 (0.86–1.13), per interquartile shift
		Subclinical hyperthyroidism	OR 0.80 (0.62–1.02), per interquartile shift
		TSH	β -1.0 (-0.3–2.3), per interquartile shift
		Total T4	β 1.1 (0.6–1.5), per interquartile shift
Berg et al. 2015 General population (n=391)	8.1–11.0 ng/mL (3rd PFOS quartile)	TSH	**Association (p=0.03)*, 3rd quartile**

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Berg et al. 2017 General population (n=370 pregnant women)	8.03 ng/mL (median maternal serum PFOS)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	**Association (p<0.05)*** NS (p<0.05) after adjustment for other perfluoroalkyls or persistent organic pollutants
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	19.57 ng/mL (geometric mean PFOS)	TSH	NS (p=0.896)
		Free T4	NS (p=0.623)
Chan et al. 2011 General population (n=94 women with hypothyroxinemia and 175 matched controls)	7.59 and 7.08 ng/mL (geometric mean PFOS in cases and controls)	Hypothyroxinemia risk	OR 0.88 (0.63–1.24)
Crawford et al. 2017 General population (n= 99 30–44-year-old women)	9.29 ng/mL (geometric mean serum PFOS)	Total T4	NS (p=0.28)
		Free T4	NS (p=0.42)
		T3	NS (p=0.19)
		TSH	NS (p=0.98)
Dallaire et al. 2009 General population (n=623)	18.28 ng/mL (geometric mean PFOS)	TSH	**Inverse association (p≤0.05)***
		T3	**Inverse association (p≤0.05)***
		T4-binding globulin	**Inverse association (p≤0.01)***
		Free T4	**Association (p≤0.05)***
Dufour et al. 2018 General population (n=214 pregnant women)	0.88 ng/mL (cord blood mean PFOS); 0.73–1.01 ng/mL (3rd quartile cord blood PFOS)	Hypothyroidism	**OR 3.22 (1.08–10.92)*, 3rd quartile** OR 2.95 (0.98–10.07), 4th quartile
		TSH in infants	NS (p=0.679)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	7.96 ng/mL (median PFOS)	TSH	NS (p=0.3537)
		T4	NS (p=0.1134)
Kang et al. 2018 General population (150 children, 3–18 years)	5.68 ng/mL (median serum PFOS)	Free T4	NS (p=0.987)
		TSH	NS (p=0.628)
Lewis et al. 2015 General population (NHANES) (n=1,682)	3.76–11.1 ng/mL (range of median PFOS for different age groups)	TSH	NS (p>0.05)
		Free T4	**Association (p<0.05)*, 20–<40-year-old females**
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
Melzer et al. 2010 General population (NHANES) (n=3,966)	57.73 and 50.96 ng/mL (4th PFOS quartile mean in women and men)	Thyroid disease risk	OR 1.15 (0.7–1.91, p=0.568), females OR 1.58 (0.72–3.47, p=0.251), males **OR 2.68 (1.03–6.98, p=0.043)*, males 4th quartile versus combined 1st and 2nd quartiles**
Preston et al. 2018 General population (n=732 mothers and 480 infants)	24.0 ng/mL (maternal median serum PFOS)	Total T4	β 0.01 (-0.14–0.16)
		Free T4	β -1.04 (-2.36–0.29)
		TSH	β 0.90 (-7.27–9.80) **β-16.4 (-29.8 to -0.38)*, TPOAb positive mothers**
		Neonatal T4	**β -1.1 (-2.1 to -0.1)*, 4th quartile.**

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Raymer et al. 2012 General population (n=256)	37.4 ng/mL (mean PFOS)	TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.66 ng/mL (cord blood median PFOS)	Cord blood T4	NS (p=0.10)
		Cord blood T3	NS (p=0.37)
		Cord blood TSH	NS (p=0.73)
Shrestha et al. 2015 General population (n=87 with thyroid disease)	31.6 ng/mL (geometric mean PFOS)	TSH	NS (p=0.094)
		Free T4	**Association (p=0.044)***
		T4	**Association (p=0.001)***
		T3	NS (p=0.287)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	7.24 ng/mL (mean cord blood PFOS)	Cord blood T4	**β -0.458 (-0.916 to -0.001, p<0.05)***
		Cord blood T3	β 0.027 (-0.072–0.125)
		Cord blood TSH	**β 0.346 (0.101–0.591, p<0.05)***
Wang et al. 2013a General population (903 pregnant women)	12.77 ng/mL (geometric mean PFOS)	TSH	**Association (p=0.03)***
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (285 pregnant women)	12.73 ng/mL (median PFOS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	NS (p>0.05)
Webster et al. 2016 General population (NHANES) (n=1,525)	13.9 ng/mL (geometric mean PFOS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p<0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wen et al. 2013 General population (NHANES) (n=1,181)	14.2 ng/mL (geometric mean PFOS)	TSH	NS (p=0.931), men NS (p=0.358), women
		Total T4	NS (p=0.840), men NS (p=0.433), women
		Total T3	NS (p=0.404), men NS (p=0.384), women
		Thyroglobulin	NS (p=0.342), men NS (p=0.061), women
		Subclinical hypothyroidism risk	**OR 1.98 (1.19–3.28, p<0.05)*, men** **OR 3.03 (1.14–8.07, p<0.05)*, women**
		Subclinical hyperthyroidism risk	OR 0.92 (0.19–4.46), men OR 1.90 (0.33–6.80), women
Yang et al. 2016a General population (n=157 pregnant women)	5.08 ng/mL (mean PFOS)	TSH	**Inverse association (p<0.01)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFHxS			
Berg et al. 2017 General population (n=370 pregnant women)	0.44 ng/mL (median maternal serum PFHxS)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bloom et al. 2010 General population (n=31)	0.75 ng/mL (geometric mean PFHxS)	TSH	NS (p=0.956)
		Free T4	NS (p=0.567)
Chan et al. 2011 General population (n=94 women with hypothyroxinemia and 175 matched controls)	1.28 and 1.37 ng/mL (geometric mean PFHxS in cases and controls)	Hypothyroxinemia risk	OR 1.12 (0.89–1.41)
Crawford et al. 2017 General population (n= 99 30–-44-year-old women)	1.59 ng/mL (geometric mean serum PFHxS)	Total T4	NS (p=0.50)
		Free T4	NS (p=0.84)
		T3	NS (p=0.22)
		TSH	NS (p=0.71)
Dufour et al. 2018 General population (n=214 pregnant women)	0.18 ng/mL (cord blood mean PFHxS)	Hypothyroidism	OR 1.92 (95% CI 0.87–4.25),detected versus non-detected
		TSH in infants	NS (p=0.894)
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	1.51 ng/mL (median PFHxS)	TSH	NS (p=0.8144)
		T4	NS (p=0.5147)
Kang et al. 2018 General population (150 children, 3–18 years)	0.793 ng/mL (median serum PFHxS)	Free T4	NS (p=0.308)
		TSH	NS (p=0.901)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lewis et al. 2015 General population (NHANES) (n=1,682)	0.69–1.81 ng/mL (range of median PFHxS for different age groups)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
Preston et al. 2018 General population (n=732 mothers and 480 infants)	2.4 ng/mL (maternal median serum PFHxS)	Total T4	β -0.05 (-0.14–0.04)
		Free T4	β -0.60 (-1.39–0.19)
		TSH	β 2.89 (-2.12–8.17)
		Neonatal T4	**β -1.1 (-2.1 to -0.1)*, 4th quartile**
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.38 ng/mL (cord blood median PFHxS)	Cord blood T4	NS (p=0.83)
		Cord blood T3	NS (p=0.15)
		Cord blood TSH	NS (p=0.15)
Wang et al. 2013a General population (n=903 pregnant women)	0.62 ng/mL (geometric mean PFHxS)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	0.81 ng/mL (median PFHxS)	TSH	**Association (p<0.05)**
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	NS (p>0.05)
Webster et al. 2016 General population (NHANES) (n=1,525)	1.9 ng/mL (geometric mean PFHxS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wen et al. 2013 General population (NHANES) (n=1,181)	2.0 ng/mL (geometric mean PFHxS)	TSH	NS (p=0.608), men NS (p=0.720), women
		Total T4	NS (p=0.641), men **Association (p=0.022)*, women**
		Total T3	NS (p=0.917), men **Association (p<0.001)*, women**
		Thyroglobulin	NS (p=0.455), men NS (p=0.725), women
		Subclinical hypothyroidism risk	OR 1.57 (0.76–3.25), men **OR 3.10 (1.22–7.86, p<0.05)*, women**
		Subclinical hyperthyroidism risk	OR 0.56 (0.24–1.20.92), men **OR 12.27 (1.07–4.80.90)*,women**
Yang et al. 2016a General population (n=157 pregnant women)	0.63 ng/mL (mean PFHxS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	TSH	Investigators noted differences between groups was small and not clinically relevant
		T4	
		T3	
Lopez-Espinosa et al. 2012 Community (n=10,725 children aged 1–17 years)	1.5 ng/mL (median PFNA)	Thyroid disease	OR 1.05 (0.78–1.41), per interquartile shift
		Hypothyroidism	OR 1.11 (0.77–1.60), per interquartile shift
		Subclinical hypothyroidism	OR 0.99 (0.88–1.12), per interquartile shift
		Subclinical hyperthyroidism	OR 0.78 (0.61–1.01), per interquartile shift

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		TSH	β -1.1 (CI 0.7–1.5), per interquartile shift
		Total T4	B 0.8 (-0.4–2.0), per interquartile shift
Berg et al. 2017 General population (n=370 pregnant women)	0.56 ng/mL (median maternal serum PFNA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	0.79 ng/mL (geometric mean PFNA)	TSH	NS (p=0.789)
		Free T4	NS (p=0.424)
Crawford et al. 2017 General population (n= 99 30–44-year-old women)	0.84 ng/mL (geometric mean serum PFNA)	Total T4	NS (p=0.34)
		Free T4	**Association (β 0.08, p<0.01)***
		T3	**Association (β 5.65, p=0.02)***
		TSH	NS (p=0.91)
Dufour et al. 2018 General population (n=214 pregnant women)	0.18 ng/mL (cord blood mean PFNA), 0.23–0.68 ng/mL (4th quartile cord blood PFNA)	Hypothyroidism	OR 1.17 (0.37–3.92), 4th quartile
		TSH in infants	NS (p=0.064)
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Ji et al. 2012 General population (n=633)	2.09 ng/mL (median PFNA)	TSH	NS (p=0.1354)
		T4	NS (p=0.7436)
Kang et al. 2018 General population (150 children, 3–18 years)	0.938 ng/mL (median serum PFNA)	Free T4	**β 0.052 (0.007–0.097, p=0.025)***
		TSH	NS (p=0.840)
Preston et al. 2018 General population (n=732 mothers and 480 infants)	0.6 ng/mL (maternal median serum PFNA)	Total T4	β -0.05 (-0.16–0.05)
		Free T4	β -0.57 (-1.52–0.40)
		TSH	β -0.27 (-6.19–6.03)
		Neonatal T4	β 0.05 (-0.29–0.39)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.2 ng/mL (cord blood median PFNA)	Cord blood T4	NS (p=0.70)
		Cord blood T3	NS (p=0.93)
		Cord blood TSH	NS (p=0.14)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	7.55 ng/mL (mean cord blood PFNA)	Cord blood T4	β -0.067 (-0.252–0.009)
		Cord blood T3	β -0.03 (0.069–0.103)
		Cord blood TSH	β 0.045 (-0.051–0.142)
Wang et al. 2013a General population (n=903 pregnant women)	0.37 ng/mL (geometric mean PFNA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	1.51 ng/mL (median PFNA)	TSH	NS (p>0.05)
		Free T4	**Inverse association (p<0.001)***
		Total T4	**Inverse association (p<0.001)***
		Total T3	NS (p>0.05)
Webster et al. 2016 General population (NHANES) (n=1,525)	1.5 ng/mL (geometric mean PFNS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wen et al. 2013 General population (NHANES) (n=1,181)	1.54 ng/mL (geometric mean PFNA)	TSH	NS (p=0.973), men NS (p=0.407), women
		Total T4	NS (p=0.097), men NS (p=0.632), women
		Total T3	NS (p=0.063), men NS (p=0.258), women
		Thyroglobulin	NS (p=0.537), men NS (p=0.395), women
		Subclinical hypothyroidism risk	OR 1.30 (0.65–2.60), men OR 2.54 (0.40–16.05), women
		Subclinical hyperthyroidism risk	OR 2.41 (0.48–12.04), men OR 1.91 (0.83–4.38), women
Yang et al. 2016a General population (n=157 pregnant women)	0.52 ng/mL (mean)	TSH	**Inverse association (p<0.05)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFDA			
Berg et al. 2015 General population (n=391 pregnant women)	0.31–2.34 ng/mL (4th PFDA quartile)	T3	**Association (p=0.03) (4th quartile)**
Berg et al. 2017 General population (n=370 pregnant women)	0.23 ng/mL (median maternal serum PFNA) and 0.32–2.34 ng/mL (4th quartile serum PFNA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	**β -0.02 (-0.044 to -0.005, p<0.05)***
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bloom et al. 2010 General population (n=31)	0.21 ng/mL (geometric mean PFDA)	TSH	NS (p=0.365)
		Free T4	NS (p=0.107)
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	0.91 ng/mL (median PFDA)	TSH	NS (p=0.2721)
		T4	NS (p=0.2176)
Kang et al. 2018 General population (150 children, 3–18 years)	0.0592 ng/mL (median serum PFDA)	Free T4	NS (p=0.153)
		TSH	NS (p=0.420)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.1 ng/mL (cord blood median PFDA)	Cord blood T4	NS (p=0.40)
		Cord blood T3	NS (p=0.07)
		Cord blood TSH	NS (p=0.22)
Wang et al. 2013a General population (903 pregnant women)	0.09 ng/mL (geometric mean PFDA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (285 pregnant women)	0.46 ng/mL (median PFDA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	**Association (p<0.01)***
Yang et al. 2016a General population (n=157 pregnant women)	0.45 ng/mL (mean PFDA)	TSH	**Inverse association (p<0.01)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Berg et al. 2015 General population (n=391)	0.4–0.96 ng/mL (4th PFUnA quartile)	Free T3	**Association (p=0.00)*, 4th quartile**
Berg et al. 2017 General population (n=370 pregnant women)	0.26 ng/mL (median maternal serum PFUnA) and 0.39–1.46 (4th quartile serum PFUnA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	**β -0.02 (-0.033 to -0.003, p<0.05)**
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	0.20 ng/mL (geometric mean PFUnA)	TSH	NS (p=0.527)
		Free T4	NS (p=0.204)
Ji et al. 2012 General population (n=633)	1.75 ng/mL (median PFUnA)	TSH	NS (p=0.5368)
		T4	NS (p=0.0642)
Kang et al. 2018 General population (150 children, 3–18 years)	0.0652 ng/mL (median serum PFUnA)	Free T4	NS (p=0.581)
		TSH	NS (p=0.510)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.26 ng/mL (cord blood median PFUnA)	Cord blood T4	NS (p=0.86)
		Cord blood T3	NS (p=0.35)
		Cord blood TSH	NS (p=0.37)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	15.94 ng/mL (mean cord blood PFNA)	Cord blood T4	β 0.045 (-0.223–0.313)
		Cord blood T3	β 0.048 (-0.008–0.104)
		Cord blood TSH	β 0.077 (0.063–0.216)
Wang et al. 2013a General population (n=903 pregnant women)	0.20 ng/mL (geometric mean PFUnA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2014 General population (n=285 pregnant women)	3.26 ng/mL (median PFUnA)	TSH	NS (p>0.05)
		Free T4	**Inverse association (p<0.001)***
		Total T4	**Inverse association (p<0.001)***
		Total T3	NS (p>0.05)
Yang et al. 2016a General population (n=157 pregnant women)	0.45 ng/mL (mean PFUnA)	TSH	**Inverse association (p<0.05)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFDoDA			
Ji et al. 2012 General population (n=633)	0.92 ng/mL (median PFDoDA)	TSH	NS (p=0.6925)
		T4	NS (p=0.7153)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.08 ng/mL (cord blood median PFDoDA)	Cord blood T4	NS (p=0.69)
		Cord blood T3	NS (p=0.30)
		Cord blood TSH	NS (p=0.20)
Wang et al. 2014 General population (285 pregnant women)	0.36 ng/mL (median PFDoDA)	TSH	NS (p>0.05)
		Free T4	**Inverse association (p<0.001)***
		Total T4	**Inverse association (p<0.01)***
		Total T3	NS (p>0.05)
Yang et al. 2016a General population (n=157 pregnant women)	0.046 ng/mL (mean PFDoDA)	TSH	**Inverse association (p<0.01)***
		Free T4	**Inverse association (p<0.05)***
		Total T4	**Inverse association (p<0.05)***
		Free T3	**Inverse association (p<0.01)***
		Total T3	**Inverse association (p<0.01)***

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Me-FOSA-AcOH			
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 9 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

sensitive targets following exposure to PFOA, PFOS, PFDA, PFBS, or PFBA. Endocrine effects have not been examined in laboratory animal studies on PFNA, PFUnA, PFHpA, or FOSA.

PFOA

Epidemiological Studies. A number of epidemiological studies have examined the potential of PFOA to damage the thyroid. Steenland et al. (2015) did not find an association between serum PFOA and the risk of thyroid disease in male or female workers at the Washington Works facility. The occupational exposure studies do not suggest an association between serum PFOA and alterations in thyroid hormone levels. One study (Olsen and Zobel 2007) reported associations between serum PFOA levels and free T4 and T3 levels in workers at 3M facilities; it is noted that the investigators did not consider the results clinically relevant since the levels were within the normal range. A study reported an association between serum PFOA and TSH, but this was only observed at one time point (Olsen et al. 1998b); another study of the 3M Cottage Grove facility, reported an association between serum fluorine levels and TSH levels (Gilliland 1992). A fifth occupational study reported that TSH, T4, and T3 levels were within the reference range (Sakr et al. 2007b).

Three studies of the community affected by the Washington Works facility reported increases in self-reported thyroid disease (Anderson-Mahoney et al. 2008), any type of functional thyroid disease (Lopez-Espinosa et al. 2012; Winquist and Steenland 2014b), or hypothyroidism (Lopez-Espinosa et al. 2012). No associations between estimated cumulative serum PFOA and hyperthyroidism or hypothyroidism were found in retrospective analysis (Winquist and Steenland 2014b). However, in prospective analysis, an association between estimated cumulative serum PFOA and hypothyroidism was found in men (Winquist and Steenland 2014b). Consistent with the occupational exposure data, no association between serum PFOA and TSH levels was found (Emmett et al. 2006b; Knox et al. 2011a; Lopez-Espinosa et al. 2012). Increases in serum PFOA were also associated with increases in T4 levels and decreases in T3 uptake in adults (Knox et al. 2011a).

A number of studies have examined the thyroid outcomes associated with serum PFOA levels in the general population. An association between serum PFOA and thyroid disease risk was found in female NHANES participants, but not in males (Melzer et al. 2010). Another study utilizing NHANES data (Wen et al. 2013) found an increased risk of subclinical hypothyroidism among women, but not men, and a decreased risk of subclinical hyperthyroidism among men, but not women. An increased risk of hypothyroidism was also observed in a study of pregnant women (DuFour et al. 2018). A case-control

study of women did not find that serum PFOA levels were associated with the risk of hypothyroxinemia (Chan et al. 2011). Although five studies found associations between serum PFOA and T3 levels (Crawford et al. 2017; Jain 2013; Lewis et al. 2015; Webster et al. 2016; Wen et al. 2013), five other studies did not find these associations (Berg et al. 2017; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2014; Yang et al. 2016a). No associations between serum PFOA and TSH or T4 levels were found in the general population studies (Berg et al. 2017; Bloom et al. 2010; Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2013a, 2014; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a), with the exception of two studies which found an association for TSH and T4 levels (Lewis et al. 2015) or free T4 (Preston et al. 2018).

Studies examining possible relationships between cord blood PFOA and cord blood thyroid hormone levels have not found associations for T4, T3, or TSH (Dufour et al. 2018; Shah-Kulkarni et al. 2016; Tsai et al. 2017). Preston et al. (2018) found an inverse association between maternal serum PFOA and neonatal T4 levels.

In a clinical trial of patients with advanced solid tumors administered 50–1,200 mg APFO (approximately 0.10–2.4 mg/kg/day) for 6 weeks, increases in free T4 levels were observed with no apparent alterations in TSH (Convertino et al. 2018).

Laboratory Animal Studies. Repeated intermittent head-only exposure of male rats to up to 84 mg/m^3 APFO dusts for 2 weeks did not result in significant gross or microscopic alterations in the thyroid or adrenal gland (Kennedy et al. 1986).

In a 2-generation study in rats, daily treatment of the parental generation with 0, 1, 3, 10, or 30 mg/kg/day APFO by gavage in water for 70–90 days produced an increased incidence of hypertrophy and/or vacuolation of the zona glomerulosa of the adrenal gland from high-dose males (Butenhoff et al. 2004b). The respective incidences were 0/10, 0/10, 0/10, 2/10, and 7/10. This effect was also observed in F1 generation males treated with the same dose level. No explanation was apparent for this finding. In rats dosed with up to 15 mg/kg/day APFO in the diet for 2 years, there were no significant morphological alterations in the adrenals (3M 1983; Butenhoff et al. 2012c). A study in monkeys treated with APFO also reported effects on the adrenal glands. Griffith and Long (1980) reported diffuse lipid depletion in the adrenals from Rhesus monkeys dosed daily for 90 days with 30 mg/kg/day APFO by gavage. This dose level was lethal to some monkeys; no such effect was seen in monkeys dosed with 10 mg/kg/day.

For the most part, morphological evaluations of other endocrine glands in animals treated with PFOA have been negative. For example, male and female rats dosed via the diet with approximately 100–110 mg/kg/day APFO for 90 days showed no gross or microscopic alterations in the pituitary or thyroid glands (Griffith and Long 1980). Similar observations were reported in the pituitary, thyroid, and parathyroid glands from male and female rats dosed with up to 15 mg/kg/day APFO in the diet for 2 years (Butenhoff et al. 2012c; 3M 1983).

Administration of up to 20 mg/kg/day PFOA administered via a capsule to Cynomolgus monkeys for 4 weeks did not significantly alter free T4, total T4, free T3, total T3, or TSH (Thomford 2001). Serum T4 and total T4 were significantly reduced in Cynomolgus monkeys dosed with 10 mg/kg/day APFO administered via a capsule for up to 6 months, but were still within the normal range (Butenhoff et al. 2002). No significant changes were seen on serum free T3, total T3, or TSH, or thyroid histology.

The only relevant dermal information is that no morphological alterations were observed in the thyroid of rats following dermal application of up to 2,000 mg/kg/day APFO for 2 weeks in the Kennedy (1985) study.

PFOS

Epidemiological Studies. A number of epidemiological studies have examined the risk of thyroid disease and alterations in thyroid hormone levels to evaluate whether the thyroid gland is a target of PFOS toxicity. In studies of NHANES participants, no increases in the risk of thyroid disease were observed in men or women (Lewis et al. 2015; Melzer et al. 2010). Melzer et al. (2010) did find an increase in the risk of having thyroid disease and currently taking thyroid medication among men, and Wen et al. (2013) found increased risks of subclinical hypothyroidism among men and women. Dufour et al. (2018) also found an association between cord blood PFOS and risk of maternal hypothyroidism. Although some studies have found alterations in thyroid hormone levels, the results are not consistent across studies. Associations between serum PFOS and TSH levels were observed in three general population studies (Berg et al. 2015, 2017; Wang et al. 2014); however, one of the studies (Berg et al. 2017) found that the association was no longer significant after adjustments for exposure to other perfluoroalkyls and persistent organic compounds. In contrast, two other studies found inverse associations for TSH (Dallaire et al. 2009; Yang et al. 2016a). A third study also found an inverse association with TSH but only among pregnant women who were positive for thyroid peroxides antibodies (Preston et al. 2018). An occupational exposure study (Olsen et al. 1998a) and ten general population studies (Bloom et al. 2010;

Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Lewis et al. 2015; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2014; Wen et al. 2013) did not find associations between serum PFOS and TSH levels. Conflicting results were also reported for T3 levels, with some studies reporting associations (Olsen et al. 2003a), inverse associations (Dallaire et al. 2009), or no association (Berg et al. 2017; Crawford et al. 2017; Jain 2013; Lewis et al. 2015; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2014; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a). Most studies did not find an association with T4 levels (Berg et al. 2017; Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Lewis et al. 2015; Raymer et al. 2012; Preston et al. 2018; Wang et al. 2014; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a), but three studies did find associations between T4 levels and serum PFOS (Dallaire et al. 2009; Lewis et al. 2015; Shrestha et al. 2015). In NHANES participants with two indicators of thyroid stress (low iodine levels and high thyroid peroxidase antibody), serum PFOS levels were significantly ($p<0.05$) associated with increases in free and total T3, decreases in free T4, and increases in TSH levels (Webster et al. 2016).

Conflicting results were also found in studies using cord blood PFOS as the biomarker of exposure. Tsai et al. (2017) found an inverse association with cord blood T4 and a positive association with cord blood TSH; no association was found for T3. Shah-Kulkarni et al. (2016) found no associations for cord blood T4, T3, or TSH. It is noted that cord blood serum PFOS levels were much higher in the Tsai et al. (2017) study compared to the Shah-Kulkarni et al. (2016) study.

Laboratory Animal Studies. Chang et al. (2008b) conducted a study of thyroid function in rats exposed to PFOS (potassium salt). Administration of a single dose of 15 mg/kg by gavage in water (only dose level tested) reduced serum total T4 significantly at 2, 6, and 24 hours after dosing. This effect was attributed to a PFOS-induced transient increase in tissue availability of thyroid hormones and turnover of T4 with a resulting reduction in serum total T4. Chang et al. (2008b) concluded that PFOS did not induce a classical hypothyroid state or alter the hypothalamic-pituitary-thyroid axis. In another acute-duration study, dosing of pregnant mice with 6 mg/kg/day PFOS (potassium salt) on GDs 6–18 did not affect maternal serum levels of free or total T3 or T4 (Fuentes et al. 2006).

Changes in thyroid hormones have also been reported following intermediate-duration exposure to PFOS. For example, in a 2-generation gavage study in which dosing of rats started before mating and continued through gestation, doses ≥0.4 mg/kg/day (the lowest dose tested) caused a significant and dose-related reduction in total T4 in maternal serum on postpartum day 5 (Luebker et al. 2005b). Free T4 and TSH were not significantly affected. Exposure of pregnant rats to ≥1 mg/kg/day PFOS on GDs 2–20 induced

significant reductions in total T4 and free T4 and less marked reductions in T3 during pregnancy, particularly on GD 7 (Thibodeaux et al. 2003); however, serum TSH values were not significantly altered. A similar study in pregnant mice reported a decrease in total T4 on GD 6 in mice dosed with 20 mg/kg/day PFOS on GDs 1–17 (Thibodeaux et al. 2003). No alterations in total T4 were reported in mice dosed with 15 mg/kg/day. No information was provided regarding other thyroid hormones. Decreases in T4 levels were observed in male and female rats exposed to PFOS in the diet for 28 days (Curran et al. 2008); T3 levels were decreased in female rats exposed to 50 or 100 mg/kg/day and in male rats at 100 mg/kg/day. No histological alterations were observed in the thyroid. Another study with PFOS found no thyroid histological effects in rats exposed to 10.3 mg/kg/day for 1 day, 8.17 mg/kg/day for 7 days, or 7.34 mg/kg/day for 28 days (Elcombe et al. 2012a). Exposure of rats to ≥0.27 mg/kg/day PFOS in drinking water for 91 days resulted in decreases in total T4 levels (Yu et al. 2009a), but no changes in T3 or TSH levels (highest dose tested was 2.37 mg/kg/day). Curran et al. (2008) suggested that the apparent decreases in T4 levels, in the absence of TSH alterations and histological alterations in the thyroid, may be a result of measurement error when analog assays (chemiluminometric immunoassay and radioimmunoassay) are used due to binding interference. A decrease in serum total T4 levels was observed in Cynomolgus monkeys administered three doses of PFOS (average dose of 13.3 mg/kg in males and 14 mg/kg in females) over 315 days (Chang et al. 2017). The investigators did not consider this an adverse effect because the values were within the normal variation and there were not changes in free T4 levels or TSH levels. In another study in Cynomolgus monkeys, T3 was numerically lower than controls in one female and one male monkey dosed with 2 mg/kg/day PFOS by capsule for 4 weeks (Thomford 2002a). However, it is difficult to determine whether the effect was treatment-related based on only two animals. In a 26-week study in Cynomolgus monkeys, the highest dose of PFOS tested, 0.75 mg/kg/day, induced a significant increase in serum TSH (approximately twice control value, but still within the reference range) and a decrease in total T3 at termination, but not at earlier time points; variations in other thyroid hormones, including T4, were inconsistent regarding dose and over time (Seacat et al. 2002). The clinical relevance of the lowered total T3 values was not apparent since there was no indication of a clinical hypothyroid response, and thyroid histology was not altered by treatment with PFOS.

Examination of the adrenal glands from rats dosed with up to 1.77 mg/kg/day PFOS via the diet for 4 or 14 weeks did not show any significant gross or microscopic alterations (Seacat et al. 2003). No significant gross or microscopic lesions were reported in the adrenals, thyroid and parathyroid, or pituitary gland from rats dosed with up to 1.04 mg/kg/day PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Epidemiological Studies. Fifteen general population studies have evaluated possible associations between serum PFHxS levels and alterations in thyroid hormone levels. With the exception of a study of pregnant women, which found an association between serum PFHxS levels and TSH levels (Wang et al. 2014), and a study of NHANES participants, which found associations between serum PFHxS and total T4 and T3 in women (Wen et al. 2013), the epidemiological studies did not find associations for TSH, T3, or T4 (Berg et al. 2017; Bloom et al. 2010; Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Lewis et al. 2015; Preston et al. 2018; Wang et al. 2013a, 2014; Webster et al. 2016; Yang et al. 2016a). No associations were also found between cord blood PFHxS levels and cord blood T4, T3, or TSH (Shah-Kulkarni et al. 2016). Chan et al. (2011) did not find an increase in the risk of hypothyroxinemia associated with serum PFHxS levels. Wen et al. (2013) found increases in the risk of subclinical hypothyroidism and subclinical hyperthyroidism among women, but not men and Dufour et al. (2018) did not find an association between cord blood PFHxS levels and risk of hypothyroidism in pregnant women.

Laboratory Animal Studies. Hypertrophy and hyperplasia of the follicular cells were observed in the thyroids of male rats treated with ≥3 mg/kg/day PFHxS for at least 42 days (Butenhoff et al. 2009a). The NOAEL was 1 mg/kg/day. The investigators noted that the observed changes in rats are consistent with the known effects of inducers of microsomal enzymes where the hepatocellular hypertrophy results in a compensatory hypertrophy and hyperplasia of the thyroid due to an increase in plasma turnover of T4 and associated stimulation of TSH. Neither thyroid hormones nor TSH were measured in the study. In studies of pregnant rats, 20–30 and 60% decreases in serum thyroxine were observed in the dams administered 5 mg/kg/day or 25 mg/kg/day PFHxS on GD 7–22 (Ramhøj et al. 2018). In mice administered up to 3 mg/kg/day PFHxS prior to mating and during mating, gestation, and lactation, no alterations in TSH were observed in the parental males or females (Chang et al. 2018); this study also found no histological alterations in the thyroid gland.

PFNA

Epidemiological Studies. Inverse associations between serum PFNA levels and T4 levels (Wang et al. 2014) and TSH levels (Yang et al. 2016a) have been reported in general population studies. However, several other studies have not found alterations in TSH, T4, or T3 levels associated with serum PFNA

levels (Berg et al. 2017; Bloom et al. 2010; Jain 2013; Ji et al. 2012; Lopez-Espinosa et al. 2012; Preston et al. 2018; Wang et al. 2013a; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a). The investigators for an occupational exposure study reported that differences in TSH, T4, and T3 levels were small and clinically insignificant in groups of workers exposed to low levels, high levels, or no PFNA (Mundt et al. 2007). Preston et al. (2018) found an inverse association between serum PFNA levels and TSH levels, but only in pregnant women who were positive for maternal thyroid peroxides antibodies. Crawford et al. (2017) found associations between serum PFNA and free T4 and T3 levels in women, but no associations with total T4 or TSH. No associations between cord blood PFNA levels and cord blood T4, T3, or TSH were found in two studies (Shah-Kulkarni et al. 2016; Tsai et al. 2017). No associations were found for thyroid disease, hypothyroidism, or subclinical hypo- or hyperthyroidism among residents living near the Washington Works PFOA facility (Lopez-Espinosa et al. 2012), in NHANES participants (Wen et al. 2013), or in pregnant women (Dufour et al. 2018).

PFDA

Epidemiological Studies. Most general population studies did not find associations between serum PFDA levels and TSH, T3, or T4 levels (Berg et al. 2017; Bloom et al. 2010; Ji et al. 2012; Kang et al. 2018; Wang et al. 2013a, 2014; Yang et al. 2016a). The exceptions were studies in pregnant women that found positive associations (Berg et al. 2015; Wang et al. 2014) or inverse associations with T3 (Berg et al. 2017), or an inverse association with TSH levels (Yang et al. 2016a). No associations between cord blood PFDA and cord blood T4, T3, or TSH were found in a study by Shah-Kulkarni et al. (2016).

Laboratory Animal Studies. Administration of a single dose of 80 mg/kg PFDA to female C57BL/6N mice by gavage resulted in 2- and 4-fold increases in serum T3 and T4, respectively, relative to controls 30 days after dosing (Harris et al. 1989). No alterations were observed in the adrenal glands of rats administered 0.5 mg/kg/day PFDA for 28 days or mice receiving weekly gavage doses of 5 mg/kg for 4 weeks (Frawley et al. 2018).

PFUnA

Epidemiological Studies. Inverse associations between serum PFUnA and serum TSH (Yang et al. 2016a) T4 (Wang et al. 2014), or T3 (Berg et al. 2015, 2017) have been reported in pregnant women. However, other general population studies have not found association between PFUnA and TSH, T4, or T3 levels (Bloom et al. 2010; Ji et al. 2012; Kang et al. 2018; Wang et al. 2013a, 2014; Yang et al. 2016a)

or between cord blood PFUnA and cord blood T4, T3, or TSH (Shah-Kulkarni et al. 2016; Tsai et al. 2017).

PFBS

Laboratory Animal Studies. Treatment of rats with up to 900 mg/kg/day PFBS by gavage for 28 days did not alter the gross or microscopic appearance of the adrenal, pituitary, or thyroid/parathyroid glands (3M 2001). Levels of thyroid hormones in serum were not available in this study. A study in pregnant mice administered ≥200 mg/kg/day PFBS on GDs 1–20 found decreases in maternal levels of total T4, free T4, and total T3 and increases in TSH levels (Feng et al. 2017).

PFBA

Laboratory Animal Studies. Treatment of rats with up to 184 mg/kg/day PFBA by gavage for 5 days did not affect the gross or microscopic morphology of the adrenal, thyroid, or pituitary glands (3M 2007a). Treatment with ≥30 mg/kg/day for 28 or 90 days significantly increased the incidence of hyperplasia/ hypertrophy of the follicular epithelium of the thyroid gland (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b). These changes were not observed following a 3-week recovery period. Van Otterdijk (2007a, 2007b; Butenhoff et al. 2012a) suggested that the thyroid lesion likely reflected an increase in T4 producing follicular cells in response to feedback mechanisms from the increased turnover of T4 by the hypertrophic hepatocytes. None of these studies measured thyroid hormones or TSH in serum.

PFDoDA

Epidemiological Studies. Four general population studies have evaluated the effect of PFDoDA on thyroid hormone levels. Wang et al. (2014) reported inverse associations between serum PFDoDA and free T4 and total T4 in pregnant women; no associations were found for TSH or total T3. In another study of pregnant women (Yang et al. 2016a), inverse associations were found for TSH, free T4, total T4, free T3, and total T3. The third study (Ji et al. 2012) found no associations between serum PFDoDA and TSH or T4. Shah-Kulkarni et al. (2016) did not find associations between cord blood PFDoDA levels and cord blood T4, T3, or TSH levels.

Laboratory Animal Studies. Histological alterations were observed in the pancreas, adrenal gland, and/or thymus of rats administered 2.5 mg/kg/day PFDoDA for 42–47 days (Kato et al. 2015). Decreases in

zymogen granules were observed in the pancreas of male rats and edema of the pancreas interstitium was observed in females (most female rats died before the end of the study). Atrophy of the adrenal cortex was observed in males and in females exposed for 42 days and allowed to recover for 14 days. Atrophy of the thymic cortex was observed in females (most dying before the end of the study). A 28-day study found a 40% reduction in serum estradiol levels in pubertal female rats administered 3 mg/kg/day for 28 days (Shi et al. 2009b).

PFHxA

Laboratory Animal Studies. An increased incidence of thyroid follicular epithelial hypertrophy was observed in female rats administered 500 mg/kg/day NaPFHx for 93 days (Loveless et al. 2009). No alterations were observed in male rats in this study or in a second 90-day study in which male and female rats were administered doses as high as 200 mg/kg/day NaPFHx.

2.14 IMMUNOLOGICAL

Overview. Epidemiological studies have evaluated three categories of altered immune response related to exposure to perfluoroalkyls: immunosuppression (altered antibody response, infectious disease resistance), hypersensitivity (asthma, wheezing, eczema, atopic dermatitis, allergies), and autoimmunity. A summary of epidemiological studies evaluating immunological endpoints is presented in Table 2-16; more detailed descriptions of individual studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 10. Epidemiological data evaluating potential immunological effects are available for all perfluoroalkyls except PFBA. In general, the epidemiological studies identify the immune system as a target of perfluoroalkyl toxicity. The strongest evidence of the immunotoxicity of perfluoroalkyls in humans comes from epidemiological studies finding associations evaluating the antibody response to vaccines. Associations have been found for PFOA, PFOS, PFHxS, and PFDA. There is also some limited evidence for decreased antibody response for PFNA, PFUnA, and PFDoDA, although many of the studies did not find associations for these compounds. In general, decreases in disease resistance have not been found for PFOA, PFOS, PFHxS, or PFNA. There is marginal evidence for associations between PFOA, PFOS, PFHxS, PFNA, PFDA, PFBS, and PFDoDA and increased risk of asthma; the evidence was considered marginal due to the small number of studies evaluating the outcome and/or conflicting study results. There are limited data of effects on

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Anderson-Mahoney et al. 2008 Community (n=566 adults)	NR	Asthma	**SPR 1.82 (1.47–2.25)***
Ashley-Martin et al. 2015 General population (1,258 women)	NR	IL-33/TSLP (cord blood)	OR 1.1 (0.6–1.8)
		IgE (cord blood)	OR 1.1 (0.6–1.9)
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	3.59 and 3.27 ng/mL (geometric mean 2005–2006 and 2007–2010) >4.47 ng/mL (4th quartile)	Food allergies	**OR 9.09 (3.52–24.90)*, 4th quartile**
		Food sensitization	NS (p=0.74 for trend)
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	2.04–10.12 ng/mL (maternal 3rd tertile PFOA)	Risk of number of days above the median	
		Fever	**OR 1.97 (1.07–3.62)*, 3rd tertile**
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
		Risk of number of days	
		Fever	OR 1.12 (0.82–1.54), 3rd tertile
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	1.5 and 1.0 ng/mL (mean serum PFOA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.67 (1.49–4.79)*, 3rd quartile**
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2010 General population. (n=1,400 pregnant women and young children)	5.6 ng/mL (maternal PFOA)	Risk of hospitalization for infectious disease in young children	IRR 0.96 (0.87–1.06) for trend **IRR 1.21 (1.04–1.42)* for trend, girls** **IRR 0.83 (0.73–0.95)* for trend, boys**
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	2.713 ng/mL (mean maternal plasma PFOA)	Prevalence of allergic disease	OR 0.830 (0.591–1.16), 4th quartile
		Prevalence of wheezing	OR 1.09 (0.729–1.65), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	2.713 ng/mL (mean maternal serum PFOA)	Risk of total infectious diseases	OR 1.11 (0.806–1.54), 4th quartile, p=0.393 for trend.
Grandjean et al. 2012; Mogensen et al. 2015a General population (n=456 and n=464 children 5 and 7 years of age)	4.1 and 4.4 ng/mL (median PFOA at age 5 and 7 years) 3.20 ng/mL (geometric mean maternal PFOA)	Tetanus antibody levels at age 5	NS, maternal PFOA NS, PFOA at age 5
		Tetanus antibody levels at age 7	NS, maternal PFOA **β -35.8% (-51.9 to -14.2)*, per 2-fold increase in PFOA levels at age 5** NS, PFOA at age 7
		Diphtheria antibody levels at age 5	NS, maternal PFOA NS (p=0.69), PFOA at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFOA **β -25.2% (-42.9 to -2.0)*, per 2-fold increase in PFOA levels at age 5** **β -25.4% (-40.9 to -5.8)*, per 2-fold increase in PFOA levels, PFOA at age 7**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	4.4 and 2.0 ng/mL (median PFOA at age 7 and 13 years)	Tetanus antibody levels at age 13	NS (p=0.637), PFOA at age 7 NS (p=0.856), PFOA at age 13
		Diphtheria antibody levels at age 13	Full cohort NS (p=0.742), PFOA at age 7 NS (p=0.129), PFOA at age 13 Cohort restricted to children without possible unscheduled booster vaccines NS (p=0.480), PFOA at age 7 **Association (p=0.029)*, PFOA at age 13**
Granum et al. 2013 General population (n=56 children age 3 years)	1.1 ng/mL (mean maternal PFOA)	Rubella antibody levels	**Inverse association (p=0.001)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	**Association (p<0.001)***
		Number of episodes of gastroenteritis	**Association (p=0.048)***
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	4.3 and 4.0 ng/mL (median PFOA in asthmatics and nonasthmatics)	Asthma episode in last 12 months	**OR 1.18 (1.01–1.39)*, per doubling PFOA**
		Current asthma	NS (p=0.26)
		Wheezing	NS (p=0.98)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Impinen et al. 2018 General population (n=641 infants followed through age 10)	**Exposure:** level 1.8 ng/mL (mean cord PFOA)	Number of common colds (0–2 years of age)	NS (p=0.089)
		Number of lower respiratory infections (0–10 years of age)	**β 0.28 (0.22–0.35; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS
Kielsen et al. 2016 General pop. (n=12 adults)	1.69 ng/mL (median PFOA)	Diphtheria antibody levels	NS (p=0.250), unadjusted
		Tetanus antibody levels	NS (p=0.970), unadjusted.
Looker et al. 2014 Community (C8) (n=411)	33.74 ng/mL (geometric mean) 13.8–31.5 ng/mL (2nd quartile)	Seroprotection from influenza A H3N2 virus	**OR 0.34 (0.14–0.83)*, 2nd quartile**
		Seroprotection from influenza A H1N1 virus	NS (p=0.02)
		Seroprotection from influenza type B virus	NS (p=0.68)
		Cold or flu infection	NS (p>0.05)
		Frequency of colds	NS (p>0.05)
Okada et al. 2012 General population (n=343 pregnant women)	1.3 ng/mL (maternal median PFOA)	Cord IgE levels Males Females	 NS (p>0.05) **Inverse association (p<0.05)***
		Infant food allergy	OR 1.67 (0.52–5.37)
		Eczema	OR 0.96 (0.23–4.02)
		Wheezing	OR 1.27 (0.27–6.05)
		Otitis media	OR 1.51 (0.45–5.12)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Okada et al. 2014 General population (n=2,603 infants)	2.67 ng/mL (maternal mean PFOA)	Risk of allergic diseases Males Females	 OR 0.63 (0.63–1.37), 4th quartile **OR 0.64 (0.42–0.97)*, 4th quartile**
		Eczema Males Females	 OR 0.75 (0.48–1.18), 4th quartile OR 0.65 (0.39–1.09), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	1.02 and 0.50 ng/mL (median serum PFOA in cases and controls)	Asthma	**OR 2.76 (1.82–4.17)***
Smit et al. 2015 General population (n=1,024 children)	0.97 and 1.79 ng/mL (maternal mean PFOA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.80 (0.62–1.04), whole cohort
		Ever having eczema	OR 0.97 (0.81–1.17), whole cohort
		Current eczema	OR 1.01 (0.79–1.29), whole cohort
		Ever having wheezing	OR 0.91 (0.76–1.10), whole cohort
		Current wheezing	OR 0.97 (0.71–1.33), whole cohort
Steenland et al. 2013 Community (C8) (28,441)	Estimated cumulative	Ulcerative colitis	**OR 1.76 (1.04–2.99)*, 2nd quartile**
		Rheumatoid arthritis	NS (p>0.05)
		Crohn's disease	NS (p>0.05)
		Type I diabetes	NS (p>0.05)
		Lupus	NS (p>0.05)
		Multiple sclerosis	NS (p>0.05)
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative	Asthma	NS (p=0.27), with no lag NS (p=0.53), with 10-year lag **Positive categorical trend (p=0.05)*, with no lag**
		Ulcerative colitis	**RR 6.57 (1.47–29.40)*, with 10-year lag**
		Rheumatoid arthritis	**Positive categorical trend (p=0.04)*, with no lag**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	4.13 ng/mL (geometric mean)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	NS (95% CI included unity), whole cohort **β -6.6% (-11.7 to -1.5)*, per 2-fold increase in PFOA levels, seropositive subcohort**
		Rubella antibody titers	NS (95% CI included unity), whole cohort **β -8.9% (-14.6 to -2.9)*, per 2-fold increase in PFOA levels, seropositive subcohort**
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	3.59 ng/mL (geometric mean)	Rhinitis	**OR 1.35 (1.10–1.66)***
		Current asthma	OR 1.28 (0.81–2.04)
		Wheeze	OR 0.94 (0.51–1.73)
		Allergy	OR 1.12 (0.85–1.47)
		Allergic sensitization	
		Plants	OR 0.88 (0.67–1.15)
		Dust mites	OR 0.93 (0.75–1.16)
		Pets	OR 1.17 0.81–1.68)
		Cockroach or shrimp	OR 0.79 (0.55–1.13)
		Rodents	OR 1.65 (0.59–4.60
		Mold	OR 1.21 (0.85–1.72)
		Food	OR 1.02 (0.60–1.73)
Stein et al. 2016b General population (n=78 adults receiving influenza vaccine)	2.28 ng/mL (geometric mean)	Seroconversion	
		Hemagglutinin	NS (p=0.07 for trend)
		Immunohistochem.	NS (p=0.27 for trend)
		Serum cytokine levels	NS (p>0.05 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2011 General population (n=244 children aged 2 years)	1.71 ng/mL (median cord PFOA)	Serum IgE levels	NS (p=0.870)
		Cord blood IgE levels	Association (p=0.047)*
		Atopic dermatitis	NS (p>0.05)
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	1.51 and 1.00 ng/mL (mean in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 4.24 (1.91–9.42)*, males 4th quartile** **OR 3.68 (1.43–9.48)*, females 4th quartile**
		T-helper cytokines	
		IL-4	**Association (p=0.001 for trend)***
		IL-5	**Association (p=0.004 for trend)***
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)
PFOS			
Ashley-Martin et al. 2015 General population (1,258 women)	NR	IL-33/TSLP (cord blood)	1.1 (0.6–1.9)
		IgE (cord blood)	OR 1.1 (0.6–1.9)
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	14.98 and 8.74 ng/mL (geometric mean PFOS 2005–2006 and 2007–2010) 9.17–13.75 ng/mL (3rd quartile)	Food allergies	**OR 2.43 (1.05–5.59)*, 3rd quartile (trend not significant, p=0.27)**
		Food sensitization	NS (p=0.49 for trend)
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	10.19–25.10 ng/mL (maternal 3rd tertile PFOS)	Risk of number of days above the median	
		Fever	**OR 2.35 (1.34–4.11)*, 3rd tertile**
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Risk of number of days	
		Fever	**OR 1.65 (1.24–2.18)*, 3rd tertile**
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	45.5 and 33.4 ng/mL (mean serum PFOS levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.63 (1.48–4.69)*, 4th quartile**
		Asthma severity	**Association (p=0.045 for trend)***
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
Fei et al. 2010 General population (n=1,400 pregnant women and young children)	35.3 ng/mL (maternal PFOS)	Risk of hospitalization for infectious disease in young children	IRR 1.00 (0.91–1.09) for trend **IRR 1.18 (1.03–1.36)* for trend, girls** IRR 0.90 (0.80–1.12) for trend, boys
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	5.456 ng/mL (mean maternal plasma PFOS)	Prevalence of allergic disease	OR 0.815 (0.596–1.11), 4th quartile
		Prevalence of wheezing	OR 0.770 (0.526–1.12), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	5.456 ng/mL (mean maternal serum PFOS)	Risk of total infectious diseases	**OR 1.44 (1.06–1.96)*, 2nd quartile; p=0.008 for trend**
Grandjean et al. 2012; Mogensen et al. 2015a General population (n=456 and n=464 children 5 and 7 years of age)	17.3 and 15.5 ng/mL (median PFOS at age 5 and 7 years) 27.3 ng/mL (geometric mean maternal PFOS)	Tetanus antibody levels at age 5	NS, maternal PFOS **β -28.5% (-45.5 to -6.1)*, per 2-fold increase in PFOS levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFOS NS, PFOS at ages 5 and 7

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Diphtheria antibody levels at age 5	NS, maternal PFOS NS, PFOS at age 5
		Diphtheria antibody levels at age 7	**β -27.6% (-45.8 to -3.3)*, per 2-fold increase in PFOS levels at age 5** **β -30.3% (47.3 to -7.8)*, per 2-fold increase in PFOS levels at age 7**
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	15.3 and 6.7 ng/mL (median at age 7 and 13 years)	Tetanus antibody levels at age 13	Full cohort NS (p=0.240), PFOS at age 7 NS (p=0.237), PFOS at age 13 Cohort restricted to children without possible unscheduled booster vaccines **Association (p=0.043)*, PFOS at age 7** NS (p=0.144), PFOS at age 13
		Diphtheria antibody levels at age 13	NS (p=0.07), age 7 NS (p=0.454), age 13
Granum et al. 2013 General population (n=56 children age 3 years)	5.6 ng/mL (mean maternal PFOS)	Rubella antibody levels	**Inverse association (p=0.007)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	NS (p=0.501)
		Number of episodes of gastroenteritis	NS (p=0.367)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	17.0 and 16.8 ng/mL (median PFOS in asthmatics and non-asthmatics)	Asthma episode in last 12 months	NS (p=0.13), per doubling PFOS
		Current asthma	NS (p=0.24)
		Wheezing	NS (p=0.08)
Impinen et al. 2018 General population (n=641 infants followed through age 10)	5.6 ng/mL (mean cord PFOS)	Number of common colds (0–2 years of age)	NS (p=0.173)
		Number of lower respiratory infections (0–10 years of age)	**β 0.50 (0.42–0.57; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS
Kielsen et al. 2016 General population (n=12 adults)	9.52 ng/mL (median PFOS)	Diphtheria antibody levels	**Inverse association (p=0.044)*, unadjusted**
		Tetanus antibody levels	NS (p=0.420), unadjusted
Looker et al. 2014 Community (C8) (n=411)	8.32 ng/mL (geometric mean PFOS)	Response to influenza A H3N2 virus vaccine	NS (p>0.05)
		Response to influenza A H1N1 virus vaccine	NS (p>0.05)
		Response to influenza type B virus vaccine	NS (p>0.05)
		Cold or flu infection	NS (p>0.05)
		Frequency of colds	NS (p>0.05)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Okada et al. 2012 General population (n=343 infants)	5.2 ng/mL (maternal median PFOS)	Cord IgE levels	NS (p>0.05)
		Infant food allergy	OR 3.72 (0.81–17.10)
		Eczema	OR 0.87 (0.15–5.08)
		Wheezing	OR 2.68 (0.39–18.30)
		Otitis media	OR 1.40 (0.33–6.00)
Okada et al. 2014 General population (n=2,603 infants)	5.56 ng/mL (maternal mean PFOS)	Risk of allergic diseases Males Females	 OR 0.95 (0.65–1.37), 4th quartile OR 0.79 (0.53–1.17), 4th quartile
		Eczema Males Females	 OR 0.98 (0.63–1.53), 4th quartile OR 0.84 (0.51–1.38), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	31.51 and 28.83 ng/mL (median serum PFOS in cases and controls)	Asthma	**OR 1.30 (1.00–1.69)***
Smit et al. 2015 General population (n=1,024 children)	4.88 and 20.6 ng/mL (maternal mean PFOS in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.86 (0.67–1.10), whole cohort
		Ever having eczema	OR 0.98 (0.88–1.18), whole cohort
		Current eczema	OR 1.05 (0.82–1.33), whole cohort
		Ever having wheezing	OR 0.83 (0.69–1.00), whole cohort
		Current wheezing	**OR 0.60 (0.38–0.92)*, Ukraine cohort** OR 0.91 (0.62–1.36) Greenland OR 0.76 (0.56–1.01), whole cohort
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	20.8 ng/mL (geometric mean PFOS)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	**β -7.4% (-12.8 to -1.7)*, per 2-fold increase in PFOS levels, whole cohort** **β -5.9% (-9.9 to -1.6)*, per 2-fold increase in PFOS levels, seropositive subcohort**
		Rubella antibody titers	NS (95% CI included unity), whole cohort **β -13.3% (-19.9–6.2)*, per 2-fold increase in PFOS levels, seropositive subcohort**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	15.0 ng/mL (geometric mean)	Rhinitis	OR 1.16 (0.90–1.50)
		Current asthma	OR 1.20 (0.88–1.63)
		Wheeze	OR 0.76 (0.45–1.19)
		Allergy	OR 1.05 (0.80–1.37)
		Allergic sensitization	
		Plants	**OR 0.17 (0.53–0.97)***
		Dust mites	OR 1.00 (0.73–1.38)
		Pets	OR 0.83 (0.56–1.22)
		Cockroach or shrimp	**OR 0.67 (0.48–0.93)***
		Rodents	OR 0.85 (0.29–2.45)
		Mold	**OR 1.33 (1.06–1.69)***
		Food	OR 0.74 (0.39–1.40)
Stein et al. 2016b General population (n=78 adults receiving influenza vaccine)	5.22 ng/mL (geometric mean)	Seroconversion	
		Hemagglutinin	NS (p=0.81 for trend)
		Immunohistochemistry	NS (p=0.12 for trend)
		Serum cytokine levels	NS (p>0.05 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)
Wang et al. 2011 General population (n=244 children aged 2 years)	5.50 ng/mL (median cord PFOS)	Serum IgE levels	NS (p=0.179)
		Cord blood IgE levels	Association (p=0.017)*
		Atopic dermatitis	NS (p>0.05)
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	45.86 and 33.9 ng/mL (mean in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 4.38 (2.02–9.50)*, males 4th quartile** NS (p=0.899 for trend), females
		T-helper cytokines	
		IL-4	NS (p>0.05 for trend)
		IL-5	NS (p>0.05 for trend)
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Ashley-Martin et al. 2015 General population (1,258 women)	NR	IL-33/TSLP (cord blood)	1.0 (0.7–1.4)
		IgE (cord blood)	OR 1.0 (0.7–1.4)
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	2.09 and 2.19 ng/mL (geometric mean 2005–2006 and 2007–2010) >4.00 ng/mL (4th quartile)	Food allergies	**OR 3.06 (1.35–6.93)*, 4th quartile** (trend not significant, p=0.11)
		Food sensitization	NS (p=0.72 for trend)
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	0.32 ng/mL (maternal median PFHxS)	Risk of number of days above the median	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
		Risk of number of days	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	3.9 and 2.1 ng/mL (mean serum PFHxS levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.94 (1.65–5.25)*, 3rd quartile**
		Asthma severity	NS (p=0.722 for trend)
		IgE	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	0.322 ng/mL (mean maternal plasma PFHxS)	Prevalence of allergic disease	OR 0.841 (0.615–1.151), 4th quartile
		Prevalence of wheezing	OR 0.728 (0.497–1.06), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	0.322 ng/mL (mean maternal serum PFHxS)	Risk of total infectious diseases	OR 0.957 (0.7.3–1.41), 4th quartile, p=0.928 for trend Females only: **OR 1.81 (1.14–2.88)*, 3rd quartile; p=0.045 for trend** Males only: p=0223 for trend
Grandjean et al. 2012; Mogensen et al. 2015a General population (n=456 and n=464 children 5 and 7 years of age)	0.6 and 0.5 ng/mL (median PFHxS at age 5 and 7 years) 4.41 ng/mL (geometric mean maternal PFHxS)	Tetanus antibody levels at age 5	NS, maternal PFHxS **-19.0% (-29.8 to -6.6)*, per 2-fold increase in PFHxS levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFHxS **β -19.7% (-31.6 to -5.7)*, per 2-fold increase PFHxS levels at age 5** **β -22.3% (-36.3 to -5.2)*, per 2-fold increase PFHxS levels at age 7**
		Diphtheria antibody levels at age 5	NS, maternal PFHxS NS, PFHxS at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFHxS NS, PFHxS at age 5 or 7
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	0.5 and 0.4 ng/mL (median PFHxS at age 7 and 13 years)	Tetanus antibody levels at age 13	NS (p=0.334), PFHxS at age 7 NS (p=0.568), PFHxS at age 13
		Diphtheria antibody levels at age 13	NS (p=0.264), PFHxS at age 7 NS (p=0.583), PFHxS at age 13

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Granum et al. 2013 General population (n=56 children age 3 years)	0.3 ng/mL (mean maternal PFHxS levels)	Rubella antibody levels	**Inverse association (p=0.008)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	NS (p=0.078)
		Number of episodes of gastroenteritis	**Association (p=0.007)***
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	2.2 and 2.0 ng/mL (median PFHxS in asthmatics and nonasthmatics)	Asthma episode in last 12 months	NS (p=0.66), per doubling PFHxS
		Current asthma	NS (p=0.99)
		Wheezing	NS (p=0.92)
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.3 ng/mL (mean cord PFHxS)	Number of common colds (0–2 years of age)	NS (p=0.530)
		Number of lower respiratory infections (0–10 years of age)	NS (p=0.119)
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kielsen et al. 2016 General population (n=12 adults)	0.37 ng/mL (median PFHxS)	Diphtheria antibody levels	NS (p=0.055), unadjusted
		Tetanus antibody levels	NS (p=0.390), unadjusted
Okada et al. 2014 General population (n=2,603 infants)	0.324 ng/mL (maternal mean PFHxS)	Risk of allergic diseases Males Females	 OR 0.81 (0.56–1.16), 4th quartile OR 1.13 (0.75–1.69), 4th quartile
		Eczema Males Females	 OR 0.78 (0.51–1.19), 4th quartile OR 0.82 (0.49–1.36), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	2.38 and 1.07 ng/mL (median serum PFHxS in cases and controls)	Asthma	**OR 2.14 (1.48–3.11)***
Smit et al. 2015 General population (n=1,024 children)	1.53 and 2.14 ng/mL (maternal mean PFHxS in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.91 (0.69–1.18), whole cohort
		Ever having eczema	OR 1.03 (0.86–1.24), whole cohort
		Current eczema	OR 0.93 (0.73–1.20), whole cohort
		Ever having wheezing	OR 0.96 (0.79–1.17), whole cohort
		Current wheezing	OR 0.93 (0.68–1.27), whole cohort
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	2.47 ng/mL (geometric mean PFHxS)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	NS (95% CI included unity)
		Rubella antibody titers	NS (95% CI included unity), whole cohort **β -6.0% (-9.6 to -2.2)*, per 2-fold increase PFHxS levels, seropositive subcohort**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	2.09 ng/mL (geometric mean PFHxS)	Rhinitis	OR 0.81 (0.57–1.16)
		Current asthma	OR 0.98 (0.51–1.87)
		Wheeze	OR 0.99 (0.68–1.44)
		Allergy	OR 0.83 (0.59–1.17)
		Allergic sensitization	
		Plants	OR 0.93 (0.62–1.39)
		Dust mites	OR 1.01 (0.84–1.22)
		Pets	OR 0.96 (0.71–1.30)
		Cockroach or shrimp	OR 0.72 (0.56–0.93)
		Rodents	OR 0.81 (0.54–1.21)
		Mold	OR 0.98 (0.65–1.47)
		Food	OR 1.03 (0.74–1.42)
Stein et al. 2016b General population (n=78 adults receiving influenza vaccine)	1.1 ng/mL (geometric mean PFHxS)	Seroconversion	
		Hemagglutinin	NS (p=0.22 for trend)
		Immunohistochem.	NS (p=0.34 for trend)
		Serum cytokine levels	
		IFN-γ	**Association (p=0.05 for trend)***
		IFN-α2	NS (p=0.09 for trend)
		TNF-α	**Association (p=0.04 for trend)***
		IP-10	NS (p=0.59 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	3.86 and 2.10 ng/mL (mean PFHxS in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 2.97 (1.33–6.64)*, males 4th quartile** **OR 5.02 (2.05–12.30)*, females 4th quartile**
		T-helper cytokines	
		IL-4	NS (p>0.05 for trend)
		IL-5	NS (p>0.05 for trend)
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)
PFNA			
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	0.93 and 1.13 ng/mL (geometric mean PFNA 2005–2006 and 2007–2010) >1.36 ng/mL (4th quartile)	Food allergies	NS (p=0.28 for trend)
		Food sensitization	**OR 0.51 (0.28–0.92)*, 4th quartile (trend not significant, p=0.15)**
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	0.56–0.81 and 0.82–3.64 ng/mL (maternal 2nd and 3rd tertile PFNA)	Risk of number of days above the median	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	**OR 0.53 (0.31–0.92)*, 2nd tertile**
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
		Risk of number of days	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	OR 1.12 (0.84–1.49), 3rd tertile
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	1.1 and 0.9 ng/mL (mean serum PFNA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.56 (1.41–4.65)*, 4th quartile**
		Asthma severity	NS (p=0.217 for trend)
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	1.402 ng/mL (mean maternal plasma PFNA)	Prevalence of allergic disease	OR 0.873 (0.562–1.35), 4th quartile
		Prevalence of wheezing	OR 1.11 (0.760–1.63), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	1.402 ng/mL (mean maternal serum PFNA)	Risk of total infectious diseases	OR 0.918 (0.672–1.25), 4th quartile, p=0.748 for trend
Grandjean et al. 2012 General population (n=456 and n=464 children 5 and 7 years of age)	1.00 ng/mL (geometric mean PFNA at age 5 years) 0.60 ng/mL (geometric mean PFNA at age 7 years)	Tetanus antibody levels at age 5	NS, maternal PFNA NS, PFNA at age 5
		Tetanus antibody levels at age 7	NS, maternal PFNA NS, PFNA at age 5
		Diphtheria antibody levels at age 5	NS, maternal PFNA **β -16.1% (-28.8 to -1.0)*, per 2-fold increase PFNA levels at age 5**
		Diphtheria antibody levels at age 7	NS, maternal PFNA NS, PFNA at age 5
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	1.1 and 0.7 ng/mL (median PFNA at age 7 and 13 years)	Tetanus antibody levels at age 13	NS (p=0.075), PFNA at age 7 NS (p=0.394), PFNA at age 13
		Diphtheria antibody levels at age 13	NS (p=0.243), PFNA at age 7 NS (p=0.693), PFNA at age 13

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Granum et al. 2013 General population (n=56 children age 3 years)	0.3 ng/mL (mean maternal PFNA levels)	Rubella antibody levels	**Inverse association (p=0.007)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	**Association (p=0.035)***
		Number of episodes of gastroenteritis	NS (p=0.883)
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	0.9 and 0.8 ng/mL (median PFNA in asthmatics and nonasthmatics)	Asthma episode in last 12 months	NS (p=0.92), per doubling PFNA
		Current asthma	NS (p=0.97)
		Wheezing	NS (p=0.94)
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.2 ng/mL (mean cord PFNA)	Number of common colds (0–2 years of age)	NS (p=0.983)
		Number of lower respiratory infections (0–10 years of age)	**β 0.0.09 (0.03–0.14;p=0.001)***
		Rhinitis	NS
		Rhinoconjuctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kielsen et al. 2016 General population (n=12 adults)	0.66 ng/mL (median PFNA)	Diphtheria antibody levels	**Inverse association (p=0.004)*, unadjusted**
		Tetanus antibody levels	NS (p=0.250), unadjusted
Okada et al. 2014 General population (n=2,603 infants)	1.36 ng/mL (maternal mean PFNA)	Risk of allergic diseases	
		Males	OR 0.95 (0.66–1.38), 4th quartile
		Females	**OR 0.55 (0.36–0.82)*, 4th quartile**
		Eczema	
		Males	OR 0.96 (0.61–1.52), 4th quartile
		Females	OR 0.63 (0.38–1.02), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	2.00 and 0.80 ng/mL (median serum PFNA in cases and controls)	Asthma	**OR 1.61 (1.12–2.31)***
Smit et al. 2015 General population (n=1,024 children)	0.62 and 0.73 ng/mL (maternal mean PFNA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.90 (0.70–1.14), whole cohort
		Ever having eczema	OR 0.94 (0.78–1.14), whole cohort
		Current eczema	OR 1.03 (0.82–1.30), whole cohort
		Ever having wheezing	OR 0.91 (0.75–1.09), whole cohort
		Current wheezing	OR 0.90 (0.66–1.23), whole cohort
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	0.765 ng/mL (geometric mean)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	NS (95% CI included unity)
		Rubella antibody titers	NS (95% CI included unity)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	0.929 ng/mL (geometric mean)	Rhinitis	OR 1.24 (0.97–1.60)
		Current asthma	OR 1.26 (0.79–2.01)
		Wheeze	OR 0.99 (0.58–1.68)
		Allergy	OR 1.12 (0.85–1.47)
		Allergic sensitization	
		Plants	OR 0.96 (0.74–1.23)
		Dust mites	OR 1.05 (0.78–1.41)
		Pets	OR 1.26 (0.85–1.87)
		Cockroach or shrimp	OR 0.86 (0.60–1.24)
		Rodents	OR 2.25 (0.83–6.10)
		Mold	OR 1.31 (0.83–2.06)
		Food	OR 0.91 (0.55–1.50)
Stein et al. 2016b General pop. (n=78 adults receiving influenza vaccine)	0.77 ng/mL (geometric mean PFNA)	Seroconversion	
		Hemagglutinin	NS (p=0.33 for trend)
		Immunohistochem.	NS (p=0.40 for trend)
		Serum cytokine levels	NS (p>0.05 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)
Wang et al. 2011 General population (n=244 children aged 2 years)	2.30 ng/mL (median cord PFNA)	Serum IgE levels	NS (p=0.837)
		Cord blood IgE levels	NS (p=0.908)
		Atopic dermatitis	NS (p>0.05)
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	1.07 and 0.87 ng/mL (mean PFNA in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 3.33 (1.46–7.58)*, males 4th quartile** NS (p=0.142 for trend), females
		T-helper cytokines	
		IL-4	**Association (p=0.031 for trend)***
		IL-5	**Association (p=0.011 for trend)***
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	**Association (p=0.008 for trend)***

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Dalsager et al. 2016 General population (n=359 children aged 1–4 years)	0.27 ng/mL (median maternal serum PFDA level)	Symptoms of infection	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	1.2 and 1.0 ng/mL (mean serum PFDA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 3.22 (1.75–5.94)*, 4th quartile**
		Asthma severity	**Association (p=0.005 for trend)***
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** **Association (p<0.05)*, non-asthmatics**
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	0.575 ng/mL (mean maternal plasma PFDA)	Prevalence of allergic disease	OR 0.906 (0.663–1.23), 4th quartile
		Prevalence of wheezing	OR 0.879 (0.602–1.28), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	0.575 ng/mL (mean maternal serum PFDA)	Risk of total infectious diseases	OR 0.799 (0.588–1.08), 4th quartile, p=0.114 for trend
Grandjean et al. 2012 General population (n=456 and n=464 children 5 and 7 years of age)	0.28 ng/mL (geometric mean PFDA at age 5 years) 0.28 ng/mL (geometric mean maternal PFDA)	Tetanus antibody levels at age 5	NS, maternal PFDA **β -19.9% (-33.1 to -3.9), per 2-fold increase PFDA levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFDA **β -22.3 (-35.8 to -5.8), per 2-fold increase PFDA levels at age 5**
		Diphtheria antibody levels at age 5	NS, maternal PFDA NS, PFDA at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFDA NS, PFDA at age 5

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	0.4 and 0.3 ng/mL (median at age 7 and 13 years)	Tetanus antibody levels at age 13	**Association (p=0.022)*, PFDA at age 7** NS (p=0.258), PFDA at age 13
		Diphtheria antibody levels at age 13	**Association (p=0.008)*, PFDA at age 7** NS (p=0.726), PFDA at age 13
Kielsen et al. 2016 General population (n=12 adults)	0.30 ng/mL (median PFDA)	Diphtheria antibody levels	**Inverse association (p=0.009)*, unadjusted**
		Tetanus antibody levels	NS (p=0.130), unadjusted
Okada et al. 2014 General population (n=2,603 infants)	0.563 ng/mL (maternal mean PFDA)	Risk of allergic diseases Males Females	 OR 1.13 (0.78–1.64), 4th quartile OR 0.70 (0.47–1.04), 4th quartile
		Eczema Males Females	 OR 0.93 (0.60–1.44), 4th quartile OR 0.78 (0.49–1.25), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	1.13 and 0.93 ng/mL (median serum PFDA in cases and controls)	Asthma	OR 1.24 (0.97–1.58)
Smit et al. 2015 General population (n=1,024 children)	0.16 and 0.42 ng/mL (maternal mean PFDA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.92 (0.73–1.16), whole cohort
		Ever having eczema	OR 0.88 (0.73–1.06), whole cohort
		Current eczema	OR 0.95 (0.75–1.20), whole cohort
		Ever having wheezing	OR 0.85 (0.70–1.01), whole cohort
		Current wheezing	OR 0.76 (0.56–1.04), whole cohort
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	1.24 and 1.02 ng/mL (mean in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 3.45 (1.51–7.88)*, males 4th quartile** **OR 3.68 (1.43–9.48)*, females 4th quartile**
		T-helper cytokines IL-4 IL-5 IFN-γ IL-2	 NS (p>0.05 for trend) NS (p>0.05 for trend) NS (p>0.05 for trend) NS (p>0.05 for trend)
		Serum IgE	**Association (p=0.002 for trend)***

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	1.534 ng/mL (mean maternal plasma PFUnA)	Prevalence of allergic disease	OR 0.736 (0.538–1.00), 4th quartile
		Prevalence of wheezing	OR 1.04 (0.714–1.51), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	1.534 ng/mL (mean maternal serum PFUnA)	Risk of total infectious diseases	OR 1.03 (0.764–1.40), 4th quartile, p=0.786 for trend
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.1 ng/mL (mean cord PFUnNA)	Number of common colds (0–2 years of age)	**β 0.11 (0.08–0.14; p=<0.0001)***
		Number of lower respiratory infections (0–10 years of age)	**β 0.18 (0.13–0.23; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS
Kielsen et al. 2016 General population (n=12 adults)	0.21 ng/mL (median PFUnA)	Diphtheria antibody levels	**Inverse association (p=0.036)*, unadjusted**
		Tetanus antibody levels	**Inverse association (p=0.039)*, unadjusted**
Okada et al. 2014 General population (n=2,603 infants)	1.50 ng/mL (maternal mean PFUnA)	Risk of allergic diseases Males Females	 OR 1.13 (0.79–1.63), 4th quartile **OR 0.58 (0.39–0.86)*, 4th quartile**
		Eczema Males Females	 OR 1.16 (0.75–10.81), 4th quartile **OR 0.50 (0.30–0.81)*, 4th quartile**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Smit et al. 2015 General population (n=1,024 children)	0.16 and 0.68 ng/mL (maternal mean PFUnA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.96 (0.77–1.21), whole cohort
		Ever having eczema	OR 0.95 (0.79–1.15), whole cohort
		Current eczema	OR 1.07 (0.85–1.34), whole cohort
		Ever having wheezing	OR 0.84 (0.70–1.00), whole cohort
		Current wheezing	OR 0.87 (0.65–1.17), whole cohort
PFHpA			
Kielsen et al. 2016 General population (n=12 adults)	0.12 ng/mL (median PFHpA)	Diphtheria antibody levels	NS (p=0.750), unadjusted
		Tetanus antibody levels	NS (p=0.280), unadjusted
Smit et al. 2015 General population (n=1,024 children)	0.03 and 0.05 ng/mL (maternal mean PFHpA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.93 (0.71–1.22), whole cohort
		Ever having eczema	OR 0.93 (0.78–1.11), whole cohort
		Current eczema	OR 0.90 (0.70–1.15), whole cohort
		Ever having wheezing	OR 1.03 (0.84–1.25), whole cohort
		Current wheezing	**OR 0.62 (0.40–0.97)*, Ukraine cohort** OR 1.24 (0.79–1.93), Greenland cohort OR 0.88 (0.64–1.20), whole cohort
PFBS			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	0.5 and 0.5 ng/mL (mean serum PFBS levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 1.90 (1.08–3.37)*, 4th quartile**
		Asthma severity	NS (p=0.092 for trend)
		IgE	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	0.48 and 0.48 ng/mL (median serum PFBS in cases and controls)	Asthma	OR 1.06 (0.93–1.20)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	0.53 and 0.48 ng/mL (mean serum PFBS in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 2.59 (1.14–5.87)*, males 4th quartile** NS (p=0.505 for trend), females
		T-helper cytokines IL-4 IL-5 IFN-γ IL-2	 NS (p>0.05 for trend) **Association (p=0.023 for trend)*** NS (p>0.05 for trend) NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)
PFDoDA			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children)	5.8 and 4.5 ng/mL (mean serum PFDoDA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 1.81 (1.02–3.23)*, 4th quartile**
		Asthma severity	**Association (p=0.024 for trend)***
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** Association (p<0.05)*, non-asthmatics
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	0.191 ng/mL (mean maternal plasma PFDoDA)	Prevalence of allergic disease	**OR 0.621 (0.454–0.847)*, 4th quartile**
		Prevalence of wheezing	OR 0.999 (0.684–1.45), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	0.191 ng/mL (mean maternal serum PFDoDA)	Risk of total infectious diseases	OR 1.07 (0.790–1.46), 4th quartile, p=0.502 for trend
Kielsen et al. 2016 General population (n=12 adults)	0.039 ng/mL (median PFDoDA)	Diphtheria antibody levels	**Inverse association (p=0.038)*, unadjusted**
		Tetanus antibody levels	**Inverse association (p=0.038)*, unadjusted**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Okada et al. 2014 General population (n=2,603 infants)	0.188 ng/mL (maternal mean PFDoDA)	Risk of allergic diseases Males Females	 OR 0.93 (0.65–1.34), 4th quartile OR 0.58 (0.39–0.85)*, 4th quartile
		Eczema Males Females	 OR 1.00 (0.64–1.55), 4th quartile OR 0.73 (0.45–1.18), 4th quartile
Smit et al. 2015 General population (n=1,024 children)	0.04 and 0.13 ng/mL (maternal mean PFDoDA in Ukraine and Greenland cohorts)	Ever having asthma	OR 1.03 (0.81–1.30), whole cohort
		Ever having eczema	OR 0.90 (0.75–1.08), whole cohort
		Current eczema	OR 0.88 (0.70–1.14), whole cohort
		Ever having wheezing	OR 0.97 (0.80–1.16), whole cohort
		Current wheezing	OR 0.87 (0.64–1.18), whole cohort
PFHxA			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children)	0.3 and 0.2 ng/mL (mean serum PFHxA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	OR 1.60 (0.90–2.86), 4th quartile
		Asthma severity	NS (p=0.854)
		IgE	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	0.20 and 0.18 ng/mL (median serum PFHxA in cases and controls)	Asthma	OR 0.99 (0.80–1.21)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.4 ng/mL (mean cord FOSA)	Number of common colds (0–2 years of age)	NS (p=0.477)
		Number of lower respiratory infections (0–10 years of age)	**β 0.10 (0.06–0.14; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 10 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk and bold indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; IFN-α-2 = interferon-α2; IFN-γ = interferon-γ; IgA = immunoglobulin A; IgE = immunoglobulin E; IP-10 = interferon-γ-inducible protein 10; IRR= incidence risk ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR= relative risk; SPR = standard prevalence ratio; TNF-α =tumor necrosis factor-α

autoimmunity; epidemiological studies provide suggestive evidence of an association between serum PFOA and the risk of ulcerative colitis. The small number of studies investigating immunotoxicity following exposure to PFHpA and PFHxA did not find associations.

Laboratory animal studies have also evaluated immunosuppression (disease resistance, antibody response, NK cell activity, delayed-type hypersensitivity response, monocyte phagocytosis), hypersensitivity (airway resistance, local lymph node assay), and autoimmunity. In addition, laboratory animal studies have examined secondary outcomes (lymphoid organ weights, lymphocyte counts or subpopulations, lymphocyte proliferation, cytokine levels, serum antibody levels, histological alterations in immune organs). Summaries of the laboratory animal studies are presented in the LSE tables for PFOA, PFOS, and other perfluoroalkyls (Tables 2-3, 2-4, 2-5, and 2-6); the NOAEL and LOAEL values are presented in Figures 2-8, 2-9, and 2-10. No laboratory animal studies were identified for PFUnA, PFHpA, PFDoDA, or FOSA. Studies in laboratory animals identify the immune system as a sensitive target of toxicity following exposure to PFOA and PFOS. The observed effects include impaired responses to T-dependent antigens, impaired response to infectious disease, and secondary outcomes (decreases in spleen and thymus weights and in the number of thymic and splenic lymphocytes). A small number of studies evaluated the immunotoxicity of other perfluoroalkyls and most did not evaluate immune function. No alterations in spleen or thymus organ weights or morphology were observed in studies on PFHxS, PFBA, and PFDA. A study on PFNA found decreases in spleen and thymus weights and alterations in splenic lymphocyte phenotypes.

The National Toxicology Program (NTP 2016b) concluded that exposure to PFOA or PFOS is presumed to be an immune hazard to humans based on a high level of evidence that PFOA and PFOS suppressed the antibody response from animals and a moderate level of evidence from studies in humans. It was noted that the strongest evidence is for suppression of the antibody response and increased hypersensitivity (PFOA only).

PFOA

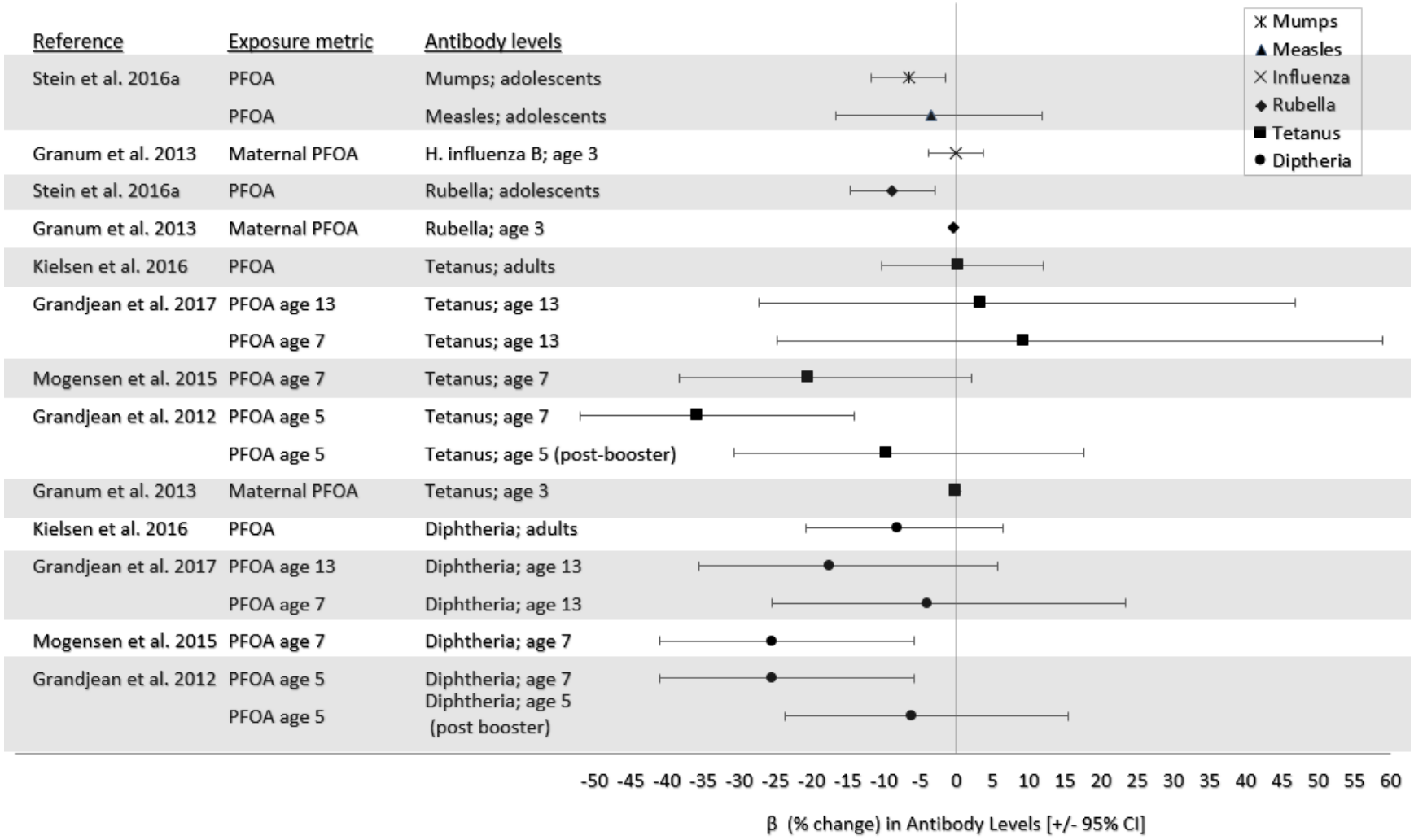
Epidemiological Studies—Immunosuppression Outcomes. Studies evaluating the immunosuppressive effects of PFOA have examined disease resistance and antibody responses. One study found associations between maternal serum PFOA and the number of episodes of the common cold and other respiratory tract infections and the number of episodes of gastroenteritis with vomiting or diarrhea in 3-year-old children (Granum et al. 2013). Another study found an association between maternal PFOA and the risk

of having a greater number of days with a fever greater than the median (Dalsager et al. 2016), although there was no increase in the number of days with a fever. A third study found an increased risk of lower respiratory tract infections associated cord PFOA from birth to 10 years of age (Impinen et al. 2018). However, other studies have not found associations between PFOA levels and the frequency of the common cold or flu in adults (Looker et al. 2014), between maternal PFOA levels and otitis media in 1.5–3-year-old children (Granum et al. 2013; Okada et al. 2012), between maternal PFOA and the risk of hospitalization for infectious diseases in young children (Fei et al. 2010), between maternal PFOA and the risk of number of days with cough, nasal discharge, diarrhea, or vomiting (Dalsager et al. 2016), between cord PFOA and number of common colds (Impinen et al. 2018), or between maternal serum PFOA and total number of infectious diseases between birth and 2 years of age (Goudarzi et al. 2017).

Several studies have evaluated the antibody response to vaccination in adults and children; the changes in the response to antibody levels relative to serum PFOA levels are graphically presented in Figure 2-21; the figure does not include data from other studies that used different statistical methods. In adults, decreases in antibody response against influenza A H3N2 virus were associated with increasing serum PFOA levels; however, there were no associations with two other strains of influenza virus (influenza A H1N1 and influenza B) (Looker et al. 2014). Another study of adults also did not find an altered immune response to influenza A H1N1 virus (Stein et al. 2016b). A small-scale study of 12 adults did not find significant alterations in the response to diphtheria or tetanus booster vaccines associated with serum PFOA levels (Kielsen et al. 2016). Increasing current serum PFOA levels were associated with lower antibody levels for mumps and rubella, but not for measles, in a cross-sectional study of adolescents (Stein et al. 2016a). A series of prospective studies by Grandjean and associates (Grandjean et al. 2012, 2017; Mogensen et al. 2015a) evaluated tetanus and diphtheria antibody levels in children at 5, 7, and 13 years of age. Diphtheria antibody levels at age 7 and 13 were inversely associated with serum PFOA levels at age 5 and 7 (Grandjean et al. 2012; Mogensen et al. 2015a) and with serum PFOA at age 13 (Grandjean et al. 2017), respectively. Decreases in tetanus antibody levels at age 7 were associated with increases in serum PFOA levels at age 5, but not at age 7 (Grandjean et al. 2012; Mogensen et al. 2015a) and tetanus antibody levels were not associated with serum PFOA at age 7 or 13 (Grandjean et al. 2017). In studies comparing maternal serum PFOA with antibody levels in children, no associations were found for tetanus antibodies at age 3 (Granum et al. 2013), age 5 (Grandjean et al. 2012), or age 7 (Grandjean et al. 2012) or for diphtheria at age 5 or 7 (Grandjean et al. 2012). It is noted that Grandjean and associates also found an inverse association between serum polychlorinated biphenyls (PCBs) and serum antibody concentrations against tetanus and diphtheria in children living in the Faroe

Figure 2-21. Antibody Responses Relative to Serum PFOA Levels in Epidemiological Studies

(Presented as percent difference in antibody concentration per 2-fold increase in serum PFOA)



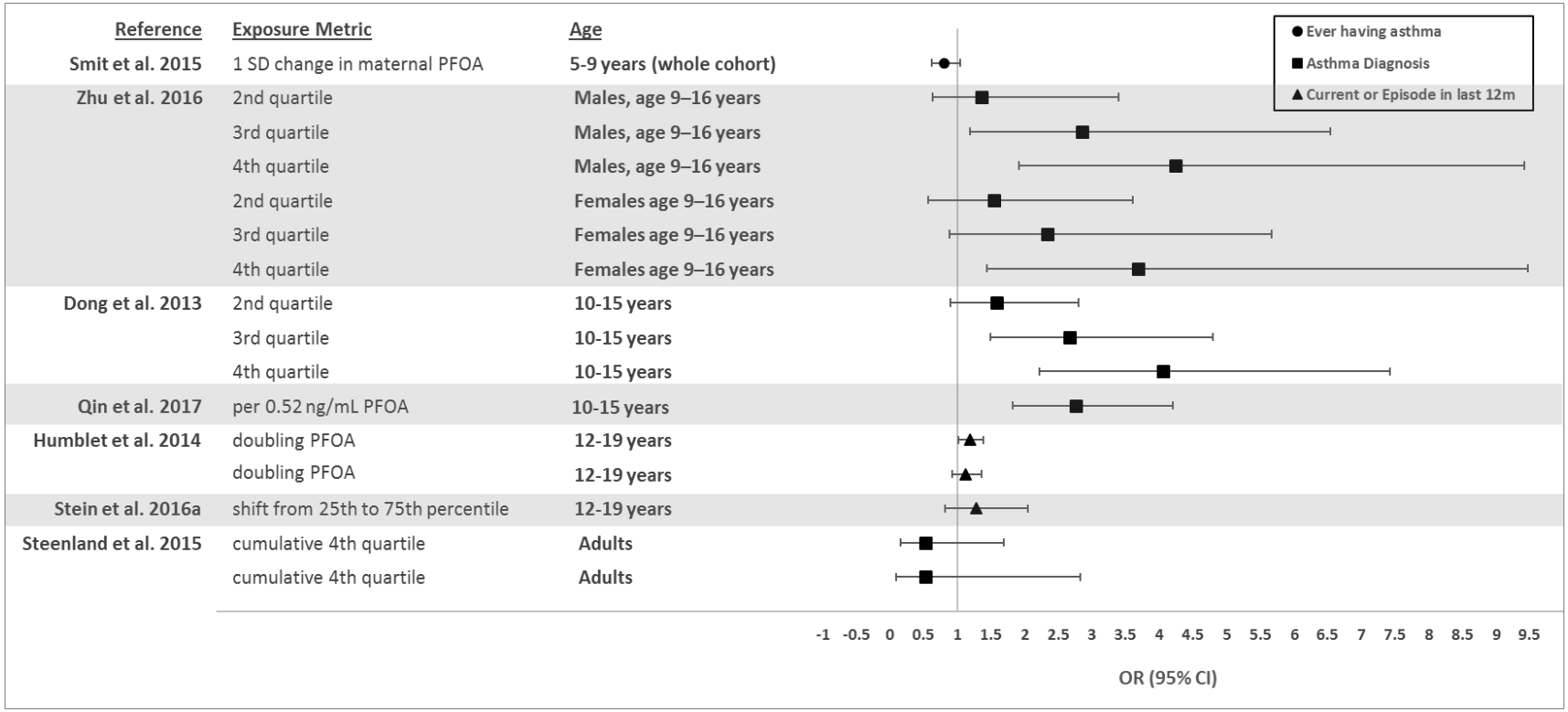
Islands (Heilmann et al. 2010). Statistically adjusting for PCB exposure (milk and serum PCB levels) did not alter the results (Grandjean et al. 2012). Lower levels of rubella antibodies at age 3 were associated with increasing maternal PFOA (Granum et al. 2013).

NTP (2016b) concluded that there is moderate confidence that exposure to PFOA is associated with suppression of the antibody response based on the available human studies. NTP (2016b) also concluded that there is low confidence that exposure to PFOA is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease).

Epidemiological Studies—Hypersensitivity Outcomes. Of the different types of hypersensitivity effects, the most widely studied endpoint is asthma; the possible association between exposure to PFOA and asthma has been studied in occupational, community, and general population studies. Several studies have found associations between current serum PFOA levels and diagnosis of asthma in children (Dong et al. 2013; Humblet et al. 2014; Qin et al. 2017) and adults (Anderson-Mahoney et al. 2008; Zhu et al. 2016). A case-control study found significantly higher serum PFOA levels in asthmatic adolescents as compared to adolescents without asthma (Zhou et al. 2017).

However, other studies have found no association between estimated cumulative serum PFOA levels and incidence of asthma being treated with medication in workers (Steenland et al. 2015) or asthma in the general population (Stein et al. 2016a). In children, no associations between maternal serum PFOA levels and asthma-related health outcomes were observed in 3-year-old children (Granum et al. 2013), 5–9-year-old children (Smit et al. 2015) or 1–10-year-old children (Impinen et al. 2018), or between current PFOA levels and current asthma in adolescents (Stein et al. 2016a). However, the Stein et al. (2016a) study did find an association with rhinitis in adolescents. No associations between maternal PFOA and wheezing were found in infants up to 18 months of age (Okada et al. 2012), infants 12 or 24 months of age (Okada et al. 2014), children 3 years of age (Granum et al. 2013), children 5–9 years of age (Smit et al. 2015), children 2–10 years old (Impinen et al. 2018), or between current serum PFOA levels and wheezing in adults (Stein et al. 2016a). The ORs for asthma diagnosis relative to serum PFOA levels are graphically presented in Figure 2-22; studies using different statistical methods are not included. No associations between maternal PFOA and prevalence of allergic diseases or wheezing were found in 4-year-old children (Goudarzi et al. 2016a). No associations between maternal PFOA and eczema were found in infants up to 18 months of age (Okada et al. 2012), children 3 years of age (Granum et al. 2013), or children 5–9 years of age (Smit et al. 2015). Similarly, no association was found between cord blood PFOA and atopic dermatitis in children 2 years of age (Wang et al. 2011).

Figure 2-22. Risk of Asthma Diagnosis Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



No associations were found between risks of allergy or allergic sensitization and current serum PFOA levels in adults (Stein et al. 2016a) or between cord PFOA in 2–10-year-old children (Impinen et al. 2018). Two studies examining the possible association between current serum PFOA levels in adults and food allergies have found mixed results, with one study finding an association (Buser and Scinicariello 2016) and one not finding an association (Stein et al. 2016a); a study in infants did not find an association between the risk of food allergy and maternal serum PFOA levels (Okada et al. 2012). It is noted that IgE levels, which were used to assess food allergies, is not a sensitive measure of clinical food allergy. No association was found for food sensitization (Buser and Scinicariello 2016).

Associations between serum PFOA and IgE, eosinophil counts, and eosinophil cationic protein levels were observed in asthmatic children (9–16 years of age), but not in non-asthmatic children (Dong et al. 2013; Zhu et al. 2016). Significantly higher IL-4 and IL-5 levels were observed in male children with asthma with the highest PFOA levels (Zhu et al. 2016). Two studies found associations between PFOA and IgE levels in infants. An inverse association was found between maternal PFOA and IgE levels in female infants but not in male infants (Okada et al. 2012), whereas Wang et al. (2011) found a correlation between cord blood PFOA and child IgE levels in males only or in males and females combined. A third study did not find an association between cord blood PFOA and IgE levels in infants (Ashley-Martin et al. 2015). NTP (2016b) concluded that there is low confidence that exposure to PFOA during childhood is associated with increased hypersensitivity responses.

Epidemiological Studies—Autoimmune Outcomes. There are limited data that can be used to evaluate the possible association between PFOA exposure and the risk of autoimmune diseases. Significant increases in the risk of ulcerative colitis were observed in an occupational exposure study (Steenland et al. 2015) and a C8 Science Panel study (Steenland et al. 2013). Although both studies found consistent results, it should be noted that the community study also included participants with occupational exposure to PFOA. The occupational study also found an association between PFOA exposure and rheumatoid arthritis; this was not observed in the community study. The community study (Steenland et al. 2013) also found no associations for other autoimmune diseases (Crohn's disease, Type I diabetes, lupus, and multiple sclerosis). A third study examined neural- and non-neural-specific antibodies and found no associations with cord blood PFOA or current serum PFOA in 7-year-old children (Osuna et al. 2014).

NTP (2016b) concluded that there is low confidence that exposure to PFOA is associated with ulcerative colitis.

Laboratory Animal Studies. The results of several mouse studies support the epidemiological data suggesting that exposure to PFOA can result in immunosuppression. Significant alterations in IgM levels in response to T-dependent antigens, such as sheep red blood cells (sRBCs) or horse red blood cells were observed in acute and intermediate oral mouse studies (DeWitt et al. 2008, 2009, 2016; Loveless et al. 2008; Yang et al. 2002a); the lowest-adverse-effect level was 3.75 mg/kg/day in mice exposed to PFOA in the drinking water for 15 days (DeWitt et al. 2008). Rats appear to be less sensitive than mice; no alterations in IgM levels were observed in rats administered PFOA via gavage for 28 days (Loveless et al. 2008). In a mouse developmental toxicity study, exposure to PFOA on GDs 6–17 was not associated with alterations in IgM or IgG levels in the offspring (Hu et al. 2010). Limited data suggest that alterations in NK cells or delayed type hypersensitivity are not sensitive endpoints for PFOA in laboratory animals. Exposure of male rats to 50 mg/kg/day PFOA by gavage for 14 days did not significantly affect the numbers of T cells, NK cells, or helper T cells (Iwai and Yamashita 2006), and tests for delayed-type hypersensitivity response in mice challenged with bovine serum albumin following exposure to 30 mg/kg/day PFOA via drinking water for 15 days were negative (DeWitt et al. 2008).

Two studies have evaluated hypersensitivity in mice. Application of ≥18.8 mg/kg/day PFOA to the dorsal surface of the ears of mice and subsequently injected with ovalbumin resulted in a significant increase in serum total IgE compared to mice exposed only to ovalbumin (Fairley et al. 2007). Ovalbumin-specific airway hyperreactivity also increased in mice co-exposed to ovalbumin and 25 mg/kg PFOA relative to mice exposed to ovalbumin alone. The investigators suggested that PFOA exposure may increase the IgE response to environmental allergens (Fairley et al. 2007). In contrast to the results of the dermal study, no increases in airway hyperresponsiveness were observed in ovalbumin-sensitized mice exposed *in utero* and post-weaning to PFOA in the diet (Ryu et al. 2014). In nonsensitized mice, PFOA did induce airway hyperresponsiveness in 12-week-old pups.

Numerous studies have evaluated secondary outcomes in monkeys, rats, and mice. In the spleen and thymus, exposure to PFOA resulted in decreases in organ weight, decreases in the number of cells, and/or atrophy (DeWitt et al. 2008; Loveless et al. 2008; Qazi et al. 2009a, 2012; Son et al. 2009; Yang et al. 2000, 2001, 2002b). Acute exposure resulted in decreases in absolute thymus weight at 11.5 mg/kg/day (Yang et al. 2001), decreases in spleen weight at 30 mg/kg/day (Qazi et al. 2012; Yang et al. 2000), and severe thymic atrophy at 30 mg/kg/day (Qazi et al. 2012; Yang et al. 2000). Exposure of male rats to 50 mg/kg/day PFOA by gavage for 14 days did not significantly affect the absolute or relative spleen weight nor did it alter lymphocyte subsets (Iwai and Yamashita 2006).

Decreases in relative spleen weight were observed at ≥0.96 mg/kg/day PFOA, and absolute spleen weight and absolute and relative thymus weights were decreased at 9.6 and 29 mg/kg/day (Loveless et al. 2008). The lowest-adverse-effect levels for spleen and thymus weight changes identified in mouse intermediate studies were 3.75 mg/kg/day PFOA for decreases in absolute spleen weight (DeWitt et al. 2008) and 9.6 mg/kg/day for decreases in absolute and relative thymus weight (Loveless et al. 2008). In rats, no alterations in spleen weight were observed following chronic exposure to 15 mg/kg/day in the diet (3M 1983; Butenhoff et al. 2012c).

Decreases in the number of splenic and thymic lymphocytes were observed in mice administered via gavage ≥9.6 mg/kg/day PFOA for 28 days (Loveless et al. 2008). In contrast, administration of 29 mg/kg/day PFOA by gavage for 28 days did not result in alterations in the number of splenic or thymic lymphocytes in rats (Loveless et al. 2008). A 10-day exposure of mice to 3.0 mg/kg/day PFOA resulted in decreases in the number of bone marrow B-lymphoid cells (Qazi et al. 2012); a decrease in bone marrow myeloid cells was also observed at 30 mg/kg/day. Examination of the B-lymphoid cell subpopulations showed decreases in pro/pre B cells, immature B cells, and early mature B cells, with the greatest reductions observed for pro/pre B cells. When mice were allowed to recover for 10 days following a 10-day exposure to 30 mg/kg/day PFOA in the diet, only a partial recovery of B-lymphoid cells was observed. Significant increases in CD4-CD8- and CD4-CD8+ thymic lymphocytes were observed in mice exposed to 47.21 mg/kg/day for 21 days; increases in CD4+CD8+ lymphocytes were observed at 17.63 and 47.21 mg/kg/day (Son et al. 2009). Similarly, there were decreases in splenic CD4-CD8- lymphocytes at 47.21 mg/kg/day and CD4-CD8+ lymphocytes at ≥0.49 mg/kg/day and increases in splenic CD4+CD8- lymphocytes at 17.63 and 47.21 mg/kg/day.

Two studies examined the immune response to mitogens in mice exposed to PFOA. Marked decreases in total leukocytes, lymphocytes, and neutrophils levels and increases in tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were observed in the peritoneal cavity, bone marrow, and spleen cells in response to lipopolysaccharide (LPS) stimulation in mice exposed to approximately 40 mg/kg/day PFOA for 10 days (Qazi et al. 2009a). Exposure of splenic lymphocytes isolated from PFOA-exposed mice to concavalin A (ConA) or LPS resulted in decreases in lymphocyte proliferation (Yang et al. 2002a).

A number of studies have evaluated the potential of PFOA to induce histological alterations in immune organs. In monkeys, administration of approximately 20 mg/kg/day PFOA administered via a capsule to Cynomolgus monkeys for 4 or 26 weeks did not affect the gross or microscopic morphology of the spleen (Butenhoff et al. 2002; Thomford 2001). Administration via gavage of 30 mg/kg/day PFOA to Rhesus

monkeys for 90 days induced atrophy of lymphoid follicles in the spleen and lymph nodes and slight to moderate hypocellularity of the bone marrow (Griffith and Long 1980). No histological alterations were observed in the spleen or thymus of rats exposed intermittently to ≤84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986), ≤29 mg/kg/day administered via gavage for 28 days (Loveless et al. 2008), or dermal doses of ≤2000 mg/kg/day for 2 weeks (Kennedy 1985) or in the spleen and mesenteric lymph nodes of rats exposed to ≤110 mg/kg/day PFOA in the diet for 90 days (Griffith and Long 1980) or ≤15 mg/kg/day PFOA in the diet for 2 years (3M 1983; Butenhoff et al. 2012c).

Studies in wild-type mice and PPARα-null mice demonstrate that PFOA-induced immunomodulation results from PPARα-dependent and -independent mechanisms (DeWitt et al. 2016; Yang et al. 2002b). Exposure to 30 or 33 mg/kg/day PFOA resulted in decreases in spleen weight, thymus weight, number of splenic lymphocytes, number of thymic lymphocytes, and CD4+ and CD8+ splenic and thymic lymphocytes in wild-type mice. Similar exposures of PPARα knockout mice did not result in alterations in spleen weight, number of splenic lymphocytes, or their phenotypes. Although decreases in thymus weight, number of thymic lymphocytes, and their phenotypes were observed in the knockout mice, the magnitudes of the changes were lower in the knockout mice than in the wild-type mice. However, similar responses were observed in T-cell-dependent antibody responses. Exposure to 30 mg/kg/day PFOA resulted in 16 and 14% decreases in the response to sRBCs in wild-type and knockout mice, respectively (DeWitt et al. 2016).

In a systematic review of the available laboratory animal data, NTP (2016b) concluded that there is high confidence that exposure to PFOA is associated with suppression of the antibody response, very low confidence that PFOA is associated with the ability to respond to infectious disease, and moderate confidence that PFOA is associated with increased hypersensitivity.

Summary. Epidemiological studies have evaluated several aspects of immunotoxicity including immunosuppression, hypersensitivity, and autoimmunity. A number of general population studies have found significant inverse associations between serum PFOA levels and antibody responses to vaccines. However, no consistent associations were found between serum PFOA and disease resistance, as measured by episodes of the common cold, cough, fever, or hospitalization for infectious disease. In tests of hypersensitivity, there is some evidence of an association between serum PFOA and asthma diagnosis in children and adults, although this finding was not consistent across studies; increased risk of allergy or allergic sensitization does not appear to be associated with serum PFOA. Based on the findings of an occupational exposure and community exposure study, there is some suggestive association between

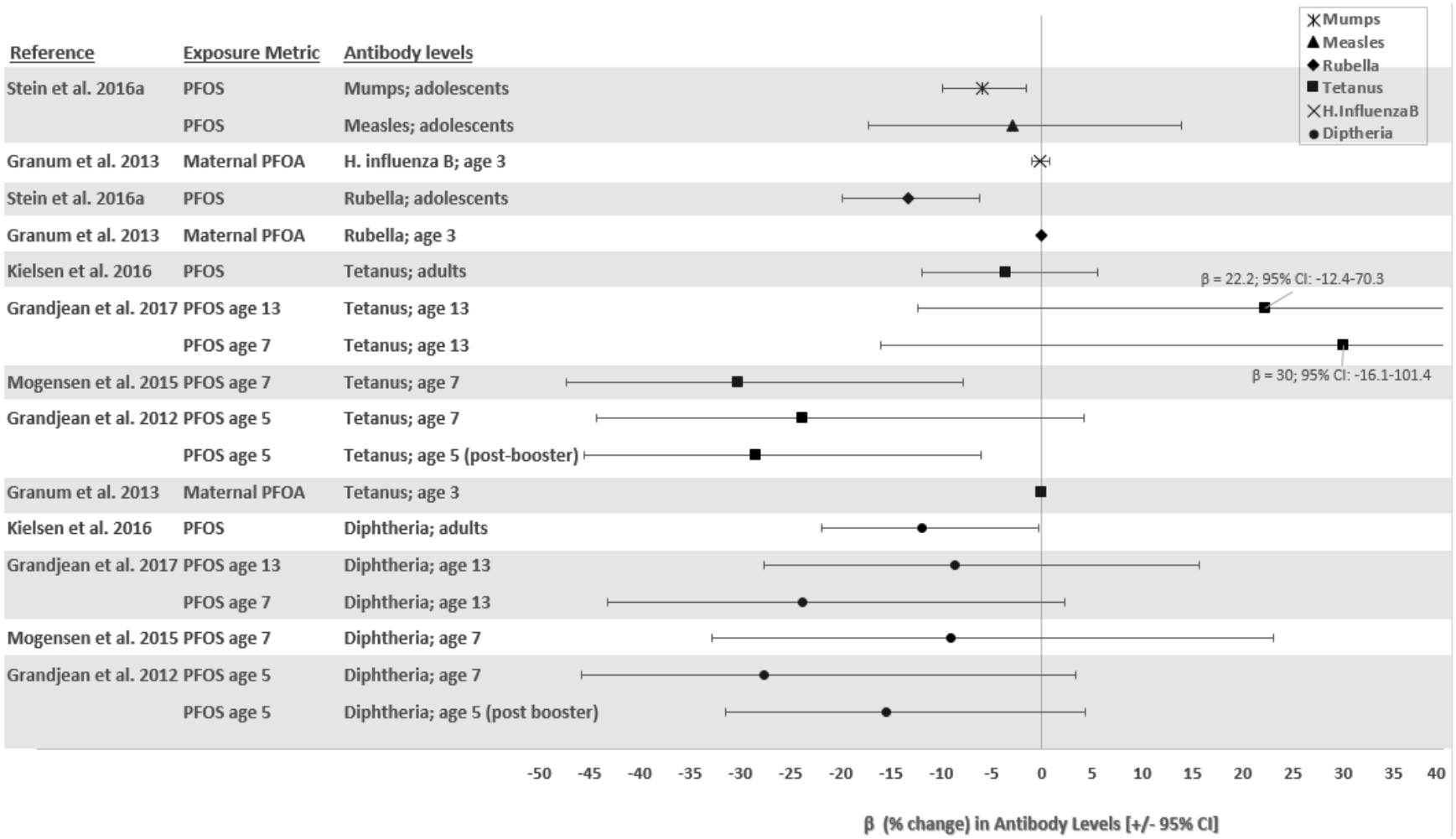
serum PFOA and an increased risk of ulcerative colitis, but not for other autoimmune diseases. Animal studies suggest that the immune system is a sensitive target of PFOA toxicity. A number of studies in mice have demonstrated evidence of immunosuppression and increased hypersensitivity. Laboratory animal studies have also found secondary immune outcomes in the spleen and thymus, which included decreases in organ weight and decreases in the number of lymphocytes.

PFOS

Epidemiological Studies—Immunosuppression Outcomes. Several epidemiological studies have evaluated the potential of PFOS to cause immunosuppression. In studies that evaluated infectious disease resistance, no alterations in the risk of otitis media were observed in infants monitored through 18 months or 3 years of age (Granum et al. 2013; Okada et al. 2012), common cold or other upper respiratory infections (Granum et al. 2013), gastroenteritis with vomiting or diarrhea (Granum et al. 2013), hospitalizations due to infectious diseases in children (Fei et al. 2010), or symptoms of infection such as nasal discharge, cough, diarrhea, or vomiting in children (Dalsager et al. 2016). In contrast, other studies have found associations between PFOS and infectious diseases. Associations between the number of days with symptoms of infection and maternal PFOS levels were observed in children (Dalsager et al. 2016) and between maternal serum PFOS and the risk of total infectious disease in early life (age 4 years) (Goudarzi et al. 2017). Associations were also found between cord PFOS levels and the number of common colds from 0 to 2 years of age and the number of lower respiratory tract infections between 0 and 10 years of age (Impinen et al. 2018).

Other studies evaluating immunosuppression found significant alterations in the response to vaccines; the changes in the response to antibody levels relative to serum PFOS levels are graphically presented in Figure 2-23; studies utilizing different statistical methods are not included in this figure. In children receiving a tetanus vaccination at age 5, there were associations between serum PFOS levels at age 5 and tetanus antibody levels at age 5 (Grandjean et al. 2012) and between serum PFOS levels at age 7 and tetanus antibody levels at age 7 when the analysis was restricted to children who were not likely to have had a booster vaccine after age 5 (Grandjean and Budtz-Jorgensen 2013). However, no associations were found between tetanus antibody levels at age 5 and maternal PFOS or child PFOS levels (Grandjean et al. 2012), between tetanus antibody levels at age 7 and maternal PFOS or child PFOS levels at age 5 or 7 years (Grandjean et al. 2012; Mogensen et al. 2015a), or between tetanus antibody levels at age 14 and child PFOS levels at age 13 (Grandjean et al. 2017). Similarly, diphtheria antibody levels at age 7 were

Figure 2-23. Antibody Responses Relative to Serum PFOS Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFOS)

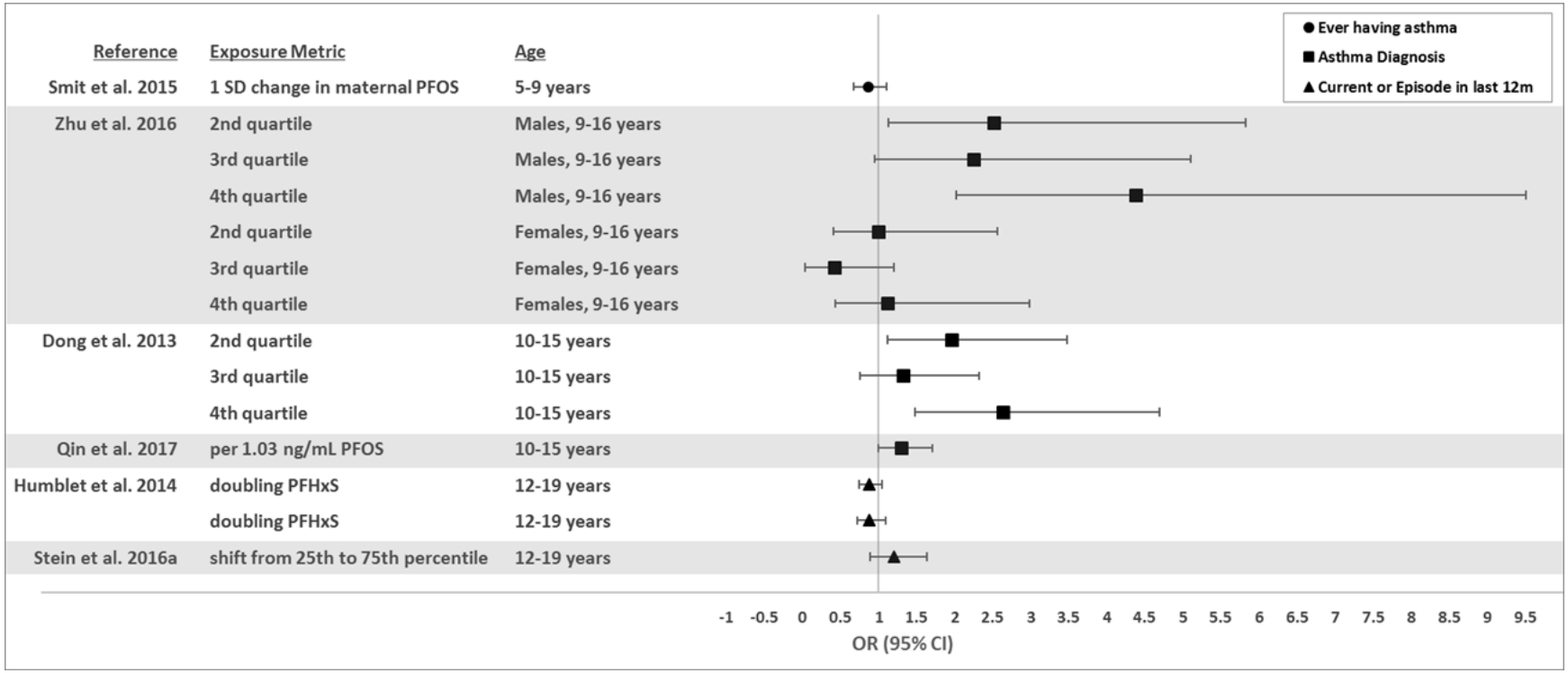


significantly associated with serum PFOS levels at age 5 and 7 (Grandjean et al. 2012; Mogensen et al. 2015a), but antibody levels at age 5 were not associated with maternal PFOS or child PFOS at age 5 years (Grandjean et al. 2012) and antibody levels at age 13 were not associated with child PFOS levels at age 7 or 13 years (Grandjean et al. 2017). In another study of children (Granum et al. 2013), decreased rubella antibody levels were associated with higher maternal PFOS levels, but no associations were found for tetanus or Haemophilus influenza type B antibodies. In adolescents, recent serum PFOS levels were inversely associated with mumps and rubella antibody levels, but not with measles antibody levels (Stein et al. 2016a). In studies in adults, recent PFOS levels were inversely associated with diphtheria antibody levels 30 days after booster administration (Kielsen et al. 2016), but not with tetanus antibody levels 30 days after booster administration (Kielsen et al. 2016) or influenza types A H3N2, A H1N1, or B antibody levels 21 days post-vaccination (Looker et al. 2014).

NTP (2016b) concluded that there is moderate confidence that exposure to PFOS is associated with suppression of the antibody response and that there is low confidence that exposure to PFOS is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease).

Epidemiological Studies—Hypersensitivity Outcomes. Several studies examined the risk of hypersensitivity associated with serum PFOS in children and adolescents; however, the results are inconsistent. In three case-control studies, increased risks of asthma were observed. Qin et al. (2017) reported increased risk of asthma in children associated with serum PFOS levels. Dong et al. (2013) reported an increased risk of asthma diagnosis and increased severity of asthma episodes in children with PFOS levels in the 4th quartile. Zhu et al. (2016) also reported an association between asthma diagnosis and serum PFOS levels in the 4th quartile; however, the association was only significant in males. A third case-control study found significantly elevated serum PFOS levels in asthmatic adolescents (Zhu et al. 2016). Prospective and cross-sectional studies in children (Granum et al. 2013) did not find an association between maternal PFOS levels and the risk of asthma diagnosis in 3-year-old children; between cord PFOS and asthma diagnosis, current asthma, or ever having asthma in 2–10-year-old children (Impinen et al. 2018); or between maternal PFOS and asthma diagnosis in adolescents (Humblet et al. 2014; Stein et al. 2016a). Data evaluating associations between serum PFOS and the risk of asthma diagnosis are presented in Figure 2-24.

Figure 2-24. Risk of Asthma Diagnosis Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



No associations between maternal PFOS or cord PFOS and eczema, atopic dermatitis, or wheezing or total allergic diseases have been found in children (Goudarzi et al. 2016a; Granum et al. 2013; Impinen et al. 2018; Okada et al. 2012, 2014; Smit et al. 2015; Wang et al. 2011). Similarly, no associations between recent serum PFOS levels in adolescents and food allergies or sensitizations (Buser and Scinicariello 2016; Stein et al. 2016a) or maternal PFOS levels and food allergies in infants (Okada et al. 2012) were observed. However, in a cross-sectional study of adolescents, recent PFOS levels were associated with mold allergies and inversely associated with the risk of plant or cockroach or shrimp allergies (Stein et al. 2016a). In related studies, cord blood PFOS levels were associated with an increase in cord IgE levels, but not in infant serum IgE levels (Wang et al. 2011). Two other studies did not find associations between maternal PFOS levels and cord IgE levels (Ashley-Martin et al. 2015; Okada et al. 2012).

NTP (2016b) concluded that there is very low confidence that exposure to PFOS is associated with changes in the hypersensitivity response in children.

Laboratory Animal Studies. A limited number of laboratory animal studies examined PFOS-induced immunosuppression. Guruge et al. (2009) reported an impaired response to an influenza A virus challenge in mice administered 0.025 mg/kg/day PFOS via gavage for 21 days (Guruge et al. 2009). Several studies have found an impaired response to sRBCs (Dong et al. 2009, 2011; Peden-Adams et al. 2008); however, decreases in NK cell activity were observed at higher doses (0.83–2.08 mg/kg/day) (Dong et al. 2009). Qazi et al. (2009a) reported several alterations in parameters associated with the innate immune system in mice exposed to approximately 40 mg/kg/day PFOS in the diet for 10 days. These alterations included marked decreases in total leukocyte and lymphocyte levels and increases in TNF-α and IL-6 levels in the peritoneal cavity and bone marrow in response to LPS stimulation; no alterations were observed in mice exposed to a 20-fold lower dose. As discussed in Section 2.17, a developmental toxicity study (Keil et al. 2008) found an altered response to sRBCs in mice exposed to PFOS *in utero*.

No alterations in spleen or thymus weights were observed in mice exposed to 0.025 mg/kg/day PFOS (Guruge et al. 2009); at a higher dose (0.42 mg/kg/day), significant decreases in relative spleen and thymus weights were observed (Dong et al. 2009; Zheng et al. 2009). Decreases in splenic and thymic cellularity were also observed at ≥0.42 mg/kg/day PFOS (Dong et al. 2009; Qazi et al. 2009b, 2012; Zheng et al. 2009). Bone marrow cells (B-lymphoid and myeloid cells) were also significantly decreased in mice exposed to 30 mg/kg/day PFOS for 10 days (Qazi et al. 2012). Within the B-lymphoid cell population, there were decreases in the number of pro/pre B cells and immature cells (Qazi et al. 2012).

Significant alterations in all splenic T cell CD4 and CD8 subpopulations were observed at ≥0.00331 mg/kg/day PFOS (Peden-Adams et al. 2008) and thymic lymphocyte phenotypes were altered at 0.42 mg/kg/day PFOS (Dong et al. 2009).

Rats treated with 1.77 mg/kg/day PFOS for 4 weeks, 6.34 mg/kg/day for 28 days, 1.56 mg/kg/day for 14 weeks, or 1.04 mg/kg/day for 2 years did not show significant morphological alterations in the spleen, thymus, or mesenteric lymph nodes (Butenhoff et al. 2012b; Lefebvre et al. 2008; Seacat et al. 2003; Thomford 2002b). Similar findings were reported in Cynomolgus monkeys dosed with up to 2 mg/kg/day for 4 weeks or up to 0.75 mg/kg/day PFOS for 26 weeks (Seacat et al. 2002; Thomford 2002a).

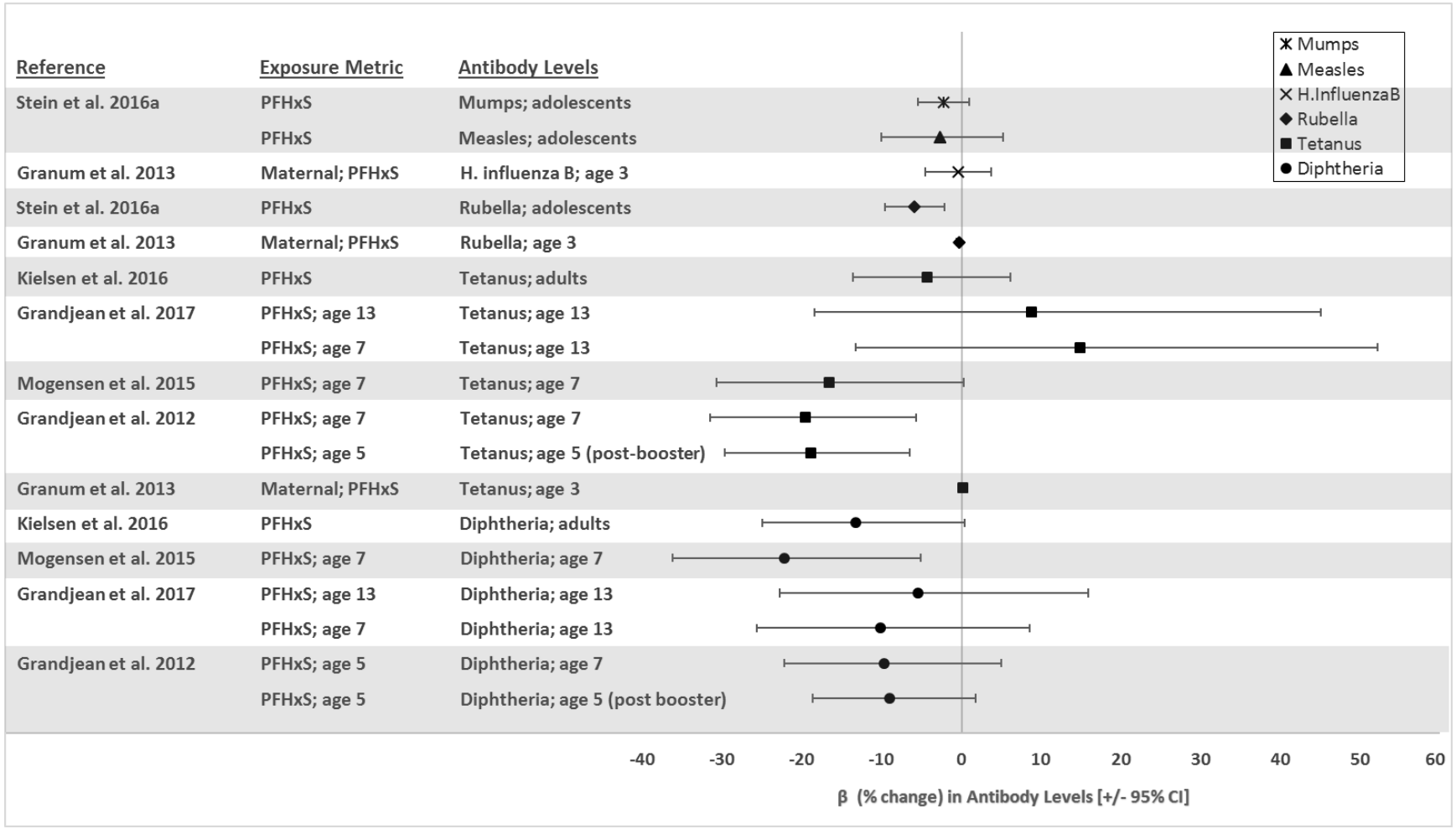
In a systematic review of the available laboratory animal data, NTP (2016b) concluded that there is high confidence that exposure to PFOS is associated with suppression of the antibody response, moderate confidence that PFOS is associated with the ability to respond to infectious disease, and low confidence that PFOS is associated with increased hypersensitivity.

Summary. A number of epidemiological studies have examined the potential immunotoxicity of PFOS. The database provides convincing evidence of an association between serum PFOS levels and immunosuppression, particularly impaired antibody responses to vaccines in adults and children. Mixed results have been observed in studies evaluating infectious disease resistance. Similarly, inconsistent results have been examined in studies evaluating associations between serum PFOS and hypersensitivity outcomes, such as asthma; no associations were found for eczema, dermatitis, food allergies/ sensitizations. Laboratory animal studies, particularly studies in mice, provide strong evidence of the immunotoxicity of PFOS. The strongest evidence comes from studies reporting impaired antibody responses resulting from oral exposure to relatively low doses of PFOS. Other immune effects include decreased response to infectious disease, decreases in spleen and thymus weights, and decreases in splenic and thymic cellularity and bone marrow cells.

PFHxS

Epidemiological Studies—Immunosuppression Outcomes. Several epidemiological studies have examined the potential of PFHxS to suppress the immune system. Altered antibody responses relative to serum PFHxS levels are graphically presented in Figure 2-25. Inverse associations were observed between tetanus antibody levels in 5- and 7-year-old children and serum PFHxS levels at age 5 or 7 years

Figure 2-25. Antibody Responses Relative to Serum PFHxS Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFHxS)

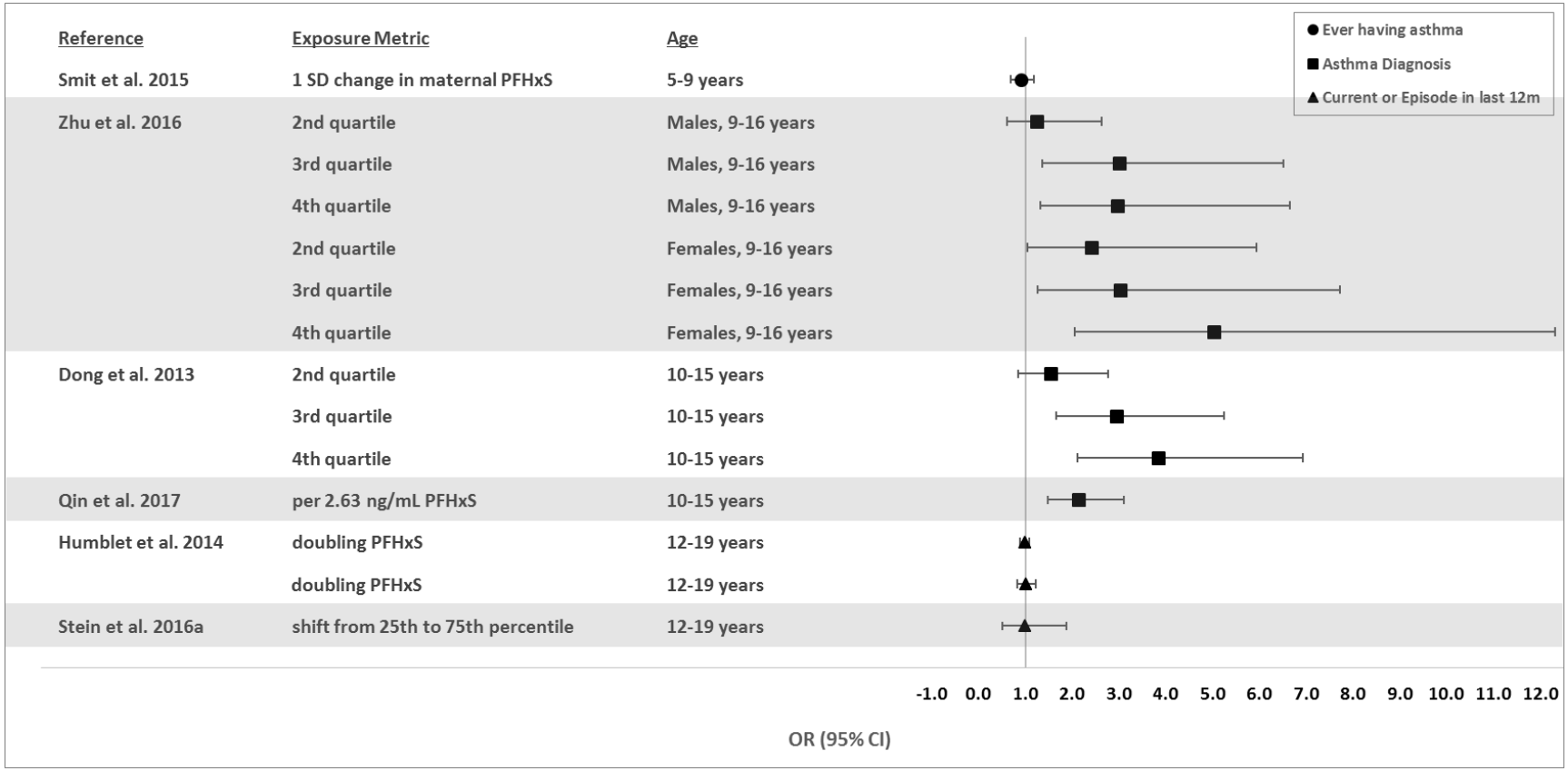


(Grandjean et al. 2012; Mogensen et al. 2015a); but there were no associations between serum PFHxS levels at age 7 or 13 and tetanus antibody levels at age 13 (Grandjean et al. 2017). No associations were found between maternal PFHxS levels and tetanus antibody levels in the children. These studies found no associations between diphtheria antibody levels at ages 5, 7, or 13 and serum PFHxS levels in the mother or in the children. A study in 3-year-old children found an inverse association between maternal PFHxS levels and rubella antibody levels, but no association with influenza type B or tetanus antibody levels (Granum et al. 2013). In adolescents, serum PFHxS levels were also inversely associated with rubella antibody titers in a seropositive subcohort (Stein et al. 2016a); no associations were found for measles or mumps antibody titers. Another study in adolescents did not find associations between recent serum PFHxS levels and tetanus or diphtheria antibody levels (Kielsen et al. 2016). A study in adults did not find associations between PFHxS levels and response to influenza vaccine; some alterations in serum cytokine levels were observed, but chemokine and IgA levels were not altered (Stein et al. 2016b).

In general, the available studies do not suggest an association between serum PFHxS and decreased infectious disease resistance. No alterations in the frequency of fever, cough, nasal discharge, otitis media, diarrhea, or vomiting were observed in children (Dalsager et al. 2016; Granum et al. 2013). Cord PFHxS levels were not associated with increased prevalence of common colds in children 0–2 years of age or lower respiratory tract infections in children 0–10 years of age (Impinen et al. 2018). No association between maternal PFHxS levels and total infectious disease prevalence was found in children up to the age of 4 years (Goudarzi et al. 2017); however, when boys and girls were analyzed separately, an association was found in girls. An association between maternal PFHxS levels and the number of episodes of gastroenteritis was found in children (Granum et al. 2013).

Epidemiological Studies—Hypersensitivity Outcomes. Data evaluating associations between serum PFHxS and the risk of asthma diagnosis are presented in Figure 2-26. No associations were observed between asthma diagnosis, wheezing, and/or eczema or total allergic diseases in children and maternal serum PFHxS levels (Goudarzi et al. 2016a; Granum et al. 2013; Smit et al. 2015) or with recent PFHxS levels in adolescents (Humblet et al. 2014; Okada et al. 2014). In contrast, case-control studies in asthmatic children did find associations between recent PFHxS serum levels and asthma diagnosis (Dong et al. 2013; Qin et al. 2017; Zhu et al. 2016), but no association with asthma severity (Dong et al. 2013). Another case-control study found significantly elevated serum PFHxS levels in adolescents with asthma (Zhu et al. 2016). Dong et al. (2013) also reported associations between serum PFHxS levels and

Figure 2-26. Risk of Asthma Diagnosis Relative to PFHxS Levels (Presented as Adjusted Odds Ratios)



eosinophil counts and eosinophil cationic protein levels in asthmatic children, but not in non-asthmatics. No associations were found with IgE levels in either case-control study (Dong et al. 2013; Zhu et al. 2016) or in a study measuring cord blood IgE (Ashley-Martin et al. 2015).

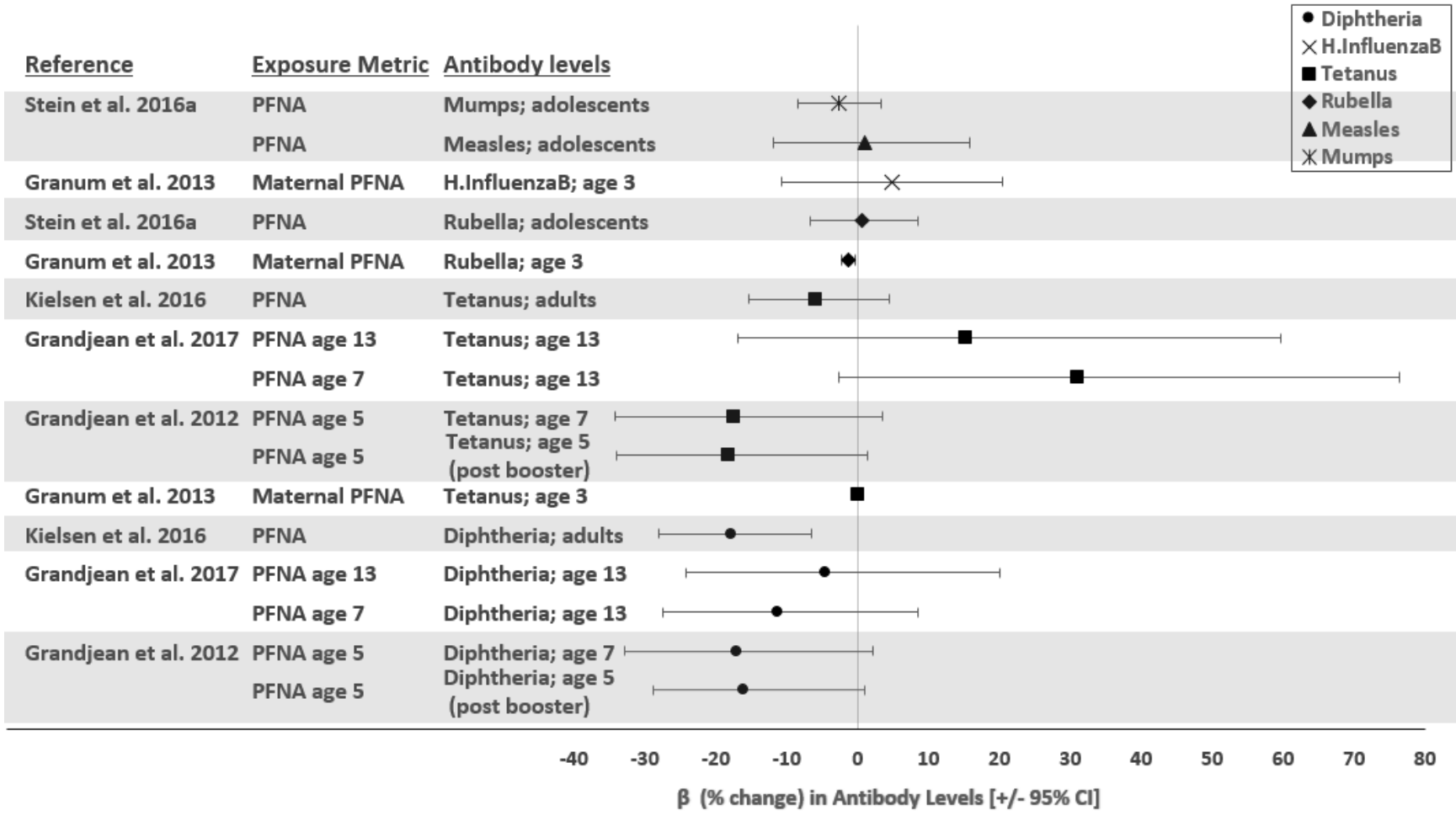
An increased risk of food allergies associated with serum PFHxS levels, but not increased sensitivity to foods, was found in adolescents (Buser and Scinicariello 2016). Another study found no associations between serum PFHxS levels and allergic sensitization to plants, dust mites, pets, cockroaches/shrimp, rodents, mold, or food in adolescents (Stein et al. 2016a).

Laboratory Animal Studies. In the only available study evaluating immunotoxicity for PFHxS, Butenhoff et al. (2009a) did not find histological alterations in the spleen, thymus, or lymph nodes of rats administered 10 mg/kg/day PFHxS via gavage for 42–56 days.

PFNA

Epidemiological Studies—Immunosuppression Outcomes. Most studies examining a possible association between serum PFNA levels and immunosuppression have not found associations. No associations were found between maternal or child PFNA levels and tetanus antibody levels at ages 3, 5, 7, or 13 (Grandjean et al. 2012, 2017; Granum et al. 2013) or in adults (Kielsen et al. 2016). Some studies have found associations between serum PFNA and diphtheria antibody levels, but the results were not consistent. Grandjean and associates found a significant inverse association between diphtheria antibodies levels at age 5 (Grandjean et al. 2012) and serum PFNA levels at age 5, but not for antibody levels at age 13 and PFNA levels at age 7 or 13 (Grandjean et al. 2017). Kielsen et al. (2016) also reported an inverse association (unadjusted for potential confounders) between serum PFNA and diphtheria antibody levels in a small study of adults. An inverse association between maternal serum PFNA and rubella antibody levels was observed in children (Granum et al. 2013), but there was no association for influenza type B antibody levels. Similarly, no associations were found between recent PFNA serum levels and measles, mumps, or rubella antibody titers in adolescents (Stein et al. 2016a). Data evaluating associations between serum PFNA and altered antibody response are presented in Figure 2-27.

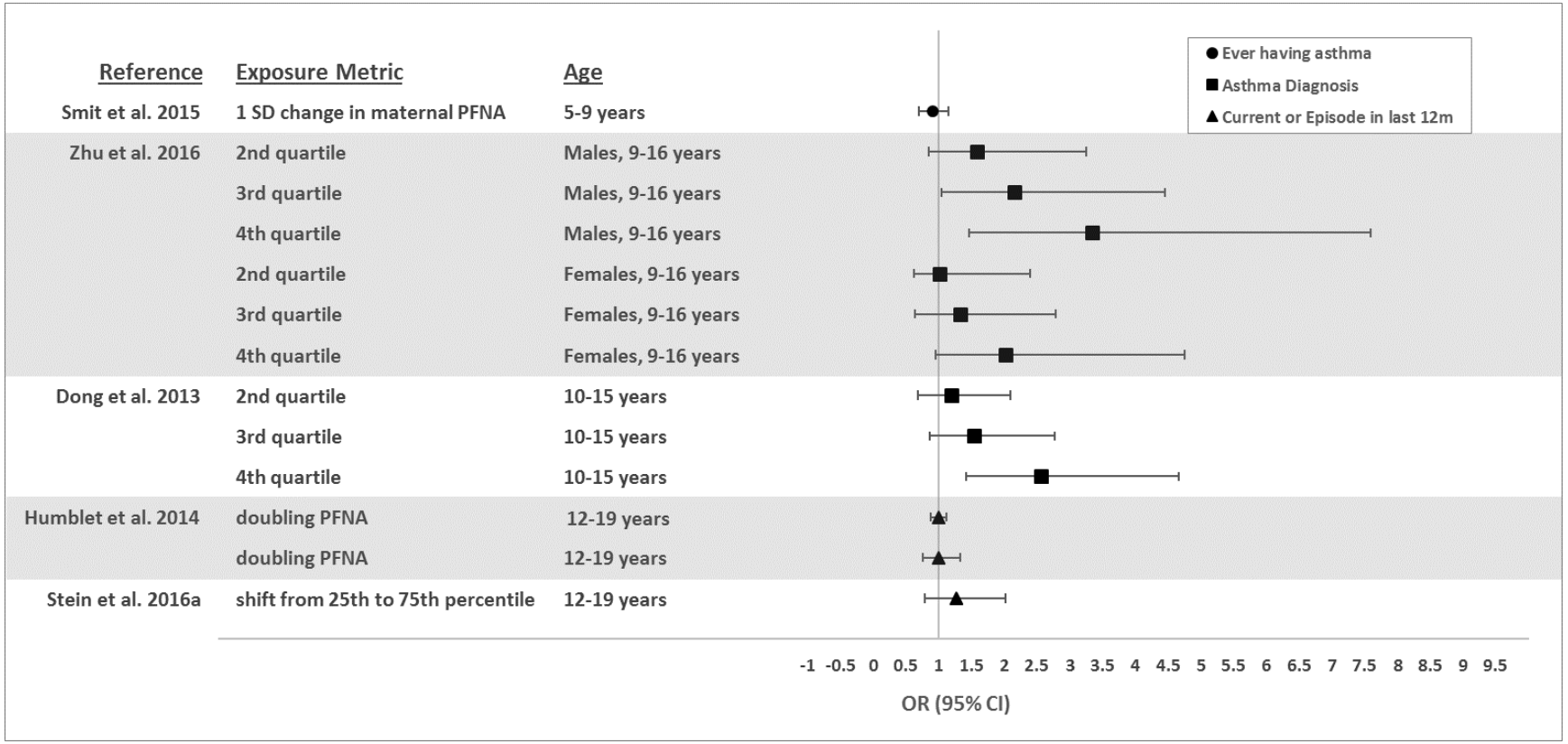
Figure 2-27. Antibody Responses Relative to Serum PFNA Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFNA)



The epidemiological data provide mixed results on whether there are associations between decreased infectious disease resistance and PFNA levels. No alterations in the risk of increased number of days with fever, cough, nasal discharge, diarrhea, or vomiting were observed in children (Dalsager et al. 2016), although the study did find a significant increase in the number of days above the median for nasal discharge. In a prospective study of children to the age of 4 years, no associations between maternal PFNA levels and prevalence of total infectious diseases were found (Goudarzi et al. 2017). Another study found that the number of episodes of the common cold in children was associated with maternal serum PFNA; no associations were found for otitis media or gastroenteritis (Granum et al. 2013). No associations between cord PFNA levels and the prevalence of common colds were found in children up to 2 years of age (Impinen et al. 2018), but cord PFNA levels were positively associated with the prevalence of lower respiratory infections in children up to the age of 10 years (Impinen et al. 2018).

Epidemiological Studies—Hypersensitivity Outcomes. Case-control studies of asthmatic children have reported associations between serum PFNA and asthma diagnosis (Dong et al. 2013; Qin et al. 2017; Zhu et al. 2016), but no association with asthma severity (Dong et al. 2013); another study found significantly higher serum PFNA levels in adolescents with asthma (Zhu et al. 2016). However, cross-sectional or retrospective studies (Humblet et al. 2014; Smit et al. 2015; Stein et al. 2016a) have not found associations. A prospective study of children to the age of 10, did not find associations between cord PFNA levels and current asthma, ever having asthma, asthma diagnosis, or wheezing (Impinen et al. 2018). Data evaluating associations between serum PFNA and the risk of asthma diagnosis are presented in Figure 2-28. Another study found no associations between maternal PFNA levels and prevalence of total allergic diseases or wheezing (Goudarzi et al. 2016a). No associations were found in adolescents between PFNA and food allergies (Buser and Scinicariello 2016), allergies (Stein et al. 2016a), or allergic sensitizations to plants, dust mites, pets, cockroach/shrimp, rodents, mold, or food (Stein et al. 2016a). However, inverse associations between serum PFNA and food sensitizations were observed in adolescents (Buser and Scinicariello 2016) and between maternal serum PFNA and allergic diseases in infants (Okada et al. 2014). No increases in the risk of other hypersensitivity effects (wheezing, eczema, or atopic dermatitis) were observed (Humblet et al. 2014; Okada et al. 2014; Smit et al. 2015; Stein et al. 2016a; Wang et al. 2011).

Figure 2-28. Risk of Asthma Diagnosis Relative to PFNA Levels (Presented as Adjusted Odds Ratios)



Laboratory Animal Studies. Administration of PFNA for 14 days resulted in decreases in thymus and/or spleen weights at ≥3 mg/kg/day in rats and mice (Fang et al. 2008, 2009, 2010); at 1 mg/kg/day, an increase in thymus weight was observed in rats (Fang et al. 2009). Fang et al. (2009) reported increases in the ratio of thymic cortex to medulla in rats presumably administered ≥3 mg/kg/day PFNA. In the spleen, there were decreases in the percentage of F4/80+ and CD49b+ cells at ≥1 mg/kg/day and in CD11c+ cells at ≥3 mg/kg/day (Fang et al. 2008). Increases in pro-inflammatory cytokines were observed in the serum at ≥3 mg/kg/day (Fang et al. 2009) and spleen at 5 mg/kg/day (Fang et al. 2010).

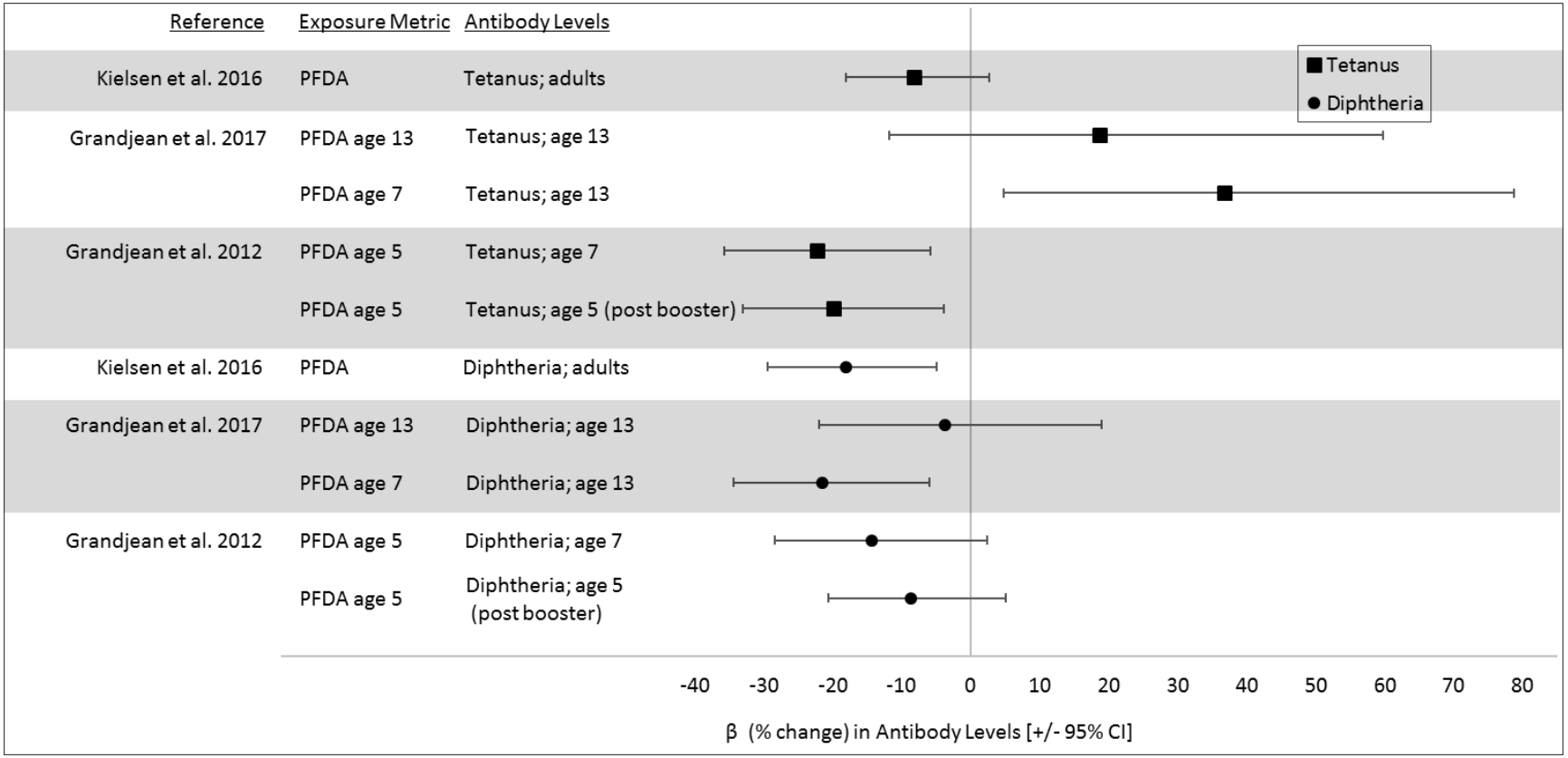
No alterations were observed in the response of splenic T lymphocytes to ConA at 5 mg/kg/day (Fang et al. 2008).

Two weeks after a single intraperitoneal administration of 46 mg/kg PFNA to male and female B57BL/6J mice, a number of immunological alterations included significant decreases in relative spleen weight and splenic leukocyte counts, alterations in splenic T-lymphocyte phenotypes (increased ratios of CD4+ and CD8+ cells), a decrease in viable thymic cells, a marked decrease in CD4+CD8+ thymic lymphocytes, and an increase in CD4+ and CD8+ thymic lymphocytes, and increased levels of tumor necrosis factor-α in response to exposure to the LPS (Rockwell et al. 2013). Similar effects were observed 4 weeks post-exposure (Rockwell et al. 2017). Comparison of the results 2 weeks post-exposure to 4 weeks post-exposure showed a partial recovery in spleen weight and specific thymic lymphocyte subpopulations, but no recovery of the ratio of specific splenic lymphocytes, thymocyte viability, or response to LPS (Rockwell et al. 2017). Some sex-related differences were noted, with females appearing to be more sensitive than males (Rockwell et al. 2017).

PFDA

Epidemiological Studies—Immunosuppression Outcomes. Studies examining possible associations between serum PFDA levels and response to vaccines have reported mixed results; see Figure 2-29 for a graphical presentation of the antibody response relative to PFDA levels. Inverse associations were observed between serum PFDA levels at age 5 and tetanus antibody levels at ages 5 and 7 (Grandjean et al. 2012) and serum PFDA levels at age 7 and antibody levels at age 13 (Grandjean et al. 2017).

Figure 2-29. Antibody Responses Relative to Serum PFDA Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFDA)

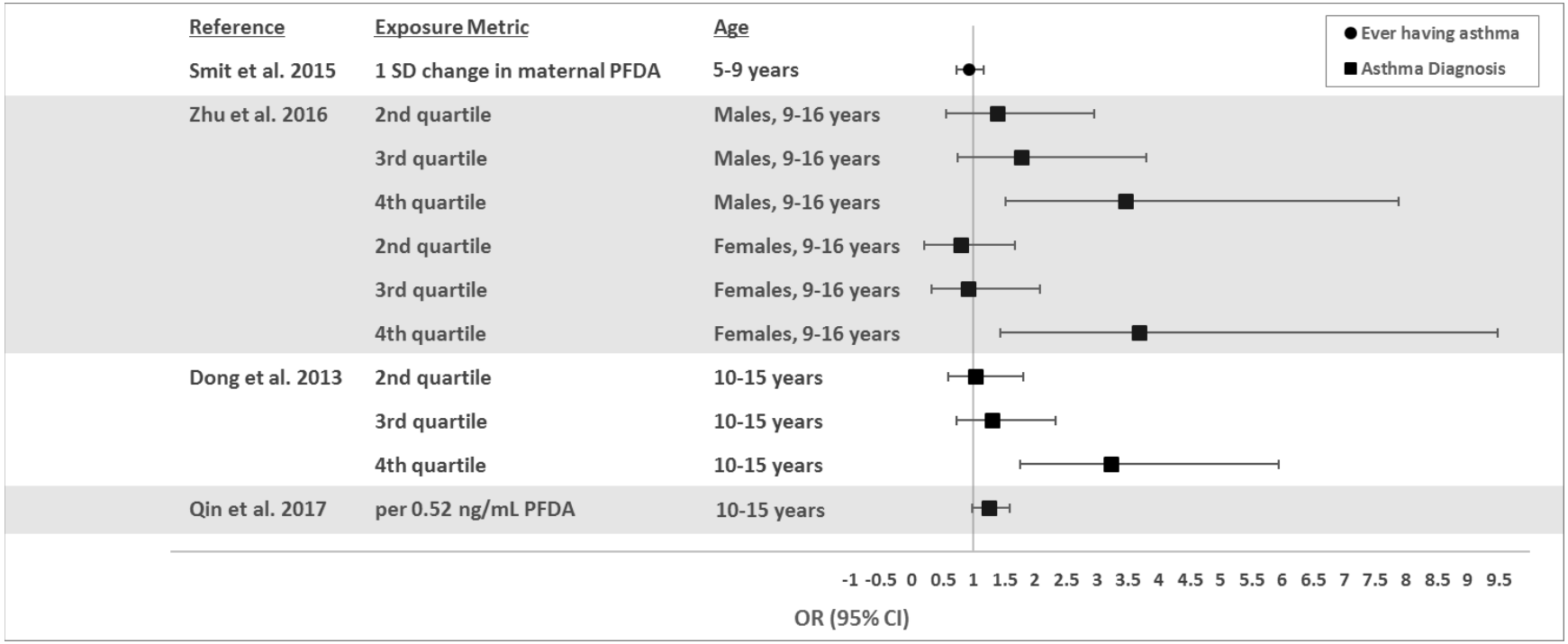


Similarly, diphtheria antibody levels at age 13 were inversely associated with serum PFDA levels at age 7 years (Grandjean et al. 2017), but no associations were observed at other time periods (Grandjean et al. 2012). In adults, diphtheria antibody levels were inversely associated with serum PFDA levels, but there was no association for tetanus antibody levels (Kielsen et al. 2016); this study did not adjust for potential confounders. Two studies examined the possible association between serum PFDA levels and infectious disease resistance, no association was found between maternal serum PFDA levels and symptoms of infection in children aged 1–4 years (Dalsager et al. 2016) and the prevalence of total infectious disease in children 0–4 years of age (Goudarzi et al. 2017).

Epidemiological Studies—Hypersensitivity Outcomes. In case-control studies, associations between asthma diagnosis and asthma severity were observed in children (Dong et al. 2013; Zhu et al. 2016); associations with serum IgE levels, absolute eosinophil counts, and eosinophil cationic protein levels were also observed. A case-control study in adolescents found significantly higher serum PFDA levels among the asthmatic cases (Zhu et al. 2016). A fourth case-control study did not find an association between serum PFDA and asthma risk in children (Qin et al. 2017). A cross-sectional study of children did not find associations between maternal PFDA levels and asthma, eczema, or wheezing in children (Smit et al. 2015). Another cross-sectional study found no association between allergic diseases or eczema in infant and maternal PFDA levels (Okada et al. 2014). In a prospective study, the prevalences of total allergic diseases or wheezing in 4-year-old children were not associated with maternal PFDA levels (Goudarzi et al. 2016a). Data evaluating associations between serum PFDA and the risk of asthma diagnosis are presented in Figure 2-30.

Laboratory Animal Studies. A single gavage dose of 80 mg/kg PFDA did not significantly alter relative thymus weight in female C57BL/6N mice, but it caused a 28% decrease in relative spleen weight 30 days after dosing (Harris et al. 1989). Lethal doses (160 and 320 mg/kg) induced atrophy and lymphoid depletion in both the thymus and spleen. No significant alterations in tests of humoral- or cell-mediated immunity, or alterations in the number of total splenic cells or splenic B-cells, T-cells, T-cell subsets, natural killer cells or macrophages were observed in rats administered up to 0.5 mg/kg/day for 28 days (Frawley et al. 2018). In tests of innate immunity, the study found decreases in the specific activity of fixed tissue macrophages in the liver in rats administered 0.25 or 0.5 mg/kg/day; the investigators suggested that interpretation of this finding may be confounded by the increased number of hepatocytes.

Figure 2-30. Risk of Asthma Diagnosis Relative to PFDA Levels (Presented as Adjusted Odds Ratios)



In mice receiving weekly doses of PFDA for 4 weeks, decreases in the number of splenic T cells, T-cell subsets, and macrophages were observed at ≥1.25 mg/kg (Frawley et al. 2018). No alterations in humoral-mediated or cell-mediated immune tests or host-resistance to the influenza virus were found.

PFUnA

Epidemiological Studies. Six epidemiological studies have evaluated the potential immunotoxicity of PFUnA in humans. Kielsen et al. (2016) reported inverse associations between serum PFUnA (unadjusted for potential confounders) and diphtheria and tetanus antibody levels in adults. Goudarzi et al. (2017) found no association between maternal PFUnA levels and the risk of total infectious diseases in children up to the age of 4 years. However, Impinen et al. (2018) found cord PFUnA levels were associated with increases in the prevalence of common colds in children up to 2 years of age and the prevalence of lower respiratory tract infections in children up to the age of 10 years.

No significant associations between maternal PFUnA levels and the risk of asthma diagnosis, eczema, or wheezing were observed in children (Smit et al. 2015). Similarly, no associations were found between cord PFUnA levels and risk of current asthma, ever having asthma, asthma diagnosis, or wheezing in children up to the age of 10 years (Impinen et al. 2018). Maternal PFUnA levels were not associated with the prevalences of total allergic diseases or wheezing in 4-year-old children (Goudarzi et al. 2016a). Okada et al. (2012) found inverse associations between maternal serum PFUnA and risk of allergies or eczema in female infants, but not in males, and Impinen et al. (2018) found no association between serum PFUnA and allergic sensitization.

PFHpA

Epidemiological Studies. In general, the two available human immunotoxicity studies did not find associations between serum PFHpA levels and diphtheria or tetanus antibody levels in adults (Kielsen et al. 2016) or risk of asthma diagnosis, eczema, or wheezing in children (Smit et al. 2015). The Smit et al. (2015) study did find an inverse association between maternal PFHpA levels and current wheezing in one subcohort; however, this was not observed in the other subcohort with higher mean maternal PFHpA levels.

PFBS

Epidemiological Studies. The epidemiological database for PFBS consists of three case-control studies in asthmatic children (Dong et al. 2013; Qin et al. 2017; Zhu et al. 2016). Two studies reported increases in asthma diagnosis, but no association with serum IgE levels (Dong et al. 2013; Zhu et al. 2016); the third study (Qin et al. 2017) did not find an association between serum PFBS and asthma risk.

Laboratory Animal Studies. No significant histological alterations were observed in spleen, thymus, or lymph nodes of rats administered via gavage 900 mg/kg/day PFBS for 28 days (3M 2001).

PFBA

Laboratory Animal Studies. No significant gross or microscopic alterations were reported in the spleen, thymus, or mesenteric lymph nodes from rats dosed with PFBA by gavage in doses of up to 184 mg/kg/day for 5 days, 150 mg/kg/day for 28 days, or 30 mg/kg/day for 90 days (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFDoDA

Epidemiological Studies. Six epidemiological studies examining potential immunotoxic endpoints were identified. Kielsen et al. (2016) found inverse associations between recent serum PFDoDA levels (not adjusted for potential confounders) and diphtheria and tetanus antibody levels in adults. No associations between maternal PFDoDA levels and the risk of total infectious diseases were found in children up to the age of 4 years (Goudarzi et al. 2017). Associations between serum PFDoDA levels and the risk of asthma diagnosis, severity of asthma, serum IgE levels, absolute eosinophil counts, and eosinophil cationic protein levels were observed in a case-control study of asthmatic children (Dong et al. 2013). A cross-sectional study of children did not find associations between maternal serum PFDoDA levels and risk of asthma diagnosis, eczema, or wheezing (Smit et al. 2015). Another study did not find associations between maternal serum PFDoDA levels and the risk of allergic disease or eczema in infants (Okada et al. 2014). In contrast, a prospective study of 4-year-old children found an inverse association between maternal PFDoDA levels and the prevalence of mother-reported total allergic diseases, but no association with the prevalence of wheezing (Goudarzi et al. 2016a).

PFHxA

Epidemiological Studies. Two epidemiological studies examined potential immunotoxic endpoints. Dong et al. (2013) found no associations between serum PFHxA levels in asthmatic and nonasthmatic children and asthma diagnosis, asthma severity, or IgE levels. Qin et al. (2017) did not find an association between serum PFHxA levels and asthma risk in children.

Laboratory Animal Studies. Thymic atrophy was observed in 3/9 female rats administered a TWA dose of 315 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005). Thymic atrophy and necrosis was also observed in most male and female rats administered 450 mg/kg/day PFHxA for 4 days; all animals died early or were sacrificed *in extremis* (Kirkpatrick 2005).

FOSA

Epidemiological Studies. The only available epidemiological study found an association between cord FOSA levels and an increased prevalence of lower respiratory tract infections in children up to the age of 10 years (Impinen et al. 2018); no association was found for common colds in children up to the age of 2 years. This study also found no associations between cord FOSA and current asthma, ever having asthma, asthma diagnosis, wheezing, or allergic sensitization (Impinen et al. 2018).

2.15 NEUROLOGICAL

Overview. There are limited data on the neurotoxicity of perfluoroalkyls in humans or laboratory animals; epidemiological data come from three studies examining memory and animal studies primarily evaluated for morphological alterations; the results of these human studies are summarized in Table 2-17 with more detailed descriptions in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 11. The epidemiological studies found decreases in the risk of memory loss associated with serum PFOA, PFOS, PFHxS, and PFNA. The potential to induce neurodevelopmental effects (including the risk of attention deficit hyperactivity disorder [ADHD]) has been more widely studied; these data are discussed in Section 2.17, Developmental. No epidemiological studies examining potential neurological effects were found for PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, or FOSA.

Table 2-17. Summary of Neurological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	14.1–27.0 ng/mL (2nd PFOA quintile)	Memory loss (self-reported)	**OR 0.88 (0.79–0.97)*, 2nd quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	4.08 ng/mL (median PFOA)	Difficulty remembering or periods of confusion (self-reported)	OR 0.92 (0.78–1.09)
Shrestha et al. 2017 General population (n=126 older adults, aged 55–74 years)	8.1 ng/mL (median serum PFOA)	Memory and learning scores	**Association (p=0.03)***
		Executive function scores	**Inverse association (p=0.04, p=0.03)***
		Visual and spatial function scores	NS (p>0.05)
		Reaction time	NS (p>0.05)
		Motor function	NS (p>0.05)
PFOS			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	20.5–27.1 ng/mL (3rd PFOS quintile)	Memory loss (self-reported)	**OR 0.86 (0.78–0.96)*, 3rd quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	22.63 ng/mL (median PFOS)	Difficulty remembering or periods of confusion (self-reported)	OR 0.90 (0.78–1.03)

Table 2-17. Summary of Neurological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shrestha et al. 2017 General population (n=126 older adults, aged 55–74 years)	33.7 ng/mL (median serum PFOS)	Memory and learning scores	**Association (p=0.04)***
		Executive function scores	NS (p>0.05)
		Visual and spatial function scores	**Association (p=0.05)***
		Reaction time	NS (p>0.05)
		Motor function	NS (p>0.05)
PFHxS			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	5.7–232.6 ng/mL (5th PFHxS quintile)	Memory loss (self-reported)	**OR 0.89 (0.79–0.99)*, 5th quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	2.05 ng/mL (median PFHxS)	Difficulty remembering or periods of confusion (self-reported)	OR 0.93 (0.82–1.06)
PFNA			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	1.0–1.2 ng/mL (2nd PFNA quintile)	Memory loss (self-reported)	**OR 0.86 (0.78–0.96)*, 2nd quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	1.01 ng/mL (median PFNA)	Difficulty remembering or periods of confusion (self-reported)	OR 0.91 (0.79–1.04)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 11 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

OR = odds ratio; NHANES = National Health and Nutrition Examination Survey; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

The results of the laboratory animal studies are presented in Tables 2-1, 2-3, 2-4, 2-5, and 2-6 and in Figures 2-6, 2-8, 2-9, and 2-10. No morphological alterations in the brain and nerves were observed in studies of PFOA, PFOS, PFBS, or PFBA. No alterations in neurological function tests were observed in studies of PFOA, PFHxS, PFHxA, PFBS, PFBA, or PFDoDA. Impaired learning and memory were observed in a study of PFOS and decreases in grip strength were observed in a study of PFUnA. Potential neurological effects were not examined in animals exposed to PFNA, PFHpA, or FOSA.

PFOA

Epidemiological Studies. Gallo et al. (2013) found a decreased risk of self-reported memory loss in older adult (>50 years of age) C8 participants with serum PFOA levels in the 2nd, 3rd, 4th, or 5th quintiles. When the participants were categorized by diabetic status, the risk of memory loss was higher among the diabetics than nondiabetics (p=0.014). In sensitivity analyses, the association between serum PFOA levels and memory impairment was compared within and across water districts. Within a water district, the association between serum PFOA and memory impairment was significant, but there was no association between the geometric mean concentration of PFOA in a district and memory impairment. A general population study conducted by Shrestha et al. (2017) of 55–74-year-old participants also found higher memory and learning scores (6% increase) and 16–18% decreases in perseverative errors and responses. In a third study, no association between serum PFOA and self-reported difficulty remembering or periods of confusion was found in NHANES participants aged 60–<85 years (Power et al. 2013).

Laboratory Animal Studies. Exposure of rats to 18,600 mg/m^3 APFO dusts for 1 hour induced excessive salivation. Intermittent, head-only exposure of male rats exposed to up to 84 mg/m^3 APFO dusts for 2 weeks did not reveal gross or microscopic alterations in the brain (Kennedy et al. 1986).

A small number of studies have examined the potential toxicity of perfluoroalkyls to the nervous system in animals, but comprehensive testing has not been conducted. No alterations in performance on a novel recognition test were observed in rats administered a single 50 mg/kg dose of PFOA (Kawabata et al. 2017). No overt signs of neurotoxicity or altered response to stimuli were observed in rats and mice administered up to 1,000 mg/kg PFOA via gavage and observed for 14 days (Sato et al. 2009). Exposure of rats to up to approximately 110 mg/kg/day PFOA via the diet for 90 days did not induce gross or microscopic alterations in the brain, spinal cord, or peripheral nerves (Griffith and Long 1980). Similar results were reported in rats fed a diet that provided approximately 15 mg/kg/day PFOA for 2 years (3M

1983; Butenhoff et al. 2012c). Rhesus monkeys exposed to doses of PFOA that caused lethality (≥30 mg/kg/day by gavage) showed signs of hypoactivity and prostration, but examination of the brain did not reveal treatment-related alterations (Griffith and Long 1980). Treatment of Cynomolgus monkeys with doses of up to 20 mg/kg/day PFOA administered via a capsule did not induce morphological alterations in the brain or sciatic nerve (Butenhoff et al. 2002).

Similarly, no gross or microscopic alterations were reported in the brain from rats dermally exposed to APFO in the Kennedy (1985) study.

PFOS

Epidemiological Studies. Three studies have examined the influence of serum PFOS levels on self-reported memory in older adults. Gallo et al. (2013) found an inverse association between serum PFOS levels and the risk of memory loss in C8 Health Study participants. No association for difficulty remembering or periods of confusion was found in the second study of NHANES participants (Power et al. 2013). A second general population study of older adults found associations between serum PFOS levels and 11% higher scores on tests of visual reproduction delayed recall and 8% higher scores on tests of visual and spatial function (Shrestha et al. 2017), but found no associations on tests of executive function, reaction time, affective state, or motor function.

Laboratory Animal Studies. No histological alterations were observed in the brain, spinal cord, and/or sciatic nerve of rats administered a single gavage dose of up to 500 mg/kg PFOS (Sato et al. 2009), rats treated with up to 1.6–1.8 mg/kg/day PFOS for 4 or 14 weeks (Seacat et al. 2003), rats exposed to 8.5 mg/kg/day PFOS in the diet for 13 weeks (Kawamoto et al. 2011), rats exposed to 1.04 mg/kg/day PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b), or Cynomolgus monkeys dosed with up to 0.75 mg/kg/day PFOS for 26 weeks (Seacat et al. 2002). However, ultrasonic stimulation resulted in bursts of locomotion immediately followed by tonic convulsions in mice administered 125 mg/kg PFOS and rats administered 250 mg/kg PFOS (Sato et al. 2009); the effect was observed 1–7 days postexposure and frequently resulted in death. Similarly, tonic convulsions following ultrasonic stimulation were observed in rats exposed to 8.5 mg/kg/day PFOS in the diet for 6 weeks (Kawamoto et al. 2011); this effect was not observed at ≤2.0 mg/kg/day. Impaired spatial learning and memory, assessed using the Morris water maze test, was observed in mice administered 2.15 or 10.75 mg/kg/day PFOS, but not 0.43 mg/kg/day, for 3 months (Long et al. 2013). Similarly, impaired performance on retention tasks, as assessed by the water maze test, was observed in mice administered 3 or 6 mg/kg/day

PFOS for 4 weeks (Fuentes et al. 2007c). Histopathological examination of the hypothalamus in male Sprague-Dawley rats administered PFOS via gavage for 28 days revealed degeneration of gonadotropic cells of the pituitary gland at ≥1.0 mg/kg/day and dense chromatin, condensed ribosomes, and loss of morphology in the hypothalamus at ≥3.0 mg/kg/day (López-Doval et al. 2014).

PFHxS

Epidemiological Studies. A decrease in the risk of self-reported memory loss was observed in older adult participants of the C8 Health Study who had serum PFHxS levels in the 5th quintile (Gallo et al. 2013). No association between serum PFHxS levels and self-reported difficulty remembering or periods of confusion was reported in a study of NHANES participants (Power et al. 2013).

Laboratory Animal Studies. In a reproductive study in rats dosed with PFHxS, a functional observational battery (FOB) and motor activity tests were conducted in males on exposure days 36 and 39 and in females on postpartum day 17 (Butenhoff et al. 2009a). The battery assessed autonomic functions, reactivity and sensitivity to stimuli, excitability, gait and sensorimotor coordination, limb grip strength, and abnormal clinical signs. No significant alterations were reported in males or females dosed with up to 10 mg/kg/day PFHxS.

PFNA

Epidemiological Studies. Self-reported memory loss was shown to be inversely associated with serum PFNA levels in a study of older C8 Health Study participants (Gallo et al. 2013). Another study of NHANES participants did not find an association with self-reported difficulty remembering or periods of confusion (Power et al. 2013).

PFUnA

Laboratory Animal Studies. In the only study located for PFUnA, a decrease in grip strength was observed in male and female rats administered 1.0 mg/kg/day PFUnA for 41–46 days and allowed to recover for 14 days (Takahashi et al. 2014). No other alterations in performance on FOB tests were found.

PFBS

Laboratory Animal Studies. A significant decrease in tail flick latency to a thermal stimulus was observed in all groups of male rats administered via gavage PFBS for 28 days. However, other tests of sensory reactivity to stimuli, grip strength, and motor activity were not affected (3M 2001), and the significance of this isolated finding is difficult to ascertain. Gross and microscopic examination of the brain, spinal cord, and sciatic nerve did not show any significant alterations. In a 90-day study, no significant alterations in motor activity or performance on functional observation tests were observed in rats at PFBS doses as high as 600 mg/kg/day (Lieder et al. 2009a).

PFBA

Laboratory Animal Studies. Administration of up to 184 mg/kg/day PFBA by gavage for 5 consecutive days to rats had no significant effect on the gross or microscopic morphology of the brain or spinal cord (3M 2007a). In a 28-day gavage study, male rats dosed with 150 mg/kg/day, but not 30 mg/kg/day, showed a delay in bilateral pupillary reflex at the end of the treatment period (Butenhoff et al. 2012a; van Otterdijk 2007a). Results from other tests, including hearing ability, static righting reflex, grip strength, and motor activity, were comparable between groups, and histological examinations of the brain (including the optic nerve), spinal cord, and sciatic nerve were unremarkable. In a 90-day study, pupillary reflex tests conducted in weeks 8 and 12 showed delayed dilation under dark conditions in rats dosed with 30 mg/kg/day (2/40 in controls versus 7/39 in high-dose rats; p=0.071 according to the Fisher Exact Test) (Butenhoff et al. 2012a; van Otterdijk 2007b). Since no abnormalities were recorded during a 3-week recovery period, and there were no histopathological alterations in the eyes, the effect was not considered biologically significant by the investigators. Tests for hearing ability, static righting reflex, grip strength, and motor activity showed no associations with treatment with PFBA. In addition, there were no significant gross or microscopic alterations in the brain, spinal cord, or sciatic nerve.

PFDoDA

Laboratory Animal Studies. Single-dose administration of 50 mg/kg resulted in impaired performance on a novel object recognition test, but did not result in alterations in other tests of memory, anxiety, or open field activity (Kawabata et al. 2017). A second study conducted functional observation tests in rats administered PFDoDA for 42 days (Kato et al. 2015). No alterations in sensorimotor reactivity, grip strength, or spontaneous motor activity were observed at 2.5 mg/kg/day. However, in rats allowed to

recover for 14 days, decreases in forelimb grip strength were observed in males and females at 2.5 mg/kg/day; a decrease in motor activity was also observed in females at 2.5 mg/kg/day but this was only observed during the first week of recovery (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Administration of up to 500 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009) or 200 mg/kg/day PFHxA for 104 weeks (Klaunig et al. 2015) had no effect on locomotion or performance in the FOB test.

2.16 REPRODUCTIVE

Overview. A number of epidemiological studies have evaluated the reproductive toxicity of perfluoroalkyls; summaries of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 12. These studies have evaluated the following categories of reproductive outcomes: alterations in reproductive hormone levels; effects on sperm; effects on menopause onset, menstrual cycle length, endometriosis, and breastfeeding duration; and effects on fertility. Overviews of the studies examining these specific endpoints are presented in Tables 2-18, 2-19, 2-20, and 2-21, respectively. In addition to these reproductive outcomes, several epidemiological studies have evaluated the influence of perfluoroalkyls on sexual maturation; these data are discussed in Section 2.17, Developmental. Although some studies examining reproductive hormone levels have found associations with PFOA, PFOS, PFHxS, PFNA, PFUnA, PFDoDA, or PFHxA levels, the findings are not consistent across studies or there are too few studies to interpret the results. Alterations in reproductive hormone levels have not been found in studies of FOSA. Some associations between serum perfluoroalkyls (PFOA, PFOS, PFHxS, PFNA, PFDA) levels and sperm parameters have been found; often, only one sperm parameter was altered and it is difficult to assess the adversity of this alteration. There is some suggestive evidence of an association between serum PFOA, PFOS, PFHxS, or PFNA levels and an increased risk of early menopause; however, this may be due to reverse causation since an earlier onset of menopause would result in a decrease in the removal of perfluoroalkyls in menstrual blood. Epidemiological studies provide mixed evidence of impaired fertility (increased risks of longer time to pregnancy and infertility); there is also some evidence for PFOA, PFOS, PFHxS PFNA, PFHpA, and PFBS but the results are not consistent across studies or were only based on a single study. The small number of studies evaluating fertility for PFDA, PFUnA, PFDoDA, and FOSA did not find associations. Reproductive outcomes have not been evaluated in epidemiological studies on PFBA.

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gilliland 1992 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	Bound testosterone	**Inverse association (p=0.05)***
		Free testosterone	**Inverse association (p=0.03)***
		Estradiol	**Association (p=0.03)***
		LH	NS (p=0.93)
		FSH	NS (p=0.91)
		Prolactin	**Association (p=0.0002)***
Olsen et al. 1998b Occupational (n=111 males in 1993 and 80 males in 1995)	0–80,000 ng/mL (PFOA range)	Prolactin	**Association (p=0.01 for trend)*, 1993** NS (p=0.58 for trend), 1995
		Estradiol	NS (p=0.66 and 0.56 for trend), 1993 and 1995
		17α-Hydroxy-progesterone	NS (p=0.21 and 0.18 for trend), 1993 and 1995
		Bound testosterone	NS (p=0.07 and 0.85 for trend), 1993 and 1995
		Free testosterone.	NS (p=0.15 or 0.82 for trend), 1993 and 1995
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	Estradiol	**Association (p=0.017)*, males**
		Testosterone	**Association (p=0.034)*, males**
Knox et al. 2011 Community (C8) (n=25,957 women)	11.3–19.8 ng/mL (2nd PFOA quintile)	Estradiol concentration	NS (p>0.05), menopausal and perimenopausal subgroups
Barrett et al. 2015 General population (n=178 women)	3.61 and 2.31 ng/mL (mean PFOA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	3.46 ng/mL (mean PFOA)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Raymer et al. 2012 General population (n=256 men)	10.4 ng/mL (mean PFOA)	Estradiol	NS (p=0.751)
		Prolactin	NS (p=0.349)
		FSH	NS (p=0.581)
		LH	**Correlation (p=0.011)***
		Free testosterone	**Correlation (p=0.015)***
		Total testosterone	NS (p=0.440)
Specht et al. 2012 General population (n=604 men)	1.3–4.8 (range of PFOA means of different sites)	SHBG	NS (p=0.39 for trend)
Tsai et al. 2015 General population (n=540 adolescents and young adults aged 12–30 years)	2.74 ng/mL (geometric mean PFOA)	SHBG	**Association (p<0.05)*, females 12–17 years old**
		FSH	NS (p>0.05)
		Testosterone	NS (p>0.05)
Vested et al. 2013 General population (n=169 males aged 19–21 years)	3.8 ng/mL (median maternal PFOA)	Testosterone	NS (p>0.05)
		Estradiol	NS (p>0.05)
		Inhibin B	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free antigen index	NS (p>0.05)
		LH	**Association (p=0.03)***
		FSH	**Association (p=0.01)***

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.5 and 0.5 ng/mL (median serum PFOA in boys and girls)	Testosterone	β -0.0549 (-0.1186–0.0088), boys β -0.1627 (-0.1627–0.0233), girls
		Estradiol	**β 0.0921 (0.0186–0.1656)*, boys** β 0.1015 (-0.0023–0.0033), girls
PFOS			
Olsen et al. 1998a Occupational (n=327)	1,480–2,440 ng/mL (range of PFOS means at different time periods)	DHEAS	NS (p=0.60)
		FSH	NS (p=0.91)
		17-HP	NS (p=0.99)
		LH	NS (p=0.69)
		Prolactin	NS (p=0.25)
		SHBG	NS (p=0.77)
		Free testosterone	NS (p=0.90)
		Bound testosterone	NS (p=035)
		Estradiol	NS (p=0.14), after removal of 1 outlier
Knox et al. 2011 Community (C8) (n=25,957 women)	11.9–17.0 and 17.1–22.4 ng/mL (2nd and 3rd PFOS quintiles)	Estradiol concentration Perimenopausal subgroup Menopausal subgroup	 **Inverse association (p=0.0001)*** **Inverse association (p=0.007)***
Barrett et al. 2015 General population (n=178 women)	16.44 and 14.18 ng/mL (mean PFOS in nulliparous and parous women)	Follicular estradiol	**Inverse association (β -0.013, 95% CI -0.023 to -0.001)*, whole cohort** **Inverse association (β -0.025, 95% CI -0.043 to -0.007)*, nulliparous subcohort**
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	8.46 ng/mL (mean PFOS)	Total testosterone	**Inverse association (p<0.05)***
		Free testosterone	**Inverse association (p<0.05)***
		Free androgen index	**Inverse association (p<0.05)***
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Raymer et al. 2012 General population (n=256 men)	37.4 ng/mL (mean PFOS)	Estradiol	NS (p>0.05)
		Prolactin	NS (p>0.05)
		FSH	NS (p>0.05)
		LH	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Total testosterone	NS (p>0.05)
Tsai et al. 2015 General population (n=540 male and female adolescents and young adults aged 12–30 years)	7.78 ng/mL (geometric mean PFOS)	SHBG	NS (p>0.05)
		FSH	**Inverse association (p<0.05)*, males 12–17 years old**
		Testosterone	**Inverse association (p<0.05)*, females 12–17 years old**
Vested et al. 2013 General population (n=169 males aged 19–21 years)	21.2 ng/mL (median maternal PFOS)	Testosterone	NS (p>0.05)
		Estradiol	NS (p>0.05)
		Inhibin B	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free antigen index	NS (p>0.05)
		LH	NS (p>0.05)
		FSH	NS (p>0.05)
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	29.9 and 28.8 ng/mL (median serum PFOS in boys and girls)	Testosterone	**β -0.0029 (-0.0055 to -0.0003)*, boys** β 0.0005 (-0.0018–0.0028), girls
		Estradiol	β 0.0024 (-0.0007–0.0055), boys β 0.0005 (-0.0023–0.0033), girls

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Barrett et al. 2015 General population (n=178 women)	1.22 and 1.65 ng/mL (mean PFHxS in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	0.81 ng/mL (mean PFHxS)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	1.4 and 1.2 ng/mL (median serum PFHxS in boys and girls)	Testosterone	β 0.0173 (-0.0211–0.0588), boys β -0.0182 (-0.0451–0.0087), girls
		Estradiol	**β 0.0462 (0.0020–0.0925)*, boys** β 0.0017 (-0.0154–0.0496), girls
PFNA			
Barrett et al. 2015 General population (n=178 women)	0.67 and 0.60 ng/mL (mean PFNA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	1.23 ng/mL (mean PFNA)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	**Association (p<0.05)***
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Tsai et al. 2015 General population (n=540 male and female adolescents and young adults aged 12–30 years)	1.10 ng/mL (geometric mean PFNA)	SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
		Testosterone	NS (p>0.05)
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.8 and 0.9 ng/mL (median serum PFNA in boys and girls)	Testosterone	**β -0.4233 (-0.6998 to -0.1467)*, boys** β -0.1018 (-0.2684–0.0648), girls
		Estradiol	β 0.3204 (-0.0115–0.6522), boys β 0.1252 (-0.0758–0.3263), girls
PFDA			
Barrett et al. 2015 General population (n=178 women)	0.25 and 0.24 ng/mL (mean PFDA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	0.38 ng/mL (mean PFDA)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.3 and 1.0 ng/mL (median serum PFDA in boys and girls)	Testosterone	**β -0.2565 (-0.4135 to -0.0994)*, boys** β -0.0626 (-0.1730–0.0477), girls
		Estradiol	β 0.0734 (-0.1189–0.2657), boys β 0.0131 (-0.1208–0.1469), girls
PFUnA			
Barrett et al. 2015 General population (n=178 women)	0.40 and 0.42 ng/mL (mean PFUnA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
Tsai et al. 2015 General population (n=540 males and females aged 12–30 years)	5.84 ng/mL (geometric mean PFUnA)	SHBG	NS (p>0.05)
		FSH	**Inverse association (p<0.05)*, females 12–17 years old**
		Testosterone	NS (p>0.05)
PFBS			
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.5 and 0.5 ng/mL (median serum PFBS in boys and girls)	Testosterone	β -0.0387 (-0.3261–0.2487), boys β 0.1326 (-0.3576–0.6229), girls
		Estradiol	β 0.0149 (-0.3216–0.3513), boys β 0.3129 (-0.2771–0.9028), girls
PFDoDA			
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	2.4 and 3.1 ng/mL (median serum PFDoDA in boys and girls)	Testosterone	β 0.0056 (-0.0056–0.0168), boys **β -0.0119 (-0.0227 to -0.0010)*, girls**
		Estradiol	β -0.0007 (-0.0139–0.0124), boys β 0.0106 (-0.0026–0.0218), girls
PFHxA			
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.2 and 0.2 ng/mL (median serum PFHxA in boys and girls)	Testosterone	**β -0.3095 (0.5942 to -0.0248)*, boys** β -0.1896 (-0.4387–0.0595), girls
		Estradiol	β 0.0600 (-0.2803–0.4003), boys β -0.1492 (-0.4515–0.1531), girls

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Barrett et al. 2015 General population (n=178 women)	0.25 and 0.23 ng/mL (mean FOSA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; DHEAS = dihydroepiandrosterone sulfate; FOSA = perfluorooctane sulfonamide; FSH = follicle stimulating hormone; LH = luteinizing hormone; NS = not significant; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; SHBG = sex hormone binding globulin

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	4.6 and 5.3 ng/mL (median PFOA in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility ↑ curvilinear velocity Other parameters	 **Association (p<0.05)*** NS (p>0.05)
		Sperm morphology ↑ percentage of sperm head acrosome area ↓ percentage sperm with coiled tails Other parameters	 **Association (p<0.05)*** **Association (p<0.05)*** NS (p<0.05)
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	3.46 ng/mL (mean PFOA)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)
Kvist et al. 2012 General population (n=588 men)	1.91–5.19 ng/mL (range of PFOA means)	Y-X chromosome ratio	NS (p>0.05)
Raymer et al. 2012 General population (n=256 men)	10.4 ng/mL (mean PFOA)	Semen volume	NS (p>0.05)
		Semen pH	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
Toft et al. 2012 General population (n=588 males)	3.8 ng/mL (median PFOA)	↑ percent motile sperm	**Association (p<0.05)***
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm morphology	NS (p>0.05)

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Vested et al. 2013 General population (n=169 males aged 19–21 years)	3.8 ng/mL (median maternal PFOA)	Sperm concentration	**Inverse association (p=0.01)***
		Total sperm count	**Inverse association (p=0.001)***
		Semen volume	NS (p>0.05)
		Percentage progressive spermatozoa	NS (p>0.05)
		Percentage morphologically normal spermatozoa	NS (p>0.05)
		Mean testicular volume	NS (p>0.05)
PFOS			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	19.15 and 21.6 ng/mL (median PFOS in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility ↑ distance travelled Other parameters	 **Association (p<0.05)*** NS (p>0.05)
		Sperm morphology	NS (p<0.05)
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	8.46 ng/mL (mean PFOS)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
Kvist et al. 2012 General population (n=588 men)	8.20–51.65 ng/mL (range of mean PFOS) 51.65 ng/mL (mean for Greenland subcohort)	Y-X chromosome ratio	**Association (p<0.05)*, whole cohort Inverse association (p=0.044 for trend)*, Greenland subcohort**
Raymer et al. 2012 General population (n=256 men)	37.4 ng/mL (mean PFOS)	Semen volume	NS (p>0.05)
		Semen pH	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm concentration	NS (p>0.05)

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Toft et al. 2012 General population (n=588 males)	18.4 ng/mL (median PFOS)	Percent motile sperm	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percent normal sperm	**Inverse association (p<0.05)***
Vested et al. 2013 General population (n=169 males aged 19–21 years)	21.2 ng/mL (median maternal PFOS)	Sperm concentration	NS (p>0.05)
		Total sperm count	NS (p>0.05)
		Semen volume	NS (p>0.05)
		Percentage progressive spermatozoa	NS (p>0.05)
		Percentage morphologically normal spermatozoa	NS (p>0.05)
		Mean testicular volume	NS (p>0.05)
PFHxS			
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	0.81 ng/mL (mean PFHxS)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)
Toft et al. 2012 General population (n=588 males)	1.1 ng/mL (median PFHxS)	Percent motile sperm	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percent normal sperm	**Inverse association (p<0.05)***

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	1.0 and 1.65 ng/mL (median PFNA in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm morphology	
		↑ percentage of normal sperm	**Association (p<0.05)***
		↓ percentage sperm with coiled tails	**Association (p<0.05)***
		Other parameters	NS (p<0.05)
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	1.23 ng/mL (mean PFNA)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)
Toft et al. 2012 General population (n=588 males)	1.2 ng/mL (median PFNA)	Percent motile sperm	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percent normal sperm	NS (p>0.05)
PFDA			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	0.3 and 0.5 ng/mL (median PFDA in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm morphology	
		↑ sperm head length	**Association (p<0.05)***
		↓ percentage sperm with coiled tails	**Association (p<0.05)***
		Other parameters	NS (p<0.05)

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	0.38 ng/mL (mean PFDA)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

NS = not significant; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Dhingra et al. 2016a Community (C8) (n=8,759; retrospective analysis)	>2,130 ng/mL·year (estimated cumulative PFOA exposure 5th quintile)	Menopause age	HR 1.11 (0.97–1.26, p=0.14), 5th quintile
		Early menopause risk	NS (p=0.45), 5-year lag NS (p=0.58), 10-year lag NS (p=0.57), 15-year lag NS (p=0.20), 20-year lag
Dhingra et al. 2016a Community (C8) (n=3,334, prospective analysis)	>4,670 ng/mL·year (estimated cumulative PFOA exposure 5th quintile) >80.8 ng/mL (measured 5th PFOA quintile)	Menopause age (estimated cumulative)	HR 1.10 (0.84–1.43, p=0.51), 5th quintile
		Menopause age (measured)	HR 1.12 (0.86–1.45, p=0.40), 5th quintile
Knox et al. 2011b Community (C8) (n=25,957)	11.3–19.8 ng/mL (2nd PFOA quintile)	Early menopause risk (menopausal subgroup)	**OR 1.5 (1.1–2.1)*, 2nd quintile**
		Early menopause risk (perimenopausal subgroup)	**OR 1.4 (1.1–1.8)*, 2nd quintile**
Buck Louis et al. 2012 General population (n=473)	2.65 and 2.15 ng/mL (geometric mean PFOA in women with or without endometriosis)	Endometriosis	**OR 1.89 (1.17–3.06)*, without parity adjustment** OR 1.62 (0.99–2.66), with parity adjustment
		Risk of moderate to severe endometriosis	**OR 2.58 (1.18–5.64)*, without parity adjustment** OR 1.86 (0.81–4.24) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	2.70–3.99 and 4.00–20.60 ng/mL (3rd and 4th quartile serum PFOA)	Self-reported endometriosis	**OR 5.45 (1.19–25.04)*, 3rd quartile** OR 1.33 (0.82–2.17), 4th quartile

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2010 General population (n=1,347 pregnant women)	3.91–5.20 ng/mL (2nd quartile for maternal PFOA)	Breastfeeding duration ≤3 months	**OR 1.98 (1.17–3.24)*, 2nd quartile**
		Breastfeeding duration ≤6 months	**OR 1.88 (1.31–2.72)*, 2nd quartile**
Lum et al. 2017 General population (n=501 women)	3.1, 3.5, and 3.1 ng/mL (median serum PFOA in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 0.98 (0.96–1.00)
Lyngsø et al. 2014 General population (n=1,623 pregnant women)	1.5 ng/mL (median PFOA)	Irregular menstrual cycle	OR 1.4 (0.9–2.2)
		Long menstrual cycle	**OR 1.7 (1.1–2.6)***
		Short menstrual cycle	OR 0.7 (0.3–1.5)
Romano et al. 2016 General population (n=336 women)	5.5–7.6 ng/mL (maternal 3rd quartile PFOA)	Breastfeeding duration ≤3 months	**RR 1.63 (1.16–2.28)*, 3rd quartile**
		Breastfeeding duration ≤6 months	**RR 1.38 (1.06–1.79)*, 3rd quartile**
Taylor et al. 2014 General population (n=2,151 women)	>2.5–4.4 and >4.4 ng/mL (2nd and 3rd PFOA tertiles)	Menopause	**HR 1.36 (1.05–1.75)*, 3rd tertile**
		Hysterectomy	**HR 1.83 (1.31–2.56)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	2.40 ng/mL (median maternal PFOA)	Breastfeeding duration (in months)	**β -1.3 (-1.9 to -0.7)*, per doubling of serum PFOA levels**
		Exclusive breastfeeding (in months)	**β -0.5 (-0.7 to -0.3)*, per doubling of serum PFOA levels**
Vagi et al. 2014 General population (n=52 cases and 50 controls)	4.1 and 2.3 ng/mL (geometric mean PFOA for cases and controls)	Polycystic ovary syndrome risk	**OR 6.93 (1.79–29.92, p=0.003)*, 3rd tertile**

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Knox et al. 2011b Community (C8) (n=25,957)	11.9–17.0 and 17.1–22.4 ng/mL (2nd and 3rd PFOS quintiles)	Early menopause risk (menopausal subgroup)	**OR 1.5 (1.1–2.1)*, 2nd quintile**
		Early menopause risk (perimenopausal subgroup)	**OR 1.1 (1.1–1.8)*, 3rd quintile**
Buck Louis et al. 2012 General population (n=473)	7.20 and 6.11 ng/mL (geometric mean PFOS in women with or without endometriosis)	Endometriosis	OR 1.39 (0.98–1.98), without parity adjustment OR 1.25 (0.87–1.80), with parity adjustment
		Risk of moderate to severe endometriosis	**OR 1.86 (1.05–3.30)*, without parity adjustment** OR 1.50 (0.82–2.74) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	18.20–392.00 ng/mL (4th quartile PFOS)	Self-reported endometriosis	OR 3.48 (1.00–12.00), 4th quartile
Fei et al. 2010 General population (n=1,347 pregnant women)	3.91–5.20 ng/mL (2nd quartile for maternal PFOA)	Breastfeeding duration ≤3 months	**OR 1.89 (1.19–3.01)*, 4th quartile**
		Breastfeeding duration ≤6 months	**OR 1.56 (1.10–2.22)*, 2nd quartile**
Lum et al. 2017 General population (n=501 women)	12.3, 12.6, and 11.5 ng/mL (median serum PFOS in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 1.01 (0.98–1.03), 3rd tertile
Lyngsø et al. 2014 General population (n=1,623 pregnant women)	8.0 ng/mL (median PFOS)	Irregular menstrual cycle	OR 1.0 0.6–1.6)
		Long menstrual cycle	OR 0.7 (0.4–1.2)
		Short menstrual cycle	OR 0.7 (0.3–1.5)

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Romano et al. 2016 General population (n=336 women)	13.9 ng/mL (maternal median PFOS)	Breastfeeding duration ≤3 months	NS (p=0.065 for trend)
		Breastfeeding duration ≤6 months	NS (p=0.111 for trend)
Taylor et al. 2014 General population (n=2,151 women)	>9–18.4 and >18.4 ng/mL (2nd and 3rd PFOS tertiles)	Menopause	HR 1.16 (0.91–1.48), 3rd tertile
		Hysterectomy	**HR 1.44 (1.12–1.85)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	19.47 ng/mL (median maternal PFOS)	Breastfeeding duration (in months)	**β -1.4 (-2.1 to -0.6)*, per doubling of serum PFOS levels**
		Exclusive breastfeeding (in months)	**β -0.3 (-0.6 to -0.1)*, per doubling of serum PFOS levels**
Vagi et al. 2014 General population (n=52 cases and 50 controls)	8.2 and 4.9 ng/mL (geometric mean PFOS for cases and controls)	Polycystic ovary syndrome risk	**OR 5.79 (1.58–24.12, p=0.005)*, 3rd tertile**
PFHxS			
Buck Louis et al. 2012 General population (n=473)	0.48 and 0.43 ng/mL (geometric mean PFHxS in women with or without endometriosis)	Endometriosis	OR 1.14 (0.58–2.24), without parity adjustment OR 0.85 (0.42–1.73), with parity adjustment
		Risk of moderate to severe endometriosis	OR 2.12 (0.85–5.27), without parity adjustment OR 1.24 (0.47–3.31) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	2.20–19.40 ng/mL (4th quartile PFHxS)	Self-reported endometriosis	OR 1.47 (0.40–1.41), 4th quartile
Romano et al. 2016 General population (n=336 women)	1.5 ng/mL (maternal median PFHxS)	Breastfeeding duration ≤3 months	NS (p=0.124 for trend)
		Breastfeeding duration ≤6 months	NS (p=0.087 for trend)

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Taylor et al. 2014 General population (n=2,151 women)	>0.9–1.8 and >1.8 ng/mL (2nd and 3rd PFHxS tertiles)	Menopause	**HR 1.42 (1.08–7.87)*, 2nd tertile**
		Hysterectomy	**HR 2.22 (1.66–2.98)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	1.45 ng/mL (median maternal PFHxS)	Breastfeeding duration (in months)	β -0.2, (-0.5–0.2), per doubling of serum PFHxS levels
		Exclusive breastfeeding (in months)	β -0.1 (-0.2–0.2), per doubling of serum PFHxS levels
Vagi et al. 2014 General population (n=52 cases and 50 controls)	1.1 and 0.7 ng/mL (geometric mean PFHxS for cases and controls)	Polycystic ovary syndrome risk	OR 1.20 (0.35–4.07), 3rd tertile
PFNA			
Buck Louis et al. 2012 General population (n=473)	0.69 and 0.58 ng/mL (geometric mean PFNA in women with or without endometriosis)	Endometriosis	**OR 2.20 (1.02–4.75)*, without parity adjustment** OR 1.99, 0.91–4.33), with parity adjustment
		Risk of moderate to severe endometriosis	OR 1.21 (0.35–4.19), without parity adjustment OR 0.99 (0.27–3.65) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	1.20–15.40 ng/mL (4th quartile PFNA)	Self-reported endometriosis	OR 3.24 (0.81–12.91), 4th quartile
Lum et al. 2017 General population (n=501 women)	1.3, 1.2, and 1.1 ng/mL (median serum PFNA in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 1.01 (0.99–1.04), 3rd tertile
Romano et al. 2016 General population (n=336 women)	0.9 ng/mL (maternal median PFNA)	Breastfeeding duration ≤3 months	NS (p=0.591 for trend)
		Breastfeeding duration ≤6 months	NS (p=0.349 for trend)

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Taylor et al. 2014 General population (n=2,151 women)	>0.80–1.5 and >1.5 ng/mL (2nd and 3rd PFNA tertiles)	Menopause	**HR 1.47 (1.14–1.90)*, 3rd tertile**
		Hysterectomy	**HR 1.39 (1.08–1.80)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	0.62 ng/mL (median maternal PFNA)	Breastfeeding duration (in months)	**β -1.3, (-2.0 to -0.7)*, per doubling of serum PFNA levels**
		Exclusive breastfeeding (in months)	**β -0.2 (-0.5 to -0.0)*, per doubling of serum PFNA levels**
Vagi et al. 2014 General population (n=52 cases and 50 controls)	1.2 and 0.9 ng/mL (geometric mean PFNA for cases and controls)	Polycystic ovary syndrome risk	OR 2.25 (0.67–8.00), 3rd tertile
PFDA			
Buck Louis et al. 2012 General population (n=473)	0.20 and 0.18 ng/mL (geometric mean PFDA in women with or without endometriosis)	Endometriosis	OR 2.95 (0.72–12.1), without parity adjustment OR 2.60 (0.62–10.9), with parity adjustment
		Risk of moderate to severe endometriosis	OR 0.72 (0.06–8.09), without parity adjustment OR 0.58 (0.04–7.42) with parity adjustment
Lum et al. 2017 General population (n=501 women)	0.4, 0.4, and 0.4 ng/mL (median serum PFDA in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 1.01 (0.99–1.04), 3rd tertile

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Timmermann et al. 2017 General population (n=1,130 woman)	0.28 ng/mL (median maternal PFDA)	Breastfeeding duration (in months)	**β -0.8 (-1.4 to -0.3) *, per doubling of serum PFDA levels**
		Exclusive breastfeeding (in months)	β -0.2 (-0.4–0.0), per doubling of serum PFDA levels

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; OR = odds ratio; NS = not significant; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; RR = risk ratio

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Bach et al. 2015a General population (n=1,372 pregnant women)	2.0 ng/ml (median maternal PFOA)	Fecundability	FR 1.00 (0.99–1.01), per 0.1 ng/mL
		Infertility risk	OR 1.00 (0.98–1.01), per 0.1 ng/mL
Bach et al. 2015c, 2015d General population (n=440 pregnant women)	5.6–7.7 ng/mL (4th PFOA quartile)	Fecundability Parous subgroup Nulliparous subgroup	FR 0.86 (0.63–1.19), 4th quartile FR 0.74 (0.48–1.13), 4th quartile FR 0.99 (0.64–1.54), 4th quartile
		Infertility Parous subgroup Nulliparous subgroup	OR 1.67 (0.70–4.00), 4th quartile OR 1.74 (0.46–6.55), 4th quartile OR 1.56 (0.55–4.42), 4th quartile
Bach et al. 2015c, 2015d (re-analysis of Fei et al. 2009, 2012 data) General population (n=1,161 pregnant women)	4.1–5.4 ng/mL (2nd PFOA quartile) 7.2–41.5 ng/mL (4th PFOA quartile)	Fecundability Parous subgroup Nulliparous subgroup	**FR 0.78 (0.65–0.94)*, 2nd quartile** **FR 0.76 (0.59–0.96)*, 2nd quartile** **FR 0.74 (0.56–0.98)*, 4th quartile**
		Infertility Parous subgroup Nulliparous subgroup	**OR 1.91 (1.16–3.13)*, 2nd quartile** **OR 2.30 (1.09–4.87)*, 2nd quartile** OR 1.48 (0.80–2.75), 4th quartile
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 3.112 and 5.016 ng/mL or withdrawing from study or not pregnant 3.101 and 4.749 ng/mL (geometric mean serum PFOA in females and males)	Fecundability Female serum PFOA Male serum PFOA	 OR 0.95 (0.82–1.11) OR 1.01 (0.88–1.17)
Crawford et al. 2017 General population (n=99 30–44-year-old women)	2.79 ng/mL (geometric mean serum PFOA)	Fecundability	FR 1.15 (0.66–2.01)
Fei et al. 2009 General population (n=1,240 pregnant women)	3.91–5.20 ng/mL (2nd PFOA quartile, maternal)	Fecundability	**FOR 0.72 (0.57–0.90)*, 2nd quartile**
		Infertility	**OR 2.06 (1.22–3.51)*, 2nd quartile**

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2012 (re-analysis of Fei et al. 2009 data) General population (n=1,240 pregnant women)	3.91–5.20 and ≥6.97 ng/mL (2nd and 4th PFOA quartiles, maternal)	Fecundability Parous subgroup Nulliparous subgroup	 **FOR 0.61 (0.46–0.80)*, 2nd quartile** FOR 0.63 (0.39–1.04), 4th quartile
		Infertility Parous subgroup Nulliparous subgroup	 **OR 3.39 (1.75–6.53)*, 2nd quartile** OR 1.30 (0.52–3.21; p=0.082 for trend), 4th quartile
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	1.65 ng/mL (median PFOA)	Fecundability Primiparous subgroup	FR1.04 (0.87–1.25) **FR 1.31 (1.03–1.68)***
		Infertility	OR 1.11 (0.74–1.66)
Lum et al. 2017 General population (501 couples)	≥4.20 ng/mL (3rd tertile serum PFOA)	Probability of pregnancy	OR 0.7 (0.5–1.1)
Vélez et al. 2015 General population (n=1,743 pregnant women)	1.7 ng/mL (median maternal PFOA)	Fecundability	**FOR 0.89 (0.83–0.94, p<0.001)***
		Infertility	**OR 1.31 (1.11–1.53, p=0.001)***
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	5.58 and 5.61 ng/mL (median PFOA in women with no pregnancy and pregnant)	Fecundability	OR 1.18 (0.78–1.78)
		Not becoming pregnant within first six cycles	OR 1.21 (0.67–2.18)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>19.6–72.1 ng/mL (3rd tertile serum PFOA)	Endometriosis-related infertility	OR 1.05 (0.58–1.91)
Whitworth et al. 2012b General population (n=416 subfecund pregnant women and 474 controls)	1.66–2.24, 2.25–3.02, and ≥3.02 ng/mL (2nd, 3rd, and 4th PFOA quartiles)	Infertility Parous subgroup Primiparous subgroup	**OR 1.6 (1.1–2.3)*, 2nd quartile** **OR 2.4 (1.4–4.1)*, 3rd quartile** OR 0.5 (0.2–1.2), 4th quartile
Whitworth et al. 2016 General population (n=451 primiparous pregnant women)	2.8 ng/mL (maternal median serum PFOA)	Fecundability	OR 1.0 (0.90–1.2)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Bach et al. 2015a General population (n=1,372 pregnant women)	8.3 ng/ml (median maternal PFOS) 10.85–36.10 ng/mL (4th PFOS quartile)	Fecundability	FR 1.09 (0.92–1.30), 4th quartile
		Infertility risk	OR 0.71 (0.47–1.07), 4th quartile
Bach et al. 2015c, 2015d General population (n=440 pregnant women)	36.3–103.8 ng/mL (4th PFOS quartile)	Fecundability Parous subgroup Nulliparous subgroup	FR 0.96 (0.75–1.24), 4th quartile FR 1.04 (0.70–1.55), 4th quartile FR 0.97 (0.62–1.51), 4th quartile
		Infertility Parous subgroup Nulliparous subgroup	OR 1.03 (0.54–2.00), 4th quartile OR 0.70 (0.16–3.11), 4th quartile OR 1.23 (0.452–3.39), 4th quartile
Bach et al. 2015c, 2015d (re-analysis of Fei et al. 2009, 2012 data) General population (n=1,161)	27.0–34.2, 34.3–43.8, and 43.9–106.7 ng/mL (2nd, 3rd, and 4th PFOS quartiles)	Fecundability Parous subgroup Nulliparous subgroup	**FR 0.79 (0.66–0.95)*, 2nd quartile** FR 0.90 (0.70–1.14), 4th quartile **FR 0.68 (0.52–0.91)*, 3rd quartile**
		Infertility Parous subgroup Nulliparous subgroup	**OR 1.65 (1.01–2.68)*, 2nd quartile** OR 1.60 (0.78–3.28), 4th quartile **OR 2.71 (1.38–5.30)*, 3rd quartile**
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 11.764 and 20.867 ng/mL or withdrawing from study or not pregnant 11.088 and 19.765 ng/mL (geometric mean serum PFOS in females and males)	Fecundability Female serum PFOS Male serum PFOS	 OR 0.99 (0.85–1.17) OR 0.96 (0.80–1.15)
Crawford et al. 2017 General population (n=99 30–44-year-old women)	9.29 ng/mL (geometric mean serum PFOS)	Fecundability	FR 0.89 (0.49–1.60)
Fei et al. 2009 General population (n=1,240 pregnant women)	26.1–33.3 ng/mL (2nd PFOS quartile, maternal)	Fecundability	**OR 0.70 (0.56–0.87)*, 2nd quartile**
		Infertility	**OR 1.70 (1.01–2.86)*, 2nd quartile**

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2012 (re-analysis of Fei et al. 2009 data) General population (n=1,240 pregnant women)	26.1–33.3 and 33.4–43.2 ng/mL (2nd and 3rd PFOS quartiles, maternal)	Fecundability Parous subgroup Nulliparous subgroup	 NS (p=0.32 for trend) **FOR 0.63 (0.43–0.91)*, 3rd quartile**
		Infertility Parous subgroup Nulliparous subgroup	 NS (p=0.26 for trend) **OR 2.50 (1.16–5.37, p=0.36 for trend)*, 3rd quartile**
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	10.60 ng/mL (median PFOS)	Fecundability	FR 0.90 (0.76–1.07)
		Infertility	OR 1.39 (0.93–2.07)
Lum et al. 2017 General population (501 couples)	≥15.20 ng/mL (3rd tertile serum PFOS)	Probability of pregnancy	OR 0.9 (0.6–1.3)
Vélez et al. 2015 General population (n=1,743 pregnant women)	4.7 ng/mL (median maternal PFOS)	Fecundability	FOR 0.96 (0.91–1.02, p=0.17)
		Infertility	OR 1.14 (0.98–1.34, p=0.09)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	35.75 and 36.29 ng/mL (median PFOS in women with no pregnancy and pregnant)	Fecundability	NS (p=0.29)
		Not becoming pregnant within first six cycles	OR 0.98 (0.54–1.77)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>9.36–138 ng/mL (3rd tertile serum PFOS)	Endometriosis-related infertility	OR 0.66 (0.36–1.21)
Whitworth et al. 2012b General population (n=416 subfecund women and 474 controls)	10.34–16.60 and ≥16.61 ng/mL (3rd and 4th PFOS quartile)	Infertility Parous subgroup Primiparous subgroup	**OR 1.4 (1.0–2.0)*, 3rd quartile** **OR 2.1 (1.2–3.8)*, 4th quartile** OR 0.7 (0.4–1.3), 4th quartile
Whitworth et al. 2016 General population (n=451 primiparous pregnant women)	14.6 ng/mL (maternal median serum PFOS)	Fecundability	OR 1.00 (0.88–1.1)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.5 ng/ml (median maternal PFHxS)	Fecundability	FR 1.00 (0.99–1.01), per 0.1 ng/mL
		Infertility risk	OR 0.98 (0.93–1.03), per 0.1 ng/mL
Crawford et al. 2017 General population (n=99 30–44-year-old women)	1.59 ng/mL (geometric mean serum PFHxS)	Fecundability	0.84 (0.46–1.54)
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	1.94 ng/mL (median PFHxS)	Fecundability	FR 0.97 (0.85–1.11)
		Infertility	OR 0.99 (0.73–1.33)
Vélez et al. 2015 General population (n=1,743 pregnant women)	1 ng/mL (median maternal PFHxS)	Fecundability	**FOR 0.91 (0.86–0.97, p=0.002)***
		Infertility	**OR 1.27 (1.09–1.48, p=0.003)***
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	1.12 and 1.22 ng/mL (median PFHxS in women with no pregnancy and pregnant)	Fecundability	**OR 1.33 (1.01–1.75)***
		Not becoming pregnant within first six cycles	OR 0.67 (0.37–1.20)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.39–1.69 ng/mL (3rd tertile serum PFHxS)	Endometriosis-related infertility	**OR 0.47 (0.26–0.87)***
Whitworth et al. 2016 General population (n=450 primiparous pregnant women)	7.0 ng/mL (maternal median serum PFHxS)	Fecundability	OR 0.97 (0.90–1.1)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.8 ng/mL (median maternal)	Fecundability	FR 1.00 (0.98–1.02), per 0.1 ng/mL
		Infertility risk	OR 0.99 (0.95–1.03), per 0.1 ng/mL
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 1.176 and 1.558 ng/mL or withdrawing from study or not pregnant 1.112 and 1.422 ng/mL (geometric mean serum PFNA in females and males)	Fecundability Female serum PFNA Male serum PFNA	 OR 1.00 (0.84–1.19) OR 1.09 (0.90–1.32)
Crawford et al. 2017 General population (n=99 30–44-year-old women)	0.84 ng/mL (geometric mean serum PFNA)	Fecundability	1.40 (0.79–2.49)
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	0.64 ng/mL (median PFNA)	Fecundability Primiparous subgroup	**FR 0.80 (0.69–0.94)*** FR 0.99 (0.88–1.22)
		Infertility	**OR 1.53 (1.08–2.15)***
Lum et al. 2017 General population (501 couples)	≥1.50 ng/mL (3rd tertile serum PFNA)	Probability of pregnancy	OR 0.8 (0.6–1.2)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.45 and 0.51 ng/mL (median PFNA in women with no pregnancy and pregnant)	Fecundability	OR 1.17 (0.88–1.54)
		Not becoming pregnant within first six cycles	OR 0.67 (0.37–1.25)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>1.50–7.10 ng/mL (3rd tertile serum PFNA)	Endometriosis-related infertility	**OR 0.52 (0.28–0.95)***

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Whitworth et al. 2016 General population (n=451 primiparous pregnant women)	0.43 ng/mL (maternal median serum PFNA)	Fecundability	OR 1.1 (0.92–1.3)
PFDA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.3 ng/mL (median maternal PFDA)	Fecundability	FR 1.00 (0.97–1.03), per 0.1 ng/mL
		Infertility risk	OR 0.99 (0.92–1.07), per 0.1 ng/mL
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 0.385 and 0.448 ng/mL or withdrawing from study or not pregnant 0.349 and 0.416 ng/mL (geometric mean serum PFDA in females and males)	Fecundability Female serum PFDA Male serum PFDA	 OR 1.11 (0.95–1.29) OR 1.08 (0.93–1.26)
Lum et al. 2017 General population (501 couples)	≥0.05 ng/mL (3rd tertile serum PFDA)	Probability of pregnancy	OR 0.9 (0.6–1.3)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.10 and 0.11 ng/mL (median PFDA in women with no pregnancy and pregnant)	Fecundability	OR 1.15 (0.89–1.49)
		Not becoming pregnant within first six cycles	OR 0.61 (0.33–1.12)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>1.79–11.2 ng/mL ng/mL (3rd tertile serum PFDA)	Endometriosis-related infertility	OR 0.74 (0.40–1.35)
Whitworth et al. 2016 General population (n=429 primiparous pregnant women)	0.11 ng/mL (maternal median serum PFDA)	Fecundability	OR 1.00 (0.85–1.2)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.3 ng/mL (median maternal PFUnA)	Fecundability	FR 1.01 (0.98–1.03), per 0.1 ng/mL
		Infertility risk	OR 0.98 (0.92–1.04), per 0.1 ng/mL
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>1.42–5.34 ng/mL (3rd tertile serum PFUnA)	Endometriosis-related infertility	OR 0.61 (0.33–1.13)
Whitworth et al. 2016 General population (n=447 primiparous pregnant women)	0.23 ng/mL (maternal median serum PFUnA)	Fecundability	OR 0.93 (0.78–1.1)
PFHpA			
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.11–0.66 ng/mL (3rd tertile serum PFHpA)	Endometriosis-related infertility	**OR 0.48 (0.26–0.86)***
PFBS			
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.086–0.094 ng/mL (2nd tertile serum PFBS)	Endometriosis-related infertility	**OR 3.74 (2.04—6.84)***
PFDoDA			
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.27–1.02 ng/mL (2nd tertile serum PFDoDA)	Endometriosis-related infertility	OR 0.61 (0.34–1.11)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Whitworth et al. 2016 General population (n=410 primiparous pregnant women)	0.04 ng/mL (maternal median serum PFDoDA)	Fecundability	OR 0.91 (0.77–1.1)
FOSA			
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 0.110 and 0.112 ng/mL or withdrawing from study or not pregnant 0.126 and 0.129 ng/mL (geometric mean serum FOSA in females and males)	Fecundability Female serum FOSA Male serum FOSA	 **OR 0.81 (0.70–0.94)*** OR 0.89 (0.78–1.02)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.10 and 0.11 ng/mL (median FOSA in women with no pregnancy and pregnant)	Fecundability	OR 1.01 (0.86–1.18)
		Not becoming pregnant within first six cycles	OR 0.81 (0.45–1.46)
Whitworth et al. 2016 General population (n=226 primiparous pregnant women)	0.03 ng/mL (maternal median serum FOSA)	Fecundability	OR 0.91 (0.71–1.2)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

FOR = fecundability odds ratio; FOSA = perfluorooctane sulfonamide; FR = fecundability ratio (probability of conceiving during a given menstrual cycle); OR = odds ratio; NS = not significant; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Studies in laboratory animals have evaluated the potential histological alterations in reproductive tissues, alterations in reproductive hormones, and impaired reproductive functions. Summaries of these studies are presented in Tables 2-1, 2-3, 2-4, 2-5, and 2-6 and in Figures 2-6, 2-8, 2-9, and 2-10. Multigeneration studies on PFOA, PFOS, and PFBS have not found alterations in reproductive parameters in animals; similarly, no effect on fertility was observed for PFHxS or PFDoDA. One study found alterations in sperm parameters and decreases in fertility in mice exposed to PFNA. An increase in the incidence of Leydig cell hyperplasia (reclassified as gonadal stromal hyperplasia) has been observed in animals exposed to PFOA; one study for PFDoDA reported ultrastructural alterations in the testes. Studies on PFOS, PFHxS, PFBS, and PFBA have not found histological alterations. Delays in mammary gland development have been observed in mice exposed to PFOA; this effect has also been observed in perinatally exposed mice (see Section 2.17, Developmental). No laboratory animal studies examined reproductive endpoints for PFDA, PFUnA, PFHpA, or FOSA.

PFOA

Epidemiological Studies—Reproductive Hormone Levels. Three studies have evaluated potential effects of PFOA exposure on reproductive hormone levels in workers (Gilliland 1992; Olsen et al. 1998b; Sakr et al. 2007b). Sakr et al. (2007b) found associations between serum PFOA and estradiol and testosterone levels in male workers at the Washington Works facility. Similarly, Gilliland (1992) found associations between serum fluorine levels and estradiol and prolactin levels and inverse associations with bound and free testosterone levels in workers at the 3M Cottage Grove facility. In contrast, Olsen et al. (1998b) did not find associations between serum PFOA and estradiol or testosterone in male workers at the 3M Cottage Grove facility. The study did find an association with prolactin levels, but this was only found in workers examined in 1993, but not in those examined in 1995. In a general population study of men aged 30–66 years of age, correlations were found between serum PFOA levels and free testosterone levels and LH levels; no correlations were found for estradiol, prolactin, follicle stimulating hormone (FSH), or total testosterone levels (Raymer et al. 2012). Another study of similar aged men did not find an association between serum PFOA and sex hormone binding globulin levels (Specht et al. 2012). Studies of young men (median age 19 years) (Joensen et al. 2013) or adolescents and young men (12–30 years of age) (Tsai et al. 2015) did not find associations between serum PFOA and reproductive hormone levels. A third study (Vested et al. 2013) found an association between LH and FSH levels and maternal serum PFOA levels in young adult males; other hormones were not affected. A fourth study in adolescents (aged 13–15 years) found an association between serum PFOA and estradiol levels in boys, but not in girls, and did not find associations for testosterone levels (Zhou et al. 2016).

Two studies of women (Barrett et al. 2015; Knox et al. 2011b) did not find associations with estradiol levels or luteal progesterone levels. A third study of adolescent and young women (Tsai et al. 2015) found an association between serum PFOA and sex hormone binding globulin levels in adolescents (12–17 years), but not in young adults; no associations with FSH or testosterone were observed in either group.

Epidemiological Studies—Effects on Sperm. Six general population studies have evaluated the potential alterations in sperm parameters associated with PFOA exposure. Although some associations have been found, the results are not consistent across studies. Buck Louis et al. (2015) reported an increase in curvilinear velocity and some alterations in sperm morphology that were associated with serum PFOA levels. Toft et al. (2012) found a PFOA-related increase in the percentage of motile sperm in men with serum PFOA levels in the 3rd tertile. Vested et al. (2013) reported inverse associations between maternal serum PFOA levels and sperm concentration and total sperm count in young adults; no alterations in motility or morphology were observed. Other studies did not find alterations in sperm viability, count, concentration, motility, or morphology (Buck Louis et al. 2015; Joensen et al. 2013; Raymer et al. 2012; Toft et al. 2012) or the Y-X chromosome ratio (Kvist et al. 2012).

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. Two studies examined possible associations between serum PFOA levels and alterations in menstrual cycle length. An increased risk of a long menstrual cycle (≥32 days) was observed in women with serum PFOA levels in the 3rd tertile and when serum PFOA was used as a continuous variable (Lyngsø et al. 2014). No alterations in the risk of having a short menstrual cycle (≤24 days) or irregular menstrual cycles (≥7 days difference between cycles) were observed. The second study did not find an association between serum PFOA and menstrual cycle length (Lum et al. 2017).

Four studies have evaluated the risk of early menopause. In a study of C8 Health Study participants, increases in the risk of early menopause was observed in perimenopausal (>42–≤51 years of age) and menopausal (>51–≤65 years of age) women with serum PFOA levels in the 2nd, 3rd, 4th, and 5th quintiles (Knox et al. 2011b). An increase in menopause risk was also observed in a cross-sectional study of NHANES participants with serum PFOA levels in the 3rd tertile (Taylor et al. 2014). Taylor et al. (2014) also found a higher risk of hysterectomy among women with serum PFOA levels in the 2nd and 3rd tertiles. Findings of higher levels of PFOA (and other perfluoroalkyls) among women with hysterectomies and that serum PFOA levels increased after menopause provide suggestive evidence that at least part of the

association may be due to reverse causation (Taylor et al. 2014). In contrast, no alterations in the risk of early menopause or age of menopause were associated with estimated cumulative serum PFOA levels in retrospective and prospective studies of C8 Health Study participants (Dhingra et al. 2016a); age of menopause was also not associated with measured serum PFOA levels in the prospective study (Dhingra et al. 2016a). Cross-sectional analysis also showed that early menopause was associated with measured serum PFOA levels, but not with modeled serum PFOA levels (Dhingra et al. 2017), providing support that reverse causation may contribute to the observed association.

Buck Louis et al. (2012) showed that the risk of endometriosis and the risk of moderate-to-severe endometriosis were associated with serum PFOA levels; however, adjustment for parity resulted in confidence intervals that included unity. A second study found an increased risk of self-reported endometriosis in women with serum PFOA levels in the 3rd quartile; for the 4th quartile women, the confidence intervals included unity (Campbell et al. 2016). A case-control study (Vagi et al. 2014) found an increased risk of polycystic ovary syndrome among women with serum PFOA levels in the 3rd tertile.

Two studies utilizing pharmacokinetic modeling have investigated whether the observed associations between PFOA exposure and early onset menopause or risk of endometriosis was due to reverse causation (Ngueta et al. 2017; Ruark et al. 2017). As discussed in Section 3.1.4, menstrual blood loss is a route of elimination of perfluoroalkyls. Therefore, variability in menstruation such as menarche, menopause, and pharmacological management of menstruation (e.g., use of oral contraceptives) could affect serum perfluoroalkyl levels, and thereby contribute to observed statistical associations between serum PFOA levels and early onset menopause (Ruark et al. 2017) or endometriosis (Ngueta et al. 2017) outcomes.

Three studies evaluated a possible association between maternal PFOA levels and breastfeeding duration. Two studies found increases in the risk of breastfeeding ≤3 or 6 months that were associated with maternal PFOA levels (Fei et al. 2010; Romano et al. 2016). Timmermann et al. (2017) found an inverse association between maternal PFOA levels and the duration of breastfeeding and the amount of time the women exclusively breastfed. Fei et al. (2010) reported that when the women were segregated by parity, the associations were only found in multiparous women. In contrast, Timmermann et al. (2017) found no differences in duration or breastfeeding exclusiveness between primiparous and multiparous women. It is noted that a number of factors can influence the duration of breastfeeding including diminished milk production, inadequate lactation support from health care providers after delivery, use of medication that is not compatible with breastfeeding, lack of spousal/family support, and individual choice. In general, these studies did not consider whether these factors may have influenced the observed associations.

Epidemiological Studies—Effects on Fertility. Several general population studies have examined the possible association between female serum PFOA levels and decreased fertility or infertility; the results are graphically presented in Figures 2-31 and 2-32, respectively. With the exception of the Buck Louis et al. (2013) and Vestergaard et al. (2012) prospective studies, all of the women were pregnant; thus, couples with unresolved infertility are underrepresented in these analyses. Maternal transfer of PFOA during pregnancy and lactation can result in lower serum PFOA levels in women (see Section 3.1.2 for additional information), as compared to nulliparous women; thus, parity should be considered when evaluating potential associations between serum PFOA and infertility. The Buck Louis et al. (2013) study is the only study that used maternal and paternal serum PFOA levels as the biomarkers of exposure. Most of the studies evaluated two aspects of fertility: fecundability, which is a measure of time to pregnancy, and risk of infertility, which is typically time to pregnancy of >12 months.

In a study of pregnant women participating in the Danish National Birth Cohort study, a decrease in fecundability and an increase in infertility were observed in women with serum PFOA (measured at gestation week 12) levels in the three highest quartiles (Fei et al. 2009). When the women were categorized by parity, decreased fecundability OR and increased infertility OR were only found in the parous group; the ORs for the nulliparous women included unity (Fei et al. 2009). A second re-analysis of these data (Bach et al. 2015a) using a different statistical approach confirmed the results of the whole group and the parous subgroup; this re-analysis also found a decrease in the fecundability risk among the nulliparous women. In another set of women participating in the Danish National Birth Cohort study (Bach et al. 2015c), no alterations in fecundability or infertility risk were observed in the whole cohort or when the women were categorized into parous and nulliparous subcohorts. It was noted that the median serum PFOA levels in this second study (4.0 ng/mL) were lower than the levels in the larger study (5.4 ng/mL). A decrease in fecundability and an increase in infertility risk were also observed in a Canadian study of pregnant women (Vélez et al. 2015). An increase in infertility risk was also found in a Norwegian study of subfecund pregnant women with serum PFOA levels in the three highest quartiles (Whitworth et al. 2012b); when the women were categorized based on parity, the infertility risk was only elevated in the parous women with serum PFOA levels in the 3rd and 4th quartiles. A multinational study also found an alteration in fecundability (Jørgensen et al. 2014a); however, this study found that higher serum PFOA levels resulted in a decrease in the time to pregnancy (fecundability ratio >1) among primiparous women.

Figure 2-31. Fecundability Relative to PFOA Levels (Presented as Adjusted Fecundability Ratios)

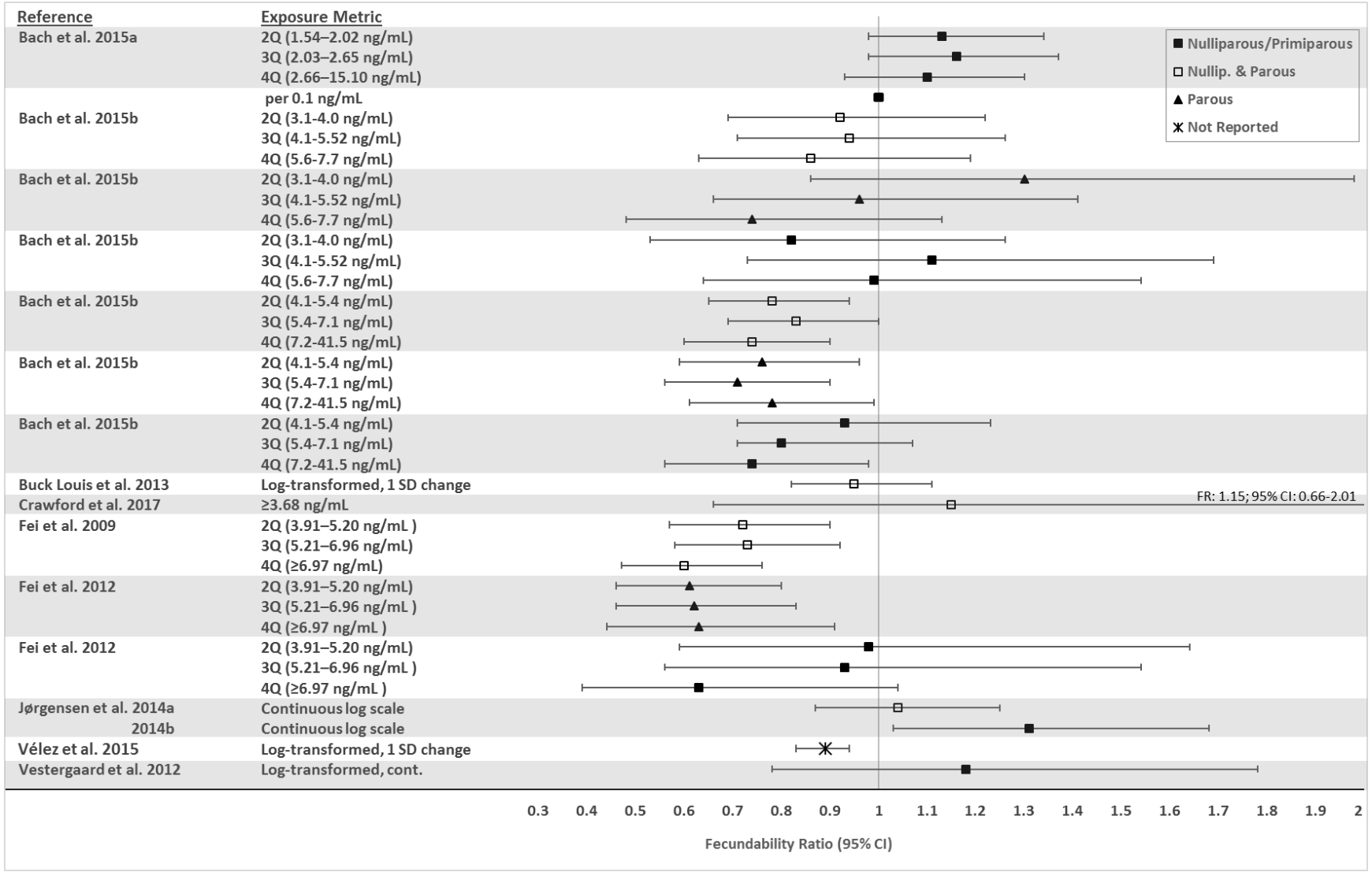
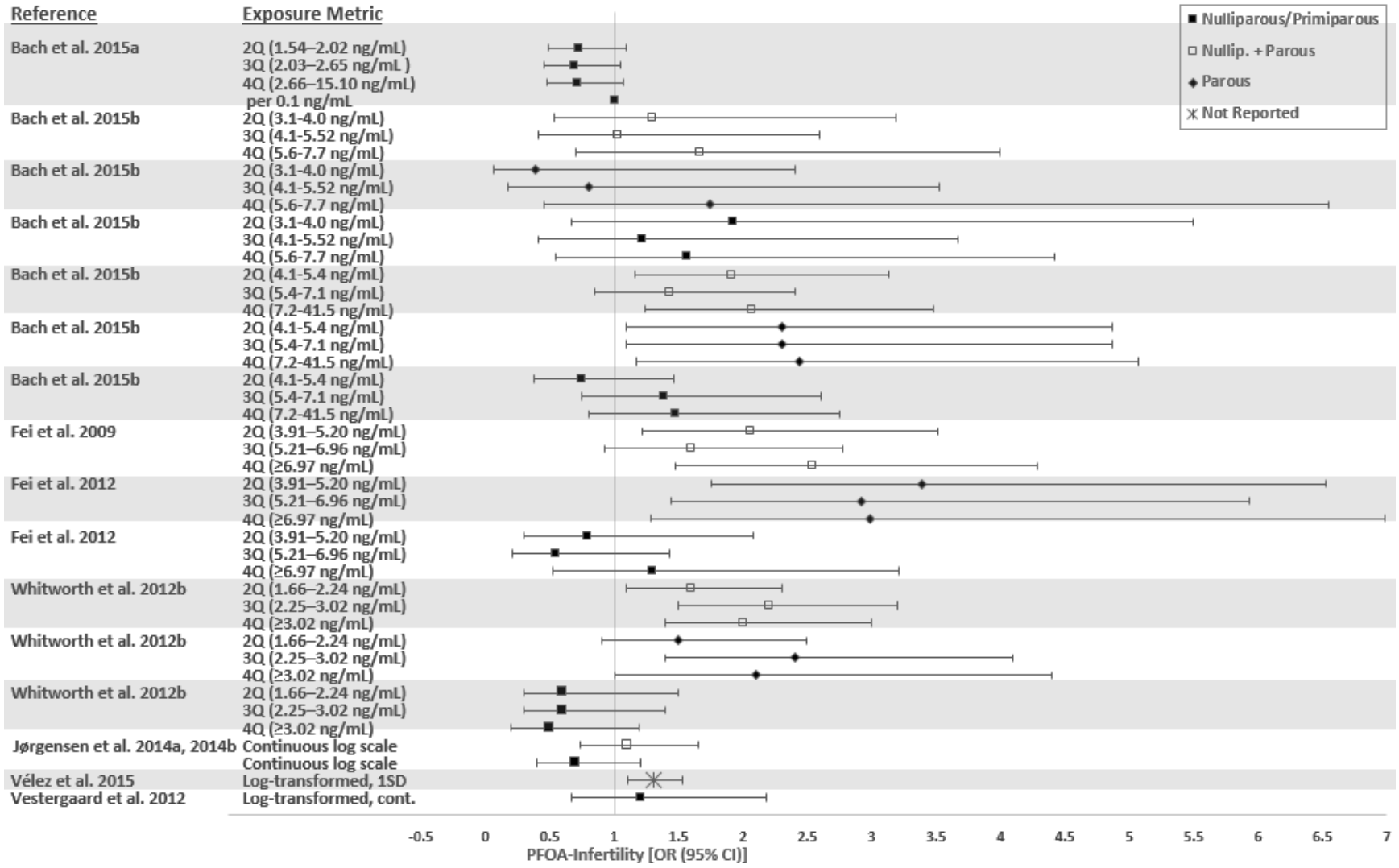


Figure 2-32. Infertility Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



Other studies of pregnant women have not found alterations in fecundability, fertility, and/or infertility (Bach et al. 2015a; Crawford et al. 2017; Jørgensen et al. 2014a; Lum et al. 2017; Wang et al. 2017; Whitworth et al. 2016). The two prospective studies which followed women intending to get pregnant for 6 months (Vestergaard et al. 2012) or 12 months (Buck Louis et al. 2013) also did not find associations between serum PFOA levels in women and fecundability; Buck Louis et al. (2013) also found no association when male serum PFOA was used as the biomarker of exposure.

Laboratory Animal Studies. Examination of the testes and epididymides of rats exposed intermittently head-only to up to 84 mg/m^3 APFO dusts for 2 weeks did not reveal any gross or microscopic treatment-related alterations (Kennedy et al. 1986).

Several studies have been conducted in rats to examine whether induction of Leydig cell tumors could be due to an endocrine-related mechanism. In a 14-day gavage study in which rats were dosed with up to 50 mg/kg/day PFOA, testes weight was not significantly affected and microscopic examination did not reveal any significant alterations (Cook et al. 1992). However, the weight of the accessory sex organ unit (ventral and dorsal lateral prostate, seminal vesicles, and coagulating glands) was significantly decreased in rats dosed with 25 mg/kg/day PFOA (17% decrease) and 50 mg/kg/day PFOA (18% decrease) relative to controls and to a pair-fed group. There was also a trend for reduced serum and interstitial fluid testosterone in PFOA-treated rats; serum LH was not altered and estradiol was significantly increased (63%) at ≥10 mg/kg/day. Challenge experiments conducted with human chorionic gonadotropin, gonadotropin-releasing hormone, or naloxone suggested that the decrease in serum testosterone was due to a lesion at the level of the testes. Serum levels of progesterone and 17α-hydroxyprogesterone were not altered by 50 mg/kg/day PFOA, but androstenedione levels were reduced 2-fold. The data suggested that the decrease in serum testosterone may be due to a decrease in the conversion of 17α-hydroxy-progesterone to androstenedione, and this could be attributed to the elevated serum levels of estradiol. The decrease in weight of the accessory sex organ unit could also be attributed to the elevated estradiol serum levels. In a subsequent study from the same group of investigators, rats dosed with 25 mg/kg/day PFOA for 14 days showed a significant increase in estradiol in serum and in testicular interstitial fluid relative to controls (Biegel et al. 1995). Treatment with PFOA for 14 days significantly increased aromatase activity in the liver (aromatase converts testosterone to estradiol), but not in testes, muscle, or adipose tissue, suggesting that PFOA increases serum estradiol by inducing aromatase activity in the liver. Treatment with PFOA also increased testicular interstitial fluid transforming growth factor α (TGFα). Collectively, the results were consistent with the hypothesis that increased estradiol levels ultimately produce Leydig cell hyperplasia and adenoma by acting as a mitogen or enhancing growth

factor secretion. A study of the dose-response relationship for PFOA and serum estradiol reported a significant increase in serum estradiol in rats dosed with ≥2 mg/kg/day, which was well correlated with total hepatic aromatase activity (Liu et al. 1996). Significant increases in serum estradiol were also reported during the first year of treatment of male rats with 13.6 mg/kg/day PFOA in a 2-year dietary study (Biegel et al. 2001).

Significant increases in the incidence of Leydig cell hyperplasia were observed in rats exposed to 13.6 mg/kg/day PFOA in the diet for 2 years (Biegel et al. 2001). Another 2-year study found an increased incidence of vascular mineralization in the testes of rats exposed to 15 mg/kg/day PFOA in the diet; no effects were observed at 1.5 mg/kg/day (3M 1983; Butenhoff et al. 2012c). In female rats, increases in the incidence of tubular hyperplasia of the ovaries were observed following a 2-year exposure to 1.5 mg/kg/day (3M 1983; Butenhoff et al. 2012c). A peer review of the histological slides from this study (3M 1983; Butenhoff et al. 2012c) concluded that the more current nomenclature for the tubular hyperplasia was gonadal stromal hyperplasia (Mann and Frame 2004). Additionally, the peer reviewers substantially disagreed with the incidence of lesions in the 1.5 mg/kg/day group and slightly disagreed with the incidence in the 15 mg/kg/day group. Based on the incidence reported by the peer reviewers, no statistically significant increases in the occurrence of gonadal stromal hyperplasia were observed in either group; a significant increase in grade 3 and above lesions were observed in the 15 mg/kg/day group.

In a 2-generation reproduction study in which male and female rats were dosed with up to 30 mg/kg/day PFOA by gavage in water for 70 days before mating and until sacrifice, there were no effects on estrous cycling, sperm number and quality, mating and fertility, or histopathology of the reproductive organs assessed in the parental and F1 generations (Butenhoff et al. 2004b). Intermediate-duration studies of rats and monkeys also did not find gross or microscopic alterations in the sex organs at termination; Cynomolgus monkeys were dosed with up to 20 mg/kg/day PFOA for 4 or 26 weeks (Butenhoff et al. 2002; Thomford 2001), Rhesus monkeys with up to 100 mg/kg/day PFOA for 13 weeks (Griffith and Long 1980), and rats with up to approximately 100–110 mg/kg/day PFOA for 13 weeks (Griffith and Long). Serum levels of estradiol and estriol were not significantly altered in the 4-week study conducted by Thomford (2001), but estrone was reduced in monkeys dosed with 2 and 20 mg/kg/day PFOA; no possible explanation was discussed. In the 26-week study (Butenhoff et al. 2002), no treatment-related alterations were reported in serum estrone, estriol, estradiol, or testosterone, indicating that the reduced serum estrone levels in the 4-week study was transitory. In 2-year dietary studies in rats, doses of 13.6 mg/kg/day PFOA significantly increased the incidence of Leydig cell hyperplasia (Biegel et al. 2001), whereas 15 mg/kg/day increased the incidence of vascular mineralization in the testes and

1.5 mg/kg/day increased the incidence of tubular hyperplasia in the ovaries (3M 1983; Butenhoff et al. 2012c).

A study in pregnant mice dosed with 5 mg/kg/day PFOA (only dose level tested) reported that the mammary gland showed changes suggesting substantial delay (possibly up to 10 days) in gland differentiation on PND 20 and alterations in milk protein gene expression on PND 20 (White et al. 2007). Subsequent studies by this group support the finding of delayed mammary gland differentiation. On PND 1, the mammary glands of mice administered 5 mg/kg/day on GDs 8–17 appeared immature; the morphology was similar to that seen in late pregnancy prior to parturition and the initiation of nursing (White et al. 2009). Another study found that the normal weaning-induced mammary gland involution was compromised on PND 22 in mice exposed to 1 mg/kg/day on GDs 1–17 or 0.001 mg/kg/day administered on GD 7–PND 22 (White et al. 2011); the investigators noted that the mammary gland structure was similar to mammary gland tissue at or near the peak of lactation (PND 10). Necrosis was observed in the placenta of mice administered via gavage 10 or 25 mg/kg/day PFOA on GDs 11–16 (Suh et al. 2011); no alterations were observed at 2 mg/kg/day.

A study of pregnant mice reported increases in serum estradiol levels, with no changes in progesterone levels, at 10 mg/kg/day when PFOA was administered on GDs 1–7 (Chen et al. 2017b); however, when PFOA was administered on GD 13, there were significant decreases in serum progesterone levels at 5 and 10 mg/kg/day with no changes in estradiol levels (Chen et al. 2017b). In peripubertal female mice, administration of 5 mg/kg PFOA 5 days/week for 4 weeks resulted in significant increases in serum progesterone levels during estrus and preestrus, but no changes in estradiol levels were observed (Zhao et al. 2010).

No gross or microscopic alterations were reported in the testes from rats dermally exposed to 2,000 mg/kg/day APFO (Kennedy 1985).

Summary. Epidemiological studies have examined a several types of reproductive endpoints. Due to inconsistent results, the available data are not suitable for determining whether there are associations between serum PFOA and reproductive hormones or effects on sperm. There is some suggestive evidence that increases in serum PFOA levels can result in earlier onset of menopause; however, this is based on the findings of two studies (a third study did not find an association) and may partially be due to reverse causation. Several general population studies found associations between serum PFOA and impaired fertility (increased time to pregnancy and/or infertility), while others have not found

associations. The available epidemiological data are considered inadequate for determining whether there is an association between serum PFOA and fertility. The database limitations include inconsistency across studies, small number of studies including measurements of male serum PFOA levels, findings in parous women but not nulliparous women, and the underrepresentation of couples not becoming pregnant. The results of a multi-generational study in rats do not suggest that the reproductive system is a sensitive target of PFOA toxicity. Additionally, histological alterations have not been observed in monkeys or rats following intermediate and/or chronic oral exposure.

PFOS

Epidemiological Studies—Reproductive Hormone Levels. In an occupational exposure study of workers at 3M Decatur and Antwerp facilities (Olsen et al. 1998a) and a general population study (Raymer et al. 2012), no associations between serum PFOS and reproductive hormones were found. Studies in adolescent and young adult males have found inverse associations between serum PFOS levels and total and free testosterone levels (Joensen et al. 2013), free androgen index (Joensen et al. 2013), and FSH levels (Tsai et al. 2015). Another study of young men did not find alterations in reproductive hormone levels (Vested et al. 2013).

In a study of females participating in the C8 Health Studies, serum PFOS levels were inversely associated with estradiol levels in both perimenopausal and menopausal women (Knox et al. 2011b). An inverse association with follicular estradiol levels was also observed in a general population study (Barrett et al. 2015); when segregated by parity, the inverse association was only found in nulliparous women. An inverse association between serum PFOS levels and testosterone levels was observed in adolescent females; no association was found in older females (Tsai et al. 2015). A general population study of adolescents (aged 13–15 years) found an inverse association between serum PFOS levels and testosterone levels in boys, but not in girls; the study also found no associations with estradiol levels in boys or girls (Zhou et al. 2016).

Epidemiological Studies—Effects on Sperm. The available general population data do not provide evidence that PFOS damages sperm. One study (Buck Louis et al. 2015) found an association for one measure of sperm motility (distance travelled) but not for other measures. Another study (Toft et al. 2012) found an inverse association between serum PFOS levels and percentage of normal sperm. Other studies have not found alterations in sperm viability, count, motility, volume, or morphology (Buck Louis et al. 2015; Joensen et al. 2013; Raymer et al. 2012; Toft et al. 2012; Vested et al. 2013). A multinational

study (Kvist et al. 2012) found a nonlinear association between serum PFOS and Y-X chromosome ratio; however, when categorized by country, the only significant trend was a negative trend in the Greenland cohort. It is noted that these are studies of individuals exposed to background levels of PFOS, involved a single measurement of PFOS, and are not adequate for establishing causality.

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. No alterations in the risk of irregular, short, or long menstrual cycle lengths associated with serum PFOS levels were observed in a study of pregnant women (Lyngsø et al. 2014). Similarly, no association between serum PFOS levels and menstrual cycle length was observed in another study (Lum et al. 2017). A study of C8 Health Study participants found increases in the risk of early menopause in perimenopausal and menopausal women with serum PFOS levels in the $\geq 3^{rd}$ and $\geq 2^{nd}$ quintiles, respectively (Knox et al. 2011b). In contrast, a study of NHANES participants did not find an association between serum PFOS and the risk of early menopause (Taylor et al. 2014). The risk of endometriosis was not associated with serum PFOS levels (Buck Louis et al. 2012; Campbell et al. 2016). However, there was a greater risk of having moderate to severe endometriosis; adjusting for parity decreased the risk and the CIs included unity. General population studies found increases in the risk of having a hysterectomy in women having serum PFOS levels in the 2^{nd} and 3^{rd} tertiles (Taylor et al. 2014) and the risk of having polycystic ovary syndrome in women with serum PFOS levels in the 3^{rd} tertile (Vagi et al. 2014). Most of these endpoints were only examined in one study and the evidence is inconclusive to determine whether there is an association between PFOS exposure and these female reproductive outcomes.

Utilizing pharmacokinetic modeling, Ruark et al. (2017) and Ngueta et al. (2017) have investigated whether the observed associations between PFOS exposure and early onset menopause or risk of endometriosis was due to reverse causation. Menstrual blood loss is a route of elimination of perfluoroalkyls (see Section 3.1.4) and variability in menstruation such as menarche, menopause, and pharmacological management of menstruation (e.g., use of oral contraceptives) could affect serum perfluoroalkyl levels, and thereby contribute to observed statistical associations between serum PFOS levels and early onset menopause (Ruark et al. 2017) or endometriosis (Ngueta et al. 2017) outcomes.

Maternal serum PFOS levels have been associated with increases in the risk of breastfeeding for ≤ 3 or 6 months (Fei et al. 2010; Romano et al. 2016) and inversely associated with the length of breastfeeding and the length of exclusive breastfeeding (Timmermann et al. 2017). When the women were segregated by parity, the associations were only found in multiparous women (Fei et al. 2010). In contrast,

Timmermann et al. (2017) found no significant alterations in breastfeeding length or exclusiveness between primiparous women and multiparous women. In general, these studies did not consider whether other factors such as the duration of breastfeeding including diminished milk production, inadequate lactation support from health care providers after delivery, use of medication that is not compatible with breastfeeding, lack of spousal/family support, and individual choice may have influenced the observed associations. Additionally, the associations between maternal PFOS and breastfeeding duration may be due to reverse causality since longer breastfeeding would likely result in lower maternal PFOS levels.

Epidemiological Studies—Effects on Fertility. Several general population studies have evaluated whether there is a possible association between serum PFOS and time-to-pregnancy (as measured using a fecundability ratio) or infertility; graphical presentations of potential associations between fecundability and infertility relative to serum PFOA levels are presented in Figures 2-33 and 2-34, respectively. A couple of studies have found associations, but most have not found associations. Fei et al. (2009) found decreases in fecundability and increases in infertility risk among pregnant women with serum PFOS levels in the top three quartiles. When the women were categorized by parity (Fei et al. 2012), the decrease in fecundability and increase in infertility risk were only observed in nulliparous women with serum PFOS levels in the 3rd and 4th quartiles; no alterations were observed among parous women. A re-analysis of these data (Bach et al. 2015c) resulted in similar associations between PFOS and fecundability and infertility. Whitworth et al. (2012b) also found an increased risk of infertility among subfecund women with serum PFOS levels in the 3rd quartile; categorizing by parity resulted in increases in only parous women with serum PFOS levels in the 4th quartile. In contrast, other studies have not found alterations in fecundability or fertility associated with maternal serum PFOS levels (Bach et al. 2015a, 2015c; Buck Louis et al. 2013; Crawford et al. 2017; Jørgensen et al. 2014a; Lum et al. 2017; Vélez et al. 2015; Vestergaard et al. 2012; Wang et al. 2016; Whitworth et al. 2016).

Laboratory Animal Studies. Significant decreases in serum testosterone levels and epididymal sperm count were observed in mice administered 10 mg/kg/day PFOS for 21 days (Wan et al. 2011), in rats administered 5 mg/kg/day for 21 days (Li et al. 2018), and in mice administered 10 mg/kg/day for 5 weeks (Qu et al. 2016). No alterations were observed in mice administered 5 mg/kg/day PFOS or in mice administered 5 or 10 mg/kg/day PFOS for 14 days (Wan et al. 2011). No alterations in reproductive performance (number of litters, gestation length, number of implantation sites, or potential resorptions) were observed in rats administered 1 mg/kg/day PFOS throughout gestation and lactation (Buttenoff et al. 2009b). Lee et al. (2015a) did find a decrease in placental weight and placental capacity (ratio of fetal weight to placental weight) in mice administered ≥0.5 mg/kg/day PFOS via gavage on GDs 11–16.

Figure 2-33. Fecundability Relative to PFOS Levels (Presented as Adjusted Fecundability Ratios)

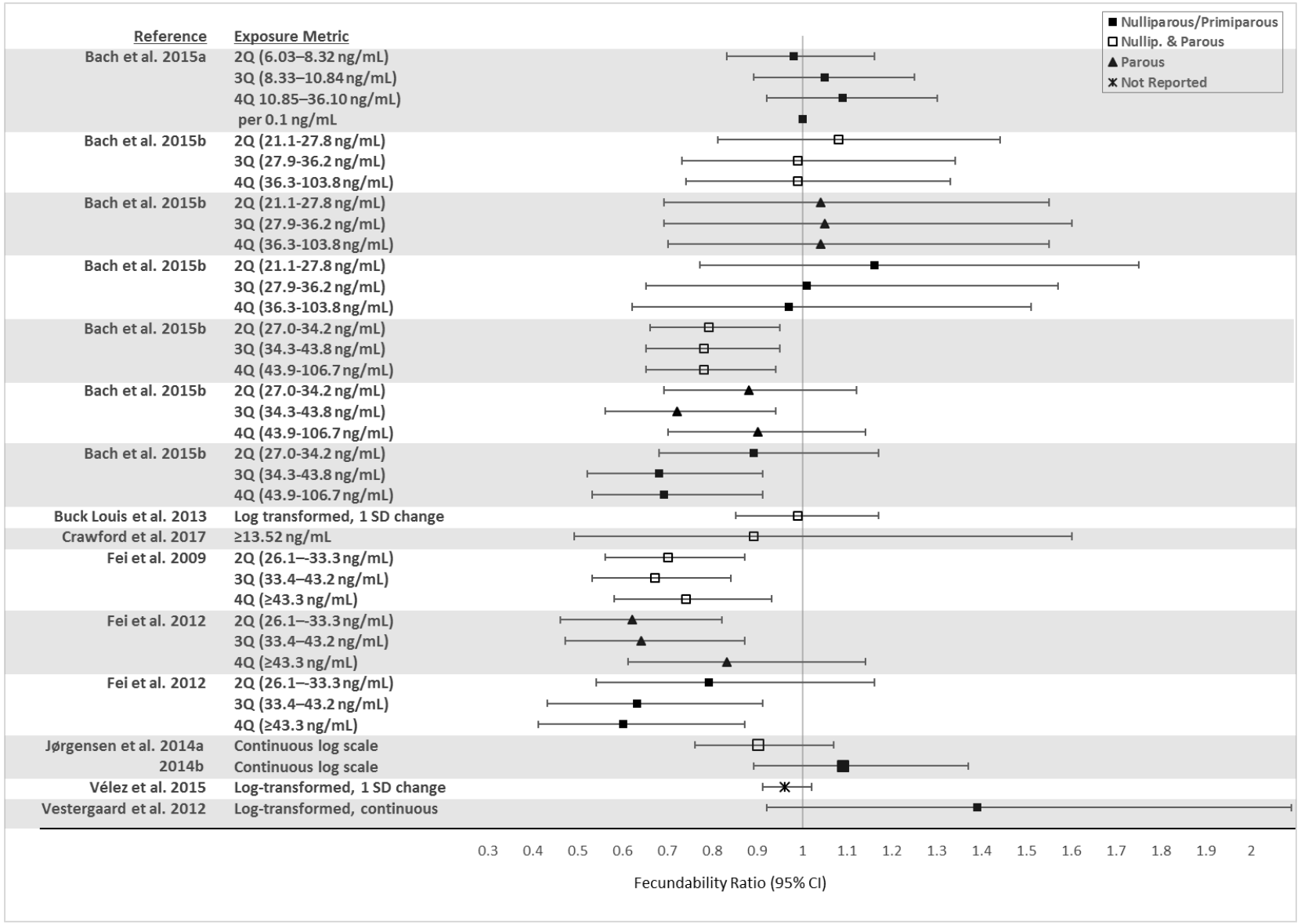
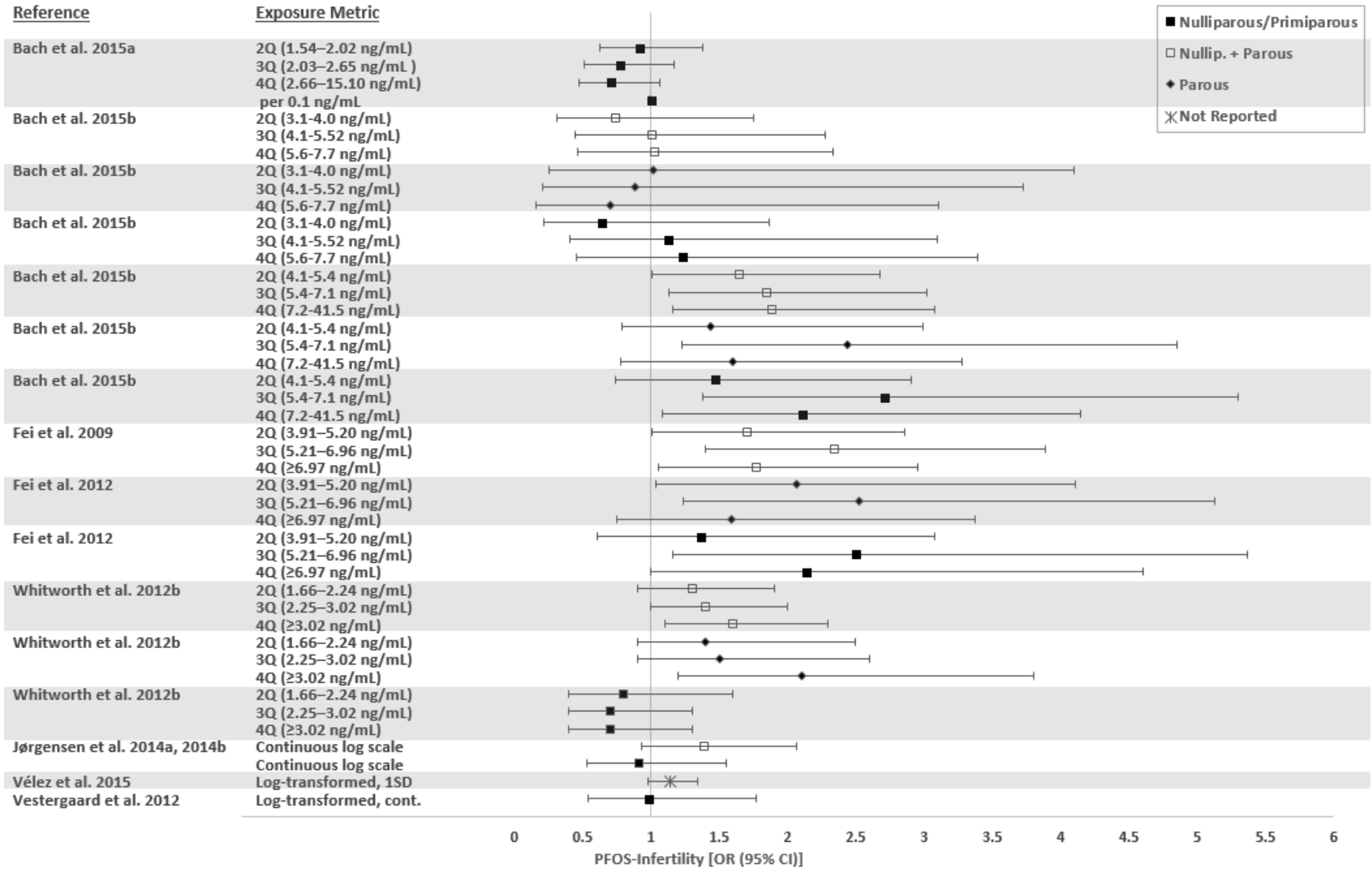


Figure 2-34. Infertility Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



Multigeneration studies with PFOS in rats did not provide indications of reproductive toxicity. Exposure of male and female rats to up to 3.2 mg/kg/day PFOS by gavage before mating and continuing during gestation did not affect mating or fertility parameters of the parental or F1 generation (Luebker et al. 2005a, 2005b). Dietary exposure of rats to 1.3–1.8 mg/kg/day PFOS for 4 or 14 weeks did not induce gross or microscopic alterations in the sex organs of males or females (Seacat et al. 2003). A similar study in Cynomolgus monkeys administered up to 0.75 mg/kg/day PFOS administered via a capsule also reported no significant morphological alterations in the sex organs, but serum estradiol was significantly decreased in males on days 62, 91, and 182 of the study (Seacat et al. 2002). In addition, treatment with PFOS had no significant effect on cell proliferation in the testes. Serum estradiol also was lower than in controls in one male and one female monkey dosed with 2 mg/kg/day PFOS for 4 weeks, but little can be concluded from results from just two animals (Thomford 2002a). In a 2-year dietary study in rats, administration of up to 1.04 mg/kg/day PFOS did not induce gross or microscopic alterations in the reproductive organs (Butenhoff et al. 2012b; Thomford 2002b). Overall, the reproductive system does not seem to be a sensitive target of PFOS toxicity, although some changes in testosterone and estradiol levels and decreases in sperm count have been observed.

PFHxS

Epidemiological Studies—Reproductive Hormone Levels. Three general population studies evaluated possible effects of PFHxS on reproductive hormone levels. In young men, no associations between serum PFHxS levels and testosterone, free androgen index, LH, estradiol, sex hormone binding globulin, or FSH levels were found (Joensen et al. 2013). Similarly, no alterations in follicular estrogen or luteal progesterone were observed in women (Barrett et al. 2015). An association between serum PFHxS levels and estradiol levels were observed in adolescent boys, but not in girls; no associations were observed for testosterone levels (Zhou et al. 2016).

Epidemiological Studies—Effects on Sperm. With the exception of the finding of an inverse association between serum PFHxS levels and percent normal sperm (Toft et al. 2012), general population studies have not found associations between PFHxS and sperm parameters (Joensen et al. 2013; Toft et al. 2012); it is noted that the Joensen et al. (2013) study of young men did not find alterations in sperm morphology.

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. Five general population studies have evaluated possible associations between serum PFHxS levels and female reproductive outcomes. Taylor et al. (2014) reported increases in the risk

of earlier menopause in women with serum PFHxS levels in the 3rd tertile and the risk of hysterectomy in women with serum PFHxS levels in the 2nd and 3rd tertiles. These findings may be due to reverse causation in that early menopause may result in higher serum PFHxS levels. Other studies did not find associations with the risk and severity of endometriosis (Buck Louis et al. 2012; Campbell et al. 2016) or polycystic ovary syndrome (Vagi et al. 2014).

Romano et al. (2016) did not find associations between maternal PFHxS levels and the risk of breastfeeding ≤3 or 6 months. Similarly, Timmermann et al. (2017) did not find associations between maternal PFHxS levels and the length of breastfeeding or length of exclusive breastfeeding.

Epidemiological Studies—Effects on Fertility. Seven studies have evaluated possible effects on fertility associated with female serum PFHxS levels. Vélez et al. (2015) found increases in time to pregnancy (measured as a decreased fecundability OR) and risk of infertility, which were associated with serum PFHxS levels in pregnant women. Vestergaard et al. (2012) reported an increase in the fecundability OR, indicating a shorter time to pregnancy, when risk was calculated using continuous serum PFHxS; however, when the subjects were divided into two groups based on serum PFHxS levels above and below the median level, the fecundability ratio included unity in the above-median group (fecundability ratio 1.29, 95% CI 0.90–1.83), as compared to the below-median group. Wang et al. (2017) found a decreased risk of endometriosis-related infertility in a case-control study. Studies by Bach et al. (2015a), Crawford et al. (2017), Jørgensen et al. (2014a), and Whitworth et al. (2016) did not find alterations in time to pregnancy, fertility, or the risk of infertility.

Laboratory Animal Studies. Exposure to 10 mg/kg/day PFHxS did not result in alterations in reproductive organ weights or histopathology in male rats exposed for a minimum of 42 days beginning 14 days prior to cohabitation and female rats sacrificed on lactation day 21 or GD 25 (rats that did not deliver a litter) (exposure began 14 days prior to cohabitation) (Butenhoff et al. 2009a). Fertility was not affected by treatment with PFHxS and there were no significant effects on sperm parameters. Also, estrous cycling was not affected by dosing with PFHxS. A similarly designed study in mice also reported no alterations in reproductive toxicity parameters (Chang et al. 2018).

PFNA

Epidemiological Studies—Reproductive Hormone Levels. Reproductive hormone alterations associated with serum PFNA levels are limited to a finding for estradiol in young men (Joensen et al. 2013); no

associations with other reproductive hormones were found in this study. In another study of adolescent and young adults, no associations between serum PFNA and sex hormone binding globulin, FSH, or testosterone were found in males or females (subjects were segregated by sex and age range) (Tsai et al. 2015). Zhou et al. (2016) found an inverse association between serum PFNA and testosterone levels in boys, but not in girls, and did not find associations for estradiol levels. Another study did not find alterations in follicular estradiol or luteal progesterone levels in women (Barrett et al. 2015).

Epidemiological Studies—Effects on Sperm. Buck Louis et al. (2015) found associations between serum PFNA and increases in the percentage of normal sperm and a decrease in the percentage of sperm with coiled tails. No associations were found for other sperm parameters (Buck Louis et al. 2015; Joensen et al. 2013; Toft et al. 2012).

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. No association between serum PFNA levels and menstrual cycle length was observed in a general population study (Lum et al. 2017). Increases in the risk of earlier menopause and hysterectomy were found in women with serum PFNA levels in the 3rd and ≥2nd serum PFNA tertiles (Taylor et al. 2014). The investigators examined the possibility that these effects may be due to reverse causation and found that serum PFNA levels increased post-menopause (Taylor et al. 2014). An increase in the risk of endometriosis was associated with serum PFNA levels in a general population study (Buck Louis et al. 2012); however, adjustment for parity resulted in OR CIs that included unity. A second study did not find an association between serum PFNA and self-reported endometriosis (Campbell et al. 2016). Vagi et al. (2014) did not find an increased risk of polycystic ovary syndrome that was associated with serum PFNA levels.

No associations between maternal PFNA levels and the risk of breastfeeding ≤3 or 6 months were found in a general population study (Romano et al. 2016). In contrast, Timmermann et al. (2017) found inverse associations between maternal PFNA levels and breastfeeding length and the length of exclusive breastfeeding. The study also found no differences in breastfeeding length or exclusiveness between primiparous and multiparous women.

Epidemiological Studies—Effects on Fertility. Jørgensen et al. (2014a) found increases in time to pregnancy (measured as a decrease in fecundability ratio) and an increase in infertility risk in a study of pregnant women. In sensitivity analysis, the fecundability ratio for primiparous women was 0.99 and the 95% CI range included unity (0.88–1.22). Wang et al. (2016) found an inverse association between

serum PFNA levels in women and the risk of endometriosis-induced infertility. Studies by Bach et al. (2015a), Buck Louis et al. (2013), Crawford et al. (2017), Lum et al. (2017), Vestergaard et al. (2012), and Whitworth et al. (2016) did not find associations between serum PFNA levels and fecundability ratio, fertility or risk of infertility.

Laboratory Animal Studies. Two acute-duration studies have evaluated the reproductive toxicity of PFNA in male rats (Feng et al. 2009, 2010). Gavage administration of 5 mg/kg/day for 14 days resulted in decreases in serum testosterone and increases in serum estradiol levels and atrophy of the seminiferous tubules (Feng et al. 2009). Electron microscopic examination of the testes revealed large vacuoles between the Sertoli cells and spermatogonia at 5 mg/kg/day; these changes as well as increases in serum Mullerian inhibiting substance and decreases in serum inhibin B cells were suggestive of damage to the secretory function of the Sertoli cells (Feng et al. 2010). In mice administered 0.5 mg/kg/day PFNA for 90 days, decreases in sperm motility, viability, and count and degenerative changes in the seminiferous tubules were observed (Singh and Singh 2018). When the mice were mated with unexposed females, significant decreases in litter size were observed at 0.5 mg/kg/day.

PFDA

Epidemiological Studies—Reproductive Hormone Levels. No associations were found between serum PFDA levels and testosterone, free androgen index, LH, estradiol, sex hormone binding globulin, or FSH levels in young men (Joensen et al. 2013). Similarly, no alterations in follicular estradiol or luteal progesterone levels were observed in women (Barrett et al. 2015). In adolescent boys, an inverse association between serum PFDA and testosterone was found; no association was found in girls (Zhou et al. 2016). This study also found no associations for estradiol levels in boys or girls.

Epidemiological Studies—Effects on Sperm. Two general population studies evaluated potential effects of PFDA exposure on sperm parameters. Buck Louis et al. (2015) found associations between serum PFDA levels and increases in sperm head length and decreases in the percentage of sperm with coiled tails. No alterations were found for sperm viability, count, volume, motility, or other morphological alterations (Buck Louis et al. 2015; Joensen et al. 2013).

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding. Three studies examined alterations in female reproductive outcomes associated with serum PFDA levels. In two studies, no associations between serum PFDA levels and the risk or severity

of endometriosis were found (Buck Louis et al. 2012; Lum et al. 2017). In the third study, an inverse association between maternal PFDA levels and duration of breastfeeding was found (Timmermann et al. 2017). No association was found for the length of exclusive breastfeeding.

Epidemiological Studies—Effects on Fertility. Six studies examined the potential for PFDA to alter fertility. No alterations in time to pregnancy (measured as fecundability ratio) or risk of infertility were observed in pregnant women (Bach et al. 2015a). Additionally, no associations with the probability of pregnancy (Lum et al. 2017), endometriosis-related infertility (Wang et al. 2017), or fecundability (Whitworth et al. 2016) were observed in other general population studies. Two prospective studies also found no association between female serum PFDA levels (Buck Louis et al. 2013; Vestergaard et al. 2012) or male serum PFDA levels (Buck Louis et al. 2013) and time to pregnancy.

PFUnA

Epidemiological Studies—Reproductive Hormone Levels. An inverse association between serum PFUnA levels and FSH levels was observed in adolescent girls (Tsai et al. 2015). The study did not find alterations in sex hormone binding globulins or testosterone levels in adolescent and young adult males or females. Another study of women did not find alterations in follicular estradiol or luteal progesterone levels (Barrett et al. 2015).

Epidemiological Studies—Effects on Fertility. Three studies evaluated possible associations between maternal serum PFUnA levels and fertility. No alterations in time to pregnancy (measured as a fecundability ratio) or infertility risk (Bach et al. 2015a), endometriosis-related infertility risk, or fecundability (Whitworth et al. (2016) were observed.

PFHpA

Epidemiological Studies. Only one study examined potential fertility associations. Wang et al. (2017) found a decreased risk of endometriosis-related infertility in a case-control study.

PFBS

***Epidemiological Studies*—**Two studies have evaluated potential association for reproductive outcomes. Zhou et al. (2016) did not find associations between serum PFBS and testosterone or estradiol levels in adolescent boys or girls. Wang et al. (2017) found an association between serum PFBS levels and endometriosis-related infertility in a case-control study.

Laboratory Animal Studies. Administration of up to 900 mg/kg/day PFBS to rats by gavage for 28 days did not cause any significant gross or microscopic alterations in primary or secondary sex organs from males or females (3M 2001). A 2-generation study in which rats were exposed to gavage doses of potassium PFBS as high as 1,000 mg/kg/day did not result in alterations in fertility, sperm parameters, estrus cycling, or histological alterations in reproductive tissues (Lieder et al. 2009b).

PFBA

Laboratory Animal Studies. No significant gross or microscopic alterations were reported in primary and secondary reproductive organs from rats dosed with PFBA by gavage in doses of up to 184 mg/kg/day for 5 days (3M 2007a), 150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or 30 mg/kg/day for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b).

PFDoDA

***Epidemiological Studies*—**A study in adolescent boys and girls found an inverse association between serum PFDoDA levels and testosterone levels in girls only; no associations were found for estradiol levels (Zhou et al. 2016). In the two studies evaluating fertility, no associations were found for endometriosis-related infertility (Wang et al. 2017) or fecundability (Whitworth et al. 2016).

Laboratory Animal Studies. Treatment of male rats with 1, 5, or 10 mg/kg/day PFDoDA by gavage for 14 days induced a dose-related decrease in testes weight, which achieved statistical significance at 10 mg/kg/day (Shi et al. 2007). Measurement of serum hormone levels showed a significant decrease in LH at 10 mg/kg/day and in testosterone at 5 and 10 mg/kg/day, no significant effect on FSH levels, and a significant decrease in serum estradiol only at 5 mg/kg/day. Alterations in the ultrastructure of the testes were seen in the 5 and 10 mg/kg/day groups and consisted of the presence of large clustered lipid droplets and enlarged mitochondria in Sertoli cells, large vacuoles, and expanded mitochondria in Leydig and

spermatogenic cells. Morphological features of apoptosis were seen in cells in the 10 mg/kg/day group. Assessment of messenger ribonucleic acid (mRNA) expression of genes involved in cholesterol transport and steroidogenesis provided evidence of altered cholesterol transport and steroid hormone synthesis, but no effects were noted for LH receptor and aromatase mRNA expression. Considering that serum total cholesterol was unaffected at 5 mg/kg/day and increased at 10 mg/kg/day and that aromatase expression was unaffected, the decrease in testosterone synthesis probably resulted from decreased steroidogenesis gene expression. In a longer-duration study (110 days) conducted by these investigators, decreased serum testosterone levels were observed at 0.2 and 0.5 mg/kg/day (Shi et al. 2009a). A third study (Kato et al. 2015) evaluated reproductive performance and found no alterations in estrous cycling during the first 14 days of exposure and no alterations in fertility, number of corpora lutea, or number of implantation sites in male and female rats administered 2.5 mg/kg/day PFDoDA for 14 days prior to mating and during gestation. In pregnant females administered 2.5 mg/kg/day, hemorrhages were observed at the implantation sites; only one female delivered live pups and 58% of the animals died or were sacrificed early. In females exposed for 42 days and not mated, continuous diestrus was observed at 2.5 mg/kg/day (Kato et al. 2015).

PFHxA

Epidemiological Studies—The only epidemiological study evaluating reproductive outcomes associated with PFHxA found an inverse association for testosterone levels in adolescent boys (Zhou et al. 2016) but did not find this association in girls and found no association with estradiol levels.

Laboratory Animal Studies. No alterations in mating, fertility, or gestation length were observed in rats administered TWA doses of 315 mg/kg/day PFHxA for 14 days prior to mating and during mating and gestation (Kirkpatrick 2005). Similarly, no alterations in mating, fertility, gestation length, number of implantation sites, estrous cycling, or sperm parameters were observed in rats administered up to 500 mg/kg/day NaPFHx for 70 days prior to mating, during the mating period, and throughout gestation and lactation (Loveless et al. 2009). A 90-day study did not find histological alterations in reproductive tissues of male or female rats administered up to 200 mg/kg/day NaPFHx (Chengelis et al. 2009b).

FOSA

Epidemiological Studies. One study examined reproductive hormone levels and did not find an association between serum FOSA and follicular estradiol or luteal progesterone levels in women (Barrett

et al. 2015). Two prospective epidemiological studies evaluated the possible association between FOSA and fertility. Vestergaard et al. (2012) did not find an increase in time to pregnancy, as measured as a fecundability ratio, or decrease in the likelihood of becoming pregnant within the first six menstrual cycles. In contrast, Buck Louis et al. (2013) found an increased time to pregnancy associated with serum FOSA levels in women, but not in men; the investigators noted that the results should be interpreted cautiously because only 10% of the blood samples had FOSA levels above the limit of detection. Another study found no association between maternal FOSA and fecundability (Whitworth et al. 2016).

2.17 DEVELOPMENTAL

Overview. A large number of epidemiological studies have examined the potential of developmental toxicity of perfluoroalkyls in the general population and in populations living in an area with high PFOA drinking water contamination. Epidemiological studies are available for 10 of the 12 perfluoroalkyls discussed in the profile; no developmental data were identified for PFHxA or PFBS. The discussion of these developmental outcomes is divided into four categories: pregnancy outcome, birth outcome, neurodevelopment, and sexual maturation. The epidemiological studies examining pregnancy outcome are summarized in Table 2-22; the pregnancy outcomes include miscarriage, stillbirth, preterm birth, and gestation age. Table 2-23 summarizes the epidemiological studies examining birth outcomes, which include birth weight, birth size, low birth weight, small for gestational age, birth defects, and sex ratio. Epidemiological studies examining neurodevelopmental endpoints, particularly risks for ADHD, are summarized in Table 2-24. Studies evaluating possible associations between serum perfluoroalkyl levels and development of the reproductive system are summarized in Table 2-25. Further details on these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 13. Studies examining childhood growth and examining the possible relationship between maternal serum perfluoroalkyl levels and body weight and BMI in children and adults are discussed in Section 2.3, Body Weight.

In general, the epidemiological studies did not find associations between perfluoroalkyl exposure and adverse pregnancy outcomes (miscarriage, preterm birth, or gestational age) for PFOA, PFOS, PFHxS, PFNA, PFDA, or PFUnA. Mixed results have been found for birth outcomes, particularly birth weight. Some epidemiological studies have found associations between maternal PFOA or PFOS exposure and decreases in birth weight, and meta-analyses of these data have found that increases in maternal PFOA or PFOS were associated with 11–19 g or 1–5 g decreases in birth weight, respectively; accounting for maternal glomerular filtration rates attenuated these results by about 50%. No consistent associations for

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Darrow et al. 2013 Community (C8) (n=1,330 women)	≥37.2 ng/mL (5th PFOA quintile)	Preterm birth	OR 1.01 (0.55–1.86)
Darrow et al. 2014 Community (C8) (n=1,129 women)	>39.4 ng/mL (5th PFOA quintile)	Miscarriage risk Parous subgroup Nulliparous subgroup	OR 1.00 (0.63–1.58), 5th quintile OR 1.06 (0.57–1.97), 5th quintile OR 0.81 (0.38–1.71), 5th quintile
Savitz et al. 2012a Community (C8) (11,737 singleton infants)	63.1–934.3 ng/mL (4th maternal PFOA quartile)	Miscarriage	OR 0.9 (0.7–1.0)
		Stillbirth	OR 1.0 (0.5–1.8)
Savitz et al. 2012b Community (13,243 cases stillbirth, preterm birth, low birth weight or small for gestational age)	21.0–717.6 ng/mL (5th maternal PFOA quintile)	Stillbirth	OR 0.8 (0.5–1.5)
		Preterm birth (<37 weeks)	OR 1.0 (0.9–1.2)
		Preterm birth (<32 weeks)	OR 1.0 (0.7–1.3)
Savitz et al. 2012b Community (4,547 infants)	83.3–921.3 ng/mL (5th maternal PFOA quintile)	Preterm birth (<37 weeks)	OR 1.2 (0.9–1.6)
		Preterm birth (<32 weeks)	OR 1.4 (0.5–3.6)
Stein et al. 2009 Community (C8) (n=1,845 pregnancies)	48.8 ng/mL (maternal mean PFOA)	Miscarriage	OR 0.9 (0.5–1.6), >90th percentile
		Preterm birth	OR 0.9 (0.6–1.5), >90th percentile
Apelberg et al. 2007b General population (n=341 singleton births)	1.6 ng/mL (cord serum median PFOA)	Gestational age	NS (p>0.05)
Buck Louis et al. 2016 General population (n=332 couples)	3.3 ng/mL (median serum PFOA in women)	Pregnancy loss	HR 0.93 (0.75–1.16)
Chen et al. 2012a General population (n=429 infants)	1.84 ng/mL (cord blood geometric mean PFOA)	Gestational age	NS (p>0.05)
		Preterm birth	OR 0.64 (0.40–1.02)
Hamm et al. 2010 General population (n=252 pregnant women)	>2.1–18 ng/mL (maternal 3rd PFOA tertile)	Preterm birth	RR 1.31 (0.38–4.45), 3rd tertile

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jensen et al. 2015 General population (n=56 cases and 336 controls)	1.58 ng/mL (maternal median PFOA)	Miscarriage before gestation week 12	OR 0.64 (0.36–1.18)
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	2.33 ng/mL (median maternal serum PFOA)	Gestational age	NS (p=0.318)
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	1.62 ng/mL (median maternal serum PFOA)	Gestational age	NS (p=0.431)
Li et al. 2017 General population (n=321 mother-infant pairs)	1.2 ng/mL (median cord serum PFOA)	Gestational age	β 0.16 (-0.02–0.33)
Lind et al. 2017a General population (n=649 pregnant women)	1.7 ng/mL (median maternal serum PFOA)	Gestational length	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	2.35 ng/mL (mean maternal serum PFOA)	Preterm	OR 0.90 (0.60–1.35)
		Gestational age	β-0.05 (-0.12–0.08)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	5.8 ng/mL (median maternal plasma PFOA)	Preterm	OR 1.0 (0.9–1.3)
		Gestation length	β -0.05 (-0.16–0.06)
Whitworth et al. 2012a General population (n=901 infants)	≥3.04 ng/mL (maternal 4th PFOA quartile)	Preterm birth	**OR 0.1 (0.03–0.6)*, 4th quartile**
Wu et al. 2012 General population (n=167 pregnant women at 2 hospitals)	18.32 and 9.76 ng/mL (mean maternal serum PFOA at each hospital)	Gestational age	**β -15.99 (-27.72 to -4.25, p<0.01)***

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Darrow et al. 2013 Community (C8) (n=1,330 women)	15.6 ng/mL (mean PFOS)	Preterm birth	OR 1.07 (0.58–1.95)
Darrow et al. 2014 Community (C8) (n=1,129 women)	>23.3 ng/mL (5th PFOS quintile)	Miscarriage risk Parous subgroup Nulliparous subgroup	OR 1.41 (0.88–2.26), 5th quintile OR 1.12 (0.58–2.17), 5th quintile OR 2.02 (0.83–4.93), 5th quintile
Stein et al. 2009 Community (C8) (n=5,262 infants)	23.2–83.4 ng/mL (>90th PFOS percentile)	Miscarriage	OR 0.9 (0.7–1.3), >90th percentile
		Preterm birth	**OR 1.4 (1.1–1.7)*, >90th percentile**
Buck Louis et al. 2016 General population (n=332 couples)	12.2 ng/mL (median serum PFOS in women)	Pregnancy loss	HR 0.81 (0.65–1.00)
Chen et al. 2012a General population (n=429 infants)	5.94 ng/mL (cord blood geometric mean PFOS)	Preterm birth	**OR 2.45 (1.47–4.08)***
Fei et al. 2007, 2008a General population (n=1,400 pregnant women)	35.3 ng/mL (maternal median PFOS)	Gestation length	NS (p>0.01)
		Preterm birth	OR 1.43 (0.50–4.11), 4th quartile
Hamm et al. 2010 General population (n=252 pregnant women)	>10–35 ng/mL (maternal 3rd tertile PFOS)	Preterm birth	RR 1.11 (0.36–3.38), 3rd tertile
Jensen et al. 2015 General population (n=56 cases and 336 controls)	8.10 ng/mL (maternal median PFOS)	Miscarriage before gestation week 12	OR 1.16 (0.59–1.29)
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	16.4 ng/mL (median maternal serum PFOS)	Gestational age	NS (p=0.201)
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	9.74 ng/mL (median maternal serum PFOS)	Gestational age	NS (p=0.952)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Li et al. 2017 General population (n=321 mother-infant pairs)	3.0 ng/mL (median cord serum PFOS)	Gestational age	β 0.11 (-0.06–0.29) **β 0.29 (0.05–0.53)*, boys only** β -0.07 (-0.35–0.20), girls only
Lind et al. 2017a General population (n=649 pregnant women)	8.1 ng/mL (median maternal serum PFOS)	Gestational length	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	6.05 ng/mL (mean maternal serum PFOS)	Preterm	OR 1.10 (0.70–1.74)
		Gestational age	β-0.06 (-0.19–0.06)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	25.7 ng/mL (median maternal plasma PFOS); 18.9–25.6 ng/mL (2nd quartile maternal PFOS)	Preterm	**OR 2.0 (1.1–3.7)*, 2nd quartile**
		Gestation length	β -0.08 (-0.17–0.02)
Whitworth et al. 2012a General population (n=901 infants)	13.0 and ≥16.59 ng/mL (maternal median and 4th quartile PFOS)	Preterm birth	**OR 0.3 (0.1–1.0, p=0.03)*, 4th quartile**
PFHxS			
Hamm et al. 2010 General population (n=252 pregnant women)	>1.4–43 ng/mL (maternal 3rd tertile PFHxS)	Preterm birth	**RR 0.31 (0.11–0.90)*, 3rd tertile**
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.298 ng/mL (maternal median PFHxS)	Miscarriage before gestation week 12	OR 1.53 (0.99–2.38)
Li et al. 2017 General population (n=321 mother-infant pairs)	3.9 ng/mL (median cord serum PFHxS)	Gestational age	β 0.12 (-0.03–0.27)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFHxS)	Gestational length	NS (p>0.05)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.58 ng/mL (mean maternal serum PFHxS)	Preterm	OR 0.85 (0.63–1.13)
		Gestational age	β -0.01 (-0.10–0.09)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	2.4 ng/mL (median maternal plasma PFHxS)	Preterm	OR 1.0 (0.9–1.1)
		Gestation length	β 0.02 (-0.04–0.07)
PFNA			
Buck Louis et al. 2016 General population (n=332 couples)	1.2 ng/mL (median serum PFNA in women)	Pregnancy loss	HR 0.86 (0.70–1.06)
Chen et al. 2012a General population (n=429 infants)	2.36 ng/mL (cord blood geometric mean PFNA)	Preterm birth	OR 0.88 (0.71–1.11)
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.72 ng/mL (maternal median PFNA)	Miscarriage before gestation week 12	**OR 16.46 (7.39–36.62)***
Li et al. 2017 General population (n=321 mother-infant pairs)	0.2 ng/mL (median cord serum PFNA)	Gestational age	β -0.02 (-0.19–0.10)
Lind et al. 2017a General population (n=649 pregnant women)	0.7 ng/mL (median maternal serum PFNA)	Gestational length	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.66 ng/mL (mean maternal serum PFNA)	Preterm	OR 0.87 (0.62–1.22)
		Gestational age	β -0.00 (-0.11–0.11)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	0.7 ng/mL (median maternal plasma PFNA)	Preterm	OR 1.2 (1.0–1.4)
		Gestation length	β-0.07 (-0.17–0.02)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Buck Louis et al. 2016 General population (n=332 couples)	1.2 ng/mL (median serum PFDA in women)	Pregnancy loss	HR 0.83 (0.66–1.04)
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.27 ng/mL (maternal median PFDA)	Miscarriage before gestation week 12	**OR 2.30 (1.18–4.47)***
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDA)	Gestational age	β 0.10 (-0.09–0.29)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFDA)	Gestational length	NS (p>0.05)
PFUnA			
Chen et al. 2012a General population (n=429 infants)	10.26 ng/mL (cord blood geometric mean PFUnA)	Preterm birth	OR 0.87 (0.64–1.16)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFUnA)	Gestational age	β 0.09 (-0.07–0.25)
PFHpA			
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFHpA)	Gestational age	β 0.14 (-0.17–0.45)
PFBA			
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFBA)	Gestational age	β 0.01 (-0.18–0.20)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDoDA			
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDoDA)	Gestational age	β 0.07 (-0.24 to 0.39)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

OR = odds ratio; NS = not significant; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR= risk ratio

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Darrow et al. 2013 Community (C8) (n=1,330 women)	≥37.2 ng/mL (5th PFOA quintile)	Birth weight	NS (p=0.70 for trend)
		Low birth weight	OR 0.92 (0.44–1.95)
Nolan et al. 2009 Community (n=1,555 singleton infants)	NR	Birth weight	NS (p>0.05)
		Low birth weight	**OR 0.37 (0.16–0.86)***
Nolan et al. 2010 Community (n=1,548 singleton infants)	NR	Congenital anomalies	OR 1.1 (0.34–3.3)
Savitz et al. 2012a Community (C8) (11,737 singleton infants)	63.1–934.3 ng/mL (4th maternal PFOA quartile)	Low birth weight	**OR 0.37 (0.16–0.86)***
		Birth defect	OR 1.0 (0.8–1.3)
Savitz et al. 2012b Community (13,243 cases stillbirth, preterm birth, low birth weight, or small for gestational age)	7.7 ng/mL (estimated maternal median PFOA)	Birth weight	β -14.80 (-42.28–13.68), per 100 ng/mL increase in PFOA
		Low birth weight	OR 1.0 (0.86–1.15), per 100 ng/mL increase in PFOA
		Small for gestational age	OR 0.86 (0.67–1.11), per 100 ng/mL increase in PFOA
Savitz et al. 2012b Community (4,547 infants)	13.4 ng/mL (estimated maternal median PFOA)	Low birth weight	OR 1.07 (0.96–1.18), per 100 ng/mL increase in PFOA
		Small for gestational age	**OR 1.08 (1.01–1.16)*, per 100 ng/mL increase in PFOA** OR 0.8 (0.6–1.2), for serum PFOA levels ≥80th percentile
		Birth weight	OR -12.76 (-26.08–0.57), per 100 ng/mL increase in PFOA
Stein et al. 2009 Community (C8) (n=1,845 pregnancies)	50.0–<120.6 and 120.6–894.4 ng/mL (3rd and 4th maternal PFOA quartile)	Low birth weight	OR 0.8 (0.3–1.9), 4th quartile
		Birth defects	OR 1.7 (0.8–3.6), 4th quartile

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2014c Community (C8) (n=10,262 infants)	61.3 ng/mL (estimated *in utero* mean PFOA)	Brain defects	OR 2.6 (1.3–5.1), interquartile range
		Gastrointestinal defects	OR 0.7 (0.3–1.4), interquartile range
		Kidney defects	OR 0.7 (0.3–1.8), interquartile range
		Craniofacial defects	OR 0.6 (0.3–1.3), interquartile range
		Eye defects	OR 1.1 (0.6–2.1), interquartile range
		Limb defects	OR 1.2 (0.7–2.0), interquartile range
		Genitourinary defects	OR 1.0 (0.6–1.7), interquartile range
		Heart defects	OR 1.2 (0.8–1.7), interquartile range
Alkhalawi et al. 2016 General population (n=156 mother-infant pairs)	2.43 ng/mL (geometric mean maternal PFOA)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Ponderal index	**β -0.412 (-0.788 to -0.037)***
Apelberg et al. 2007b General population (n=341 singleton births)	1.6 ng/mL (cord serum median PFOA)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	**Inverse association (p>0.05)***
		Ponderal index	**Inverse association (p>0.05)***
Ashley-Martin et al. 2016 General population (n=1,723 pregnant women)	1.70 and 0.39 ng/mL (maternal and cord median PFOA)	Gestational weight gain	NS (p>0.1), serum PFOA **OR 1.04 (1.02–1.06)*, cord PFOA**
Ashley-Martin et al. 2017 General population (n=1,705 mother-infant pairs)	1.7 ng/mL (median maternal plasma PFOA)	Birth weight	β -0.10 (-0.34–0.13)
		Infant leptin levels	β 0.01 (-0.15–0.13)
		Infant adiponectin levels	β 0.04 (-0.05–0.12)
Bach et al. 2016 General population (n=1,507 nulliparous women)	2.0 ng/mL (median PFOA)	Birth weight	NS, investigators noted no consistent alterations across PFOA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFOA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFOA quartiles

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bae et al. 2015 General population (n=233 couples)	5.01 and 4.05 ng/mL and 5.00 and 2.54 ng/mL (geometric mean PFOA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 0.93 (0.68–1.26), maternal PFOA OR 0.94 (0.72–1.23), paternal PFOA
Callan et al. 2016 General population (n=98 pregnant women)	0.86 ng/mL (median maternal serum PFOA)	Birth weight	β -48 g (-203–108)
		Birth length	β 0.06 (-0.70–0.81)
		Head circumference	β -0.40 (-0.96–0.16)
		Ponderal index	β-0.06 (-0.16–0.05)
Cao et al. 2018 General population (n=337 newborns)	1.59 ng/mL (mean cord serum PFOA); >1.59 ng/mL (3rd tertile cord PFOA)	Birth weight	NS (p=0.58)
		Birth length	**β -0.45 (-0.79 to -0.10)*, 3rd tertile**
		Ponderal index	NS (p=0.21)
Chen et al. 2012a General population (n=429 infants)	1.84 ng/mL (cord blood geometric mean PFOA)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Ponderal index	NS (p>0.05)
		Small for gestational age	OR 1.24 (0.75–2.05)
		Low birth weight	OR 0.53 (0.18–1.55)
Fei et al. 2007, 2008a General population (n=1,400 pregnant women)	5.6 ng/mL (maternal median PFOA)	Birth weight	**β -10.63 (-20.79 to -0.47)***
		Birth length	**β -0.069 (-0.113 to -0.024)***
		Abdominal circumference	**β -0.059 (-0.106 to -0.012)***
		Head circumference	β-0.030 (-0.064–0.004)
		Low birth weight	OR 2.44 (0.27–22.25), 4th quartile
		Small for gestational age	OR 0.97 (0.55–1.70), 4th quartile
Govarts et al. 2016 General population (n=202 infants)	1.52 ng/mL (cord blood geometric mean PFOA)	Birth weight	NS (p=0.473)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Hamm et al. 2010 General population (n=252 pregnant women)	>2.1–18 ng/mL (maternal 3rd tertile PFOA)	Birth weight	Change in weight 14.80 (-107.29–136.89), 3rd tertile
		Small for gestational age	RR 0.99 (0.25–3.92), 3rd tertile
Kim et al. 2011 General population (n=44 pregnant women)	1.46 ng/mL (maternal median PFOA)	Birth weight	NS (p>0.05)
		Cord TSH	**Association (p<0.05)***
		Cord T3	NS (p>0.05)
		Cord T4	NS (p>0.05)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	5.398 and 2.12 ng/mL (mean PFOA in cases and controls)	Thyroid stimulating immunoglobulin levels	**Inverse association (p<0.05)***
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Kobayashi et al. 2017 General population (n=177 mother-infant pairs)	1.6 ng/mL (mean maternal serum PFOA)	Birth weight	β -49.4 (-130.4–31.6)
		Birth length	β 0.01 (-0.37–0.40)
		Ponderal index	β -0.44 (-0.99–0.12)
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	2.33 ng/mL (median maternal serum PFOA)	Birth weight	β -359 (-596 to -122; p=0.003)*
		Birth length	**β -1.3 (-2.3 to -0.3, p=0.010)***
		Head circumference	NS (p=0.115)
		Small for gestational age	**OR 5.25 (1.68–16.4)*** **OR 6.55 (1.14–37.45)*, boys only** OR 4.73 (0.79–28.3), girls only
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	1.62 ng/mL (median maternal serum PFOA)	Birth weight	NS (p=0.590)
		Birth length	NS (p=0.656)
		Head circumference	NS (p=0.354)
		Small for gestational age	OR 0.66 (0.33–1.33)
Lee et al. 2013 General population (n=59 pregnant women)	2.73 ng/mL (maternal mean PFOA)	Birth weight	OR 0.54 (0.17–3.03)
		Birth length	OR 0.44 (0.12–1.58)
		Ponderal index	OR 0.56 (0.16–2.01)
		Head circumference	OR 0.82 (0.24–13.65)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lee et al. 2016 General population (n=85 infants)	1.11 ng/mL (cord blood mean PFOA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort	1.84, 0.96, and 2.51 ng/mL (maternal median PFOA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	**β -63.77 (-122.83 to -4.71, p=0.035)*, 2 SD increase in ln-transformed PFOA**
Li et al. 2017 General population (n=321 mother-infant pairs)	1.2 ng/mL (median cord serum PFOA)	Birth weight	**β -112.7 (-171.9 to -53.5)***
Lind et al. 2017a General population (n=649 pregnant women)	1.7 ng/mL (median maternal serum PFOA)	Birth weight	NS (p>0.05)
Maisonet et al. 2012 General population (n=447 girls)	3.7 ng/mL (maternal median PFOA)	Birth weight	**Inverse association (p=0.0120 for trend)***
		Birth length	NS (p=0.0978)
		Ponderal index	NS (p=0.5920)
		Body weight at 20 months	NS (p=0.4147)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	2.35 ng/mL (mean maternal serum PFOA)	Birth weight	β -9.33 (-38.81–20.16)
		Birth length	β -0.01 (-0.15–0.14)
		Head circumference	β 0.07 (-0.17–0.03)
		Small for gestational age	OR 0.92 (0.72–1.19)
		Low birth weight	OR 0.90 (0.63–1.29)
		Low birth weight at term	OR 0.85 (0.53–1.34)
Minatoya et al. 2017 General population (n=168 mother-infant pairs)	1.4 ng/mL (median maternal serum PFOA)	Birth weight	**β -197 (-391 to -3, p=0.047)***
		Ponderal index	β -1.32 (-2.66–0.02, p=0.054)
		Cord total adiponectin	NS (p=0.377)
		Cord leptin	NS (p=0.830)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Monroy et al. 2008 General population (n=101 pregnant women)	1.81 and 1.58 ng/mL (maternal and cord median PFOA)	Birth weight	NS (p>0.05), maternal serum and cord blood PFOA
Robledo et al. 2015a, 2015b General population (n=234 couples)	3.16 and 5.00 ng/mL (maternal and paternal geometric mean PFOA)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal
Sagiv et al. 2018 General population (n=1,645 pregnant women)	5.8 ng/mL (median maternal plasma PFOA)	Birth weight for gestational age	β -0.02 (-0.08–0.03)
Shi et al. 2017 General population (n= 170 infants)	1.097 ng/mL (median cord serum PFOA)	Birth weight	β 163.28 (-127.66–454.23)
		Birth length	β 0.38 (-0.41–1.17)
		Ponderal index	β 0.06 (-0.10–0.22)
Starling et al. 2017 General population (n=604 mother-infant pairs)	1.1 ng/mL (median maternal serum PFOA); 1.4–17.0 ng/mL (3rd tertile maternal PFOA)	Birth weight	**β -92.4 g (-166.2 to -18.5)*, 3rd tertile**
		Adiposity at birth	β -0.97% fat mass (-0.33–0.49), 3rd tertile
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	2.37 and 2.34 ng/mL (median maternal PFOA for boys and girls)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	NS (p>0.05)
Washino et al. 2009 General population (n=428 infants)	1.3 ng/mL (maternal median PFOA)	Birth weight	NS (p=0.207)
		Birth length	NS (p=0.631)
		Chest circumference	NS (p=0.460)
		Head circumference	NS (p=0.823)
Whitworth et al. 2012a General population (n=901 infants)	2.2 and ≥3.04 ng/mL (maternal median and 4th quartile PFOA)	Birth weight	NS (p=0.12)
		Small for gestational age	NS (p=0.92)
		Large for gestational age	NS (p=0.33)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wu et al. 2012 General population (n=167 pregnant women at 2 hospitals)	18.32 and 9.76 ng/mL (mean maternal serum PFOA at each hospital)	Birth weight	**β -267.30 (-573.27 to -37.18, p<0.05)***
		Birth length	**β -1.91 (-3.31 to -0.52, p<0.01)***
		Ponderal index	β -0.095 (-0.200–0.389)
PFOS			
Grice et al. 2007 Occupational (n=263 females)	1,300–1,970 ng/mL (range of PFOS)	Birth weight	NS (p=0.15)
Darrow et al. 2013 Community (C8) (n=1,330 women)	15.6 ng/mL (mean PFOS)	Birth weight	NS (p=0.045 for trend), whole cohort **Association (p=0.006), women (n=783) who conceived after blood sample collection**
		Low birth weight	OR 1.33 (0.60–2.96)
Stein et al. 2009 Community (C8) (n=5,262 infants)	17.7–<23.2 and 23.2–83.4 ng/mL (75th–90th and >90th PFOS percentile)	Low birth weight	**OR 1.6 (1.1–2.3)*, 75th-90th percentile**
		Birth defects	OR 1.3 (0.8–2.1)
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	9.04 ng/mL (geometric mean maternal serum PFOS)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Ponderal index	**β -0.355 (-0.702 to -0.008)***
Apelberg et al. 2007b General population (n=341 singleton births)	5 ng/mL (PFOS cord serum median)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	**Inverse association (p>0.05)***
		Ponderal index	**Inverse association (p>0.05)***
Ashley-Martin et al. 2016 General population (1,723 pregnant women)	4.60 and 0.15 ng/mL (maternal and cord PFOS median)	Gestational weight gain	**Association (p<0.1), serum PFOS in underweight/normal weight subjects** **OR 1.03 (1.00–1.05)*, cord PFOS**
Ashley-Martin et al. 2017	4.6 ng/mL (median maternal plasma PFOS)	Birth weight	β 0.05 (-0.18–0.29)
		Infant leptin levels	β -0.09 (-0.23–0.04)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
General population (n=1,705 mother-infant pairs)		Infant adiponectin levels	β 0.02 (-0.11–0.07)
Bach et al. 2016 General population (n=1,507 nulliparous women)	8.3 ng/mL (PFOS median)	Birth weight	Inverse association reported by investigators
		Birth length	NS, investigators noted no consistent alterations across PFOS quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFOS quartiles
Bae et al. 2015 General population (233 couples)	21.7 and 14.5 ng/mL and 21.5 and 10.8 ng/mL (geometric mean PFOS in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.16 (0.88–1.53), maternal PFOS OR 1.01 (0.78–1.33), paternal PFOS
Callan et al. 2016 General population (n=98 pregnant women)	1.99 ng/mL (median maternal serum PFOS)	Birth weight	β -69 g (-231–94)
		Birth length	β -0.22 (-1.0–0.57)
		Head circumference	β -0.39 (-0.98–0.20)
		Ponderal index	β -0.03 (-0.14–0.08)
Cao et al. 2018 General population (n=337 newborns)	1.43 ng/mL (mean cord serum PFOS)	Birth weight	NS (p=0.84)
		Birth length	NS (p=0.65)
		Ponderal index	NS (p=0.47)
Chen et al. 2012a General population (n=429 infants)	5.94 ng/mL (cord blood geometric mean PFOS)	Gestational age	**Inverse association (p<0.001)***
		Birth weight	**β -110.2 g (-176.0 to -44.5, p<0.001)*, per ln PFOS**
		Birth length	NS (p>0.05)
		Head circumference	**β -0.25 cm (-0.46–0.05 cm, p<0.05)*, per ln PFOS**
		Ponderal index	NS (p>0.05)
		Small for gestational age	**OR 2.27 (1.25–4.15)***
		Low birth weight	OR 2.61 (0.185–8.03)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
de Cock et al. 2014 General population (n=89 infants)	1.611 ng/mL (cord blood mean PFOS)	Weight	NS (p=0.802)
		Height	NS (p=0.975)
		BMI	NS (p=0.586)
		Head circumference	NS (p=0.649)
Fei et al. 2007, 2008a General population (n=1,400 pregnant women)	35.3 ng/mL (maternal median PFOS)	Birth weight	β -0.46 (-2.34–1.41)
		Birth length	β-0.002 (-0.011–0.006)
		Abdominal circumference	β -0.003 (-0.012–0.005)
		Head circumference	β 0.000 (-0.006–0.007)
		Gestation length	NS (p>0.01)
		Low birth weight	OR 4.82 (0.56–41.16), 4th quartile
		Small for gestation age	OR 0.98 (0.58–1.65), 4th quartile
Govarts et al. 2016 General population (n=202 infants)	2.63 ng/mL (cord blood geometric mean PFOS)	Birth weight	NS (p=0.798)
Hamm et al. 2010 General population (n=252 pregnant women)	>10–35 ng/mL (maternal 3rd tertile PFOS)	Birth weight	Change in weight 71.25 (54.97–197.48), 3rd tertile
		Small for gestational age	**RR 0.26 (0.10–0.70)*, 3rd tertile**
Kim et al. 2011 General population (n=44 pregnant women)	2.93 ng/mL (maternal median PFOS)	Birth weight	NS (p>0.05)
		Cord TSH	NS (p>0.05)
		Cord T3	**Inverse association (p<0.05)***
		Cord T4	NS (p>0.05)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; N=13 controls)	5.326 and 4.05 ng/mL (mean PFOS in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Kobayashi et al. 2017 General population (n=177 mother-infant pairs)	5.7 ng/mL (mean maternal serum PFOS)	Birth weight	β -56.0 (-162.8–50.8)
		Birth length	β 0.32 (-0.19–0.82)
		Ponderal index	**β -1.07 (-1.79 to -0.36)***

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	16.4 ng/mL (median maternal serum PFOS)	Birth weight	**β -292 (-500 to -84; p=0.006)***
		Birth length	**β -1.2 (-2.1 to -0.3, p=0.007)***
		Head circumference	NS (p=0.073)
		Small for gestational age	OR 2.51 (0.93–6.77)
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	9.74 ng/mL (median maternal serum PFOS)	Birth weight	NS (p=0.167)
		Birth length	NS (p=0.987)
		Head circumference	NS (p=0.189)
		Small for gestational age	OR 0.71 (0.42–1.20)
Lee et al. 2013 General population (n=59 pregnant women)	10.77 ng/mL (maternal mean PFOS)	Birth weight	OR 0.98 (0.32–3.03)
		Birth length	OR 0.97 (0.29–3.27)
		Ponderal index	**OR 0.22 (0.05–0.90)***
		Head circumference	OR 1.34 (0.20–8.90)
Lee et al. 2016 General population (n=85 infants)	0.87 ng/mL (cord blood mean PFOS)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	20.09, 5.04, and 7.81 ng/mL (maternal median PFOS for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.109)
Li et al. 2017 General population (n=321 mother-infant pairs)	3.0 ng/mL (median cord serum PFOS)	Birth weight	**β -95.0 (-154.0 to -36.0)*** **β -150.6 (-225.4 to -75.7)*, boys only** β -26.6 (-125.1–71.8), girls only
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	28.90 and 27.50 ng/mL (maternal median PFOS in boy and girl cases) 27.60 and 26.20 ng/mL (maternal median PFOS in boy and girl controls)	Congenital cerebral palsy Boys Girls	 **RR 1.7 (1.0–2.8)*** RR 0.7 (0.4–1.4)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lind et al. 2017a General population (n=649 pregnant women)	8.1 ng/mL (median maternal serum PFOS)	Birth weight	NS (p>0.05)
Maisonet et al. 2012 General population (n=447 girls)	19.6 ng/mL (maternal median PFOS)	Birth weight	**β -140.01 g (-238.14 to -41.89 g, p=0.0053 for trend)*, 3rd tertile**
		Birth length	**β -0.63 cm (-1.11 to -0.15 cm, p=0.103 for trend)* 3rd tertile**
		Ponderal index	NS (p=0.1120)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	6.05 ng/mL (mean maternal serum PFOS)	Birth weight	β 0.44 (-32.48–33.36)
		Birth length	β 0.03 (-0.12–0.17)
		Head circumference	β -0.00 (-0.10–0.10)
		Small for gestational age	OR 0.92 (0.70–1.22)
		Low birth weight	OR 1.06 (0.71–1.58)
		Low birth weight at term	OR 0.91 (0.55–1.50)
Minatoya et al. 2017 General population (n=168 mother-infant pairs)	5.1 ng/mL (median maternal serum PFOS)	Birth weight	β -29 (-289–232, p=0.828)
		Ponderal index	**β -2.25 (-4.01 to -0.50, p=0.012)***
		Cord total adiponectin	**β 0.12 (0.01–0.22, p=0.028)***
		Cord leptin	NS (p=0.691)
Monroy et al. 2008 General population (n=101 pregnant women)	14.54 and 6.08 ng/mL (maternal and cord median PFOS)	Birth weight	NS (p>0.05), maternal serum and cord blood PFOA
Robledo et al. 2015a, 2015b General population (n=234 couples)	12.44 and 21.6 ng/mL (maternal and paternal geometric mean PFOS)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal
Sagiv et al. 2018 General population (n=1,645 pregnant women)	25.7 ng/mL (median maternal plasma PFOS)	Birth weight for gestational age	β -0.04 (-0.08–0.01)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shi et al. 2017 General population (n= 170 infants)	0.974 ng/mL (median cord serum PFOS)	Birth weight	β 160.45 (-11.85–332.75)
		Birth length	β 0.33 (-0.14–0.79)
		Ponderal index	β 0.07 (-0.03–0.16)
Starling et al. 2017 General population (n=604 mother-infant pairs)	2.4 ng/mL (median maternal serum PFOS)	Birth weight	β -13.8 g (-53.8–26.3)
		Adiposity at birth	β 0.8 (-0.33–0.49)
Washino et al. 2009 General population (n=428 infants)	5.2 ng/mL (maternal median PFOS)	Birth weight Males Females	**β -148.8 g (-297.0 to -0.5 g, p=0.049)*, per log PFOS unit** NS (p=0.917) **β -269.4 g (-465.7 to -73.0 g, p=0.007)*, per log PFOS unit**
		Birth length	NS (p=0.167)
		Chest circumference	NS (p=0.718)
		Head circumference	NS (p=0.488)
Whitworth et al. 2012a General population (n=901 infants)	13.0 and ≥16.59 ng/mL (maternal median and 4th quartile PFOS)	Birth weight	NS (p=0.10)
		Small for gestational age	NS (p=0.51)
		Large for gestational age	NS (p=0.33)
PFHxS			
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	0.62 ng/mL (geometric mean maternal serum PFHxS)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Ponderal index	NS (p>0.05)
Ashley-Martin et al. 2016 General population (n=1,723 pregnant women)	1.00 and 0.10 ng/mL (maternal and cord PFHxS median)	Gestational weight gain	NS (p>0.1), serum PFHxS OR 1.01 (10.99–1.03), cord PFHxS
Ashley-Martin et al. 2017 General population (n=1,705 mother-infant pairs)	1.0 ng/mL (median maternal plasma PFHxS)	Birth weight	β 0.04 (-0.12–0.20
		Infant leptin levels	β 0.01 (-0.08–0.10)
		Infant adiponectin levels	β 0.02 (-0.08–0.04)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.5 ng/mL (maternal PFHxS median)	Birth weight	**Inverse association reported by investigators**
		Birth length	NS, investigators noted no consistent alterations across PFHxS quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFHxS quartiles
Callan et al. 2016 General population (n=98 pregnant women)	0.33 ng/mL (median maternal serum PFHxS)	Birth weight	β -103 g (-221–15)
		Birth length	β -0.20 (-0.78–0.38)
		Head circumference	β -0.31 (-0.74–0.12)
		Ponderal index	β -0.05 (-0.13–0.03)
Cao et al. 2018 General population (n=337 newborns)	0.16 ng/mL (mean cord serum PFHxS); 0.06–0.139 ng/mL (2nd tertile cord PFHxS)	Birth weight	NS (p=0.69)
		Birth length	NS (p=0.67)
		Head circumference	**β 1.33 (0.42–2.26)*, 2nd tertile**
		Ponderal index	NS (p=0.85)
Hamm et al. 2010 General population (n=252 pregnant women)	>1.4–43 ng/mL (maternal 3rd tertile PFHxS)	Birth weight	Change in weight (25.99, 95% CI -95.25–147.23), 3rd tertile
		Small for gestational age	RR 2.35 (0.63–8.72), 3rd tertile
Kim et al. 2011 General population (n=44 pregnant women)	0.55 ng/mL (maternal median PFHxS)	Birth weight	NS (p>0.05)
		Cord TSH	NS (p>0.05)
		Cord T3	NS (p>0.05)
		Cord T4	NS (p>0.05)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	1.228 and 1.17 ng/mL (mean PFHxS in cases and controls)	Thyroid stimulating immunoglobulin levels	**Association (p<0.05)***
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lee et al. 2013 General population (n=59 pregnant women)	1.35 ng/mL (maternal mean PFHxS)	Birth weight	OR 0.57 (0.19–1.75)
		Birth length	OR 0.44 (0.12–1.58)
		Ponderal index	OR 0.64 (0.19–2.23)
		Head circumference	OR 0.90 (0.13–6.13)
Lee et al. 2016 General population (n=85 infants)	0.60 ng/mL (cord blood mean PFHxS)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	2.05, 1.56, and 2.28 ng/mL (maternal median PFHxS for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.801)
Li et al. 2017 General population (n=321 mother-infant pairs)	3.9 ng/mL (median cord serum PFHxS)	Birth weight	β -30.0 (-83.4–23.5)
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	0.96 and 0.90 ng/mL (maternal median PFHxS in boy and girl cases) 0.92 and 0.92 ng/mL (maternal median PFHxS in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.2 (0.9–1.7) RR 1.1 (0.6–1.9)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFHxS)	Birth weight	NS (p>0.05)
Maisonet et al. 2012 General population (n=447 girls)	1.6 ng/mL (maternal median PFHxS)	Birth weight	**Inverse association (p=0.0314 for trend)***
		Birth length	**Inverse association (p=0.0008 for trend)***
		Ponderal index	NS (p=0.6802 for trend)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.58 ng/mL (mean maternal serum PFHxS)	Birth weight	β -8.60 (-32.00–14.80)
		Birth length	β -0.06 (-0.17–0.06)
		Head circumference	β -0.01 (-0.09–0.07)
		Small for gestational age	OR 0.98 (0.80–1.19)
		Low birth weight	OR 0.94 (0.71–1.23)
		Low birth weight at term	OR 0.97 (0.68–1.41)
Monroy et al. 2008 General population (n=101 pregnant women)	1.62 mg/mL (maternal median PFHxS)	Birth weight	NS (p>0.05)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	2.4 ng/mL (median maternal plasma PFHxS)	Birth weight for gestational age	β 0.00 (-0.03–0.02)
Shi et al. 2017 General population (n= 170 infants)	0.157 ng/mL (median cord serum PFHxS)	Birth weight	β 108.80 (-53.84–271.45)
		Birth length	β 0.38 (-0.06–0.82)
		Ponderal index	β 0.03 (-0.06–0.12)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.8 ng/mL (median maternal serum PFHxS); 1.1–10.9 ng/mL (3rd tertile maternal PFHxS)	Birth weight	β -31.84 g (-105.8–42.2), 3rd tertile
		Adiposity at birth	**β -0.99% fat mass (-1.75 to -0.23)*, 3rd tertile**
PFNA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.8 ng/mL (PFNA median)	Birth weight	NS, investigators noted no consistent alterations across PFNA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFNA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFNA quartiles

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bae et al. 2015 General population (233 couples)	1.60 and 1.37 ng/mL and 1.55 and 1.09 ng/mL (geometric mean PFNA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 0.94 (0.70–1.26), maternal PFNA OR 0.94 (0.71–1.24), paternal PFNA
Callan et al. 2016 General population (n=98 pregnant women)	0.30 ng/mL (median maternal serum PFNA)	Birth weight	β 14 g (-169–196)
		Birth length	β 0.20 (-0.68–1.09)
		Head circumference	β -0.14 (-0.80–0.52)
		Ponderal index	β -0.03 (-0.16–0.09)
Cao et al. 2018 General population (n=337 newborns)	0.13 ng/mL (mean cord serum PFNA)	Birth weight	NS (p=0.19)
		Birth length	NS (p=0.06)
		Ponderal index	NS (p=0.91)
Chen et al. 2012a General population (n=429 infants)	2.36 ng/mL (cord blood geometric mean PFNA)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	**Association (p<0.01)***
		Head circumference	NS (p>0.05)
		Ponderal index	**Inverse association (p<0.05)**
		Small for gestational age	OR 0.97 (0.74–1.26)
		Low birth weight	OR 0.76 (0.47–1.23)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	1.931 and 0.633 ng/mL (mean PFNA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Lee et al. 2016 General population (n=85 infants)	0.36 ng/mL (cord blood mean PFNA)	Birth weight	NS (p>0.05)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.69, 0.61, and 0.56 ng/mL (maternal median PFNA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.065)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.2 ng/mL (median cord serum PFNA)	Birth weight	β -45.6 (-106.9–15.8)
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	0.46 and 0.39 ng/mL (maternal median PFNA in boy and girl cases) 0.44 and 0.41 ng/mL (maternal median PFNA in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.2 (0.6–2.5) RR 0.6 (0.3–1.2)
Lind et al. 2017a General population (n=649 pregnant women)	0.7 ng/mL (median maternal serum PFNA)	Birth weight	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.66 ng/mL (mean maternal serum PFNA)	Birth weight	β -10.27 (-38.14–17.61)
		Birth length	β -0.00 (-0.13–0.13)
		Head circumference	β -0.04 (-0.13–0.05)
		Small for gestational age	OR 0.85 (0.68–1.07)
		Low birth weight	OR 0.86 (0.63–1.17)
		Low birth weight at term	OR 0.91 (0.60–1.38)
Monroy et al. 2008 General population (n=101 pregnant women)	0.69 mg/mL (maternal median PFNA)	Birth weight	NS (p>0.05)
Robledo et al. 2015a, 2015b General population (n=234 couples)	1.211 and 1.566 ng/mL (maternal and paternal geometric mean PFNA)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Sagiv et al. 2018 General population (n=1,645 pregnant women)	0.7 ng/mL (median maternal plasma PFNA); 0.7–0.9 ng/mL (3rd quartile maternal PFNA)	Birth weight for gestational age	**β -0.20 (-0.33 to -0.06)*, 3rd quartile**
Shi et al. 2017 General population (n= 170 infants)	0.191 ng/mL (median cord serum PFNA)	Birth weight	β 52.68 (-206.01–311.36)
		Birth length	β 0.13 (-0.57–0.83)
		Ponderal index	β 0.01 (-0.13–0.15)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.4 ng/mL (median maternal serum PFNA); 0.5–6.0 ng/mL (2nd half maternal PFNA)	Birth weight	**β -92.1 g (-150.6 to -33.6)*, 2nd half**
		Adiposity at birth	**β -0.85% fat mass (-1.46 to -0.24)*, 2nd half**
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	1.55 and 1.58 ng/mL (median maternal PFNA for boys and girls)	Birth weight	**Inverse association (p>0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	NS (p>0.05)
		Growth during childhood	NS (p>0.05)
PFDA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.3 ng/mL (PFDA median)	Birth weight	NS, investigators noted no consistent alterations across PFDA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFDA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFDA quartiles
Bae et al. 2015 General population (233 couples)	0.46 and 0.38 ng/mL and 0.49 and 0.46 ng/mL (geometric mean PFDA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.07 (0.81–1.42), maternal PFDA OR 1.02 (0.78–1.34), paternal PFDA

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Callan et al. 2016 General population (n=98 pregnant women)	0.12 ng/mL (median maternal serum PFDA)	Birth weight	β 4 g (-161–170)
		Birth length	β 0.36 (-0.44–1.15)
		Head circumference	β -0.07 (-0.67–0.53)
		Ponderal index	β -0.06 (-0.18–0.05)
Cao et al. 2018 General population (n=337 newborns)	0.12 ng/mL (mean cord serum PFDA)	Birth weight	NS (p=0.26)
		Birth length	NS (p=0.24)
		Ponderal index	NS (p=0.55)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.523 and 0.298 ng/mL (mean PFDA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Lee et al. 2016 General population (n=85 infants)	0.14 ng/mL (cord blood mean PFDA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.40, 0.16, and 0.22 ng/mL (maternal median PFDA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.158)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDA)	Birth weight	β -47.3 (-112.9–18.2)
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	0.18 and 0.16 ng/mL (maternal median PFDA in boy and girl cases) 0.17 and 0.16 ng/mL (maternal median PFDA in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.1 (0.7–1.7) RR 0.6 (0.3–1.1)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFDA)	Birth weight	NS (p>0.05)
Robledo et al. 2015a, 2015b General population (n=234 couples)	0.402 and 0.458 ng/mL (maternal and paternal geometric mean PFDA)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal
Shi et al. 2017 General population (n= 170 infants)	0.075 ng/mL (median cord serum PFDA)	Birth weight	β -3.04 (-129.67–123.59)
		Birth length	β -0.002 (-0.354–0.34)
		Ponderal index	β -0.01 (-0.08–0.06)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.1 ng/mL (median maternal serum PFDA)	Birth weight	β 11.5 g (-37.3–60.4)
		Adiposity at birth	β 0.06 (-0.45–0.56)
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.46 and 0.43 ng/mL (median maternal PFDA for boys and girls)	Birth weight	**Inverse association (p>0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	**OR 3.14 (1.07–9.19)*, girls only**
PFUnA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.3 ng/mL (PFUnA median)	Birth weight	NS, investigators noted no consistent alterations across PFUnA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFUnA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFUnA quartiles
Callan et al. 2016 General population (n=98 pregnant women)	0.08 ng/mL (median maternal serum PFUnA)	Birth weight	β 102 g (-41–245)
		Birth length	β 0.32 (-0.37–1.02)
		Head circumference	β -0.29 (-0.81–0.24)
		Ponderal index	β 0.01 (-0.09–0.11)
		Optimal body weight	**β 5.3 (1.2–9.3)***

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Cao et al. 2018 General population (n=337 newborns)	0.10 ng/mL (mean cord serum PFUnA); >0.11 ng/mL (3rd tertile cord PFUnA	Birth weight	NS (p=0.08)
		Birth length	**β 0.41 (0.06–0.77)*, 3rd tertile**
		Ponderal index	NS (p=0.56)
Chen et al. 2012a General population (n=429 infants)	10.26 ng/mL (cord blood geometric mean PFUnA)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Ponderal index	NS (p>0.05)
		Small for gestational age	OR 0.93 (0.65–1.33)
		Low birth weight	OR 1.01 (0.53–1.91)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.982 and 0.438 ng/mL (mean PFUnA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Lee et al. 2016 General population (n=85 infants)	0.22 ng/mL (cord blood mean PFUnA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.70, 0.16, and 0.13 ng/mL (maternal median PFUnA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.275)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFUnA)	Birth weight	β -29.7 (-85.7–26.3)
Shi et al. 2017 General population (n= 170 infants)	0.063 ng/mL (median cord serum PFUnA)	Birth weight	β -28.87 (-128.16–70.42)
		Birth length	β -0.20 (-0.47–0.07)
		Ponderal index	β 0.01 (-0.04–0.06).

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2016 General population (n=117 boys and 106 girls examined at age 2, 5, 8, and 11 years of age)	3.52 and 3.31 ng/mL (median maternal PFUnA for boys and girls)	Birth weight	**Inverse association (p<0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	**OR 1.83 (1.01–3.32)*, girls only**
PFHpA			
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.284 and 0.324 ng/mL (mean PFHpA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFHpA)	Birth weight	β -103.7 (-211.3–3.8) **β -266.6 (-426.8 to -106.3)*, boys only** β 15.5 (-134.1–165.1), girls only
PFBA			
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.464 and 0.220 ng/mL (mean PFBA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFBA)	Birth weight	β -46.2 (-111.3–19.0)
PFDoDA			
Cao et al. 2018 General population (n=337 newborns)	0.04 ng/mL (mean cord serum PFDoDA)	Birth weight	NS (p=0.94)
		Birth length	NS (0.51)
		Ponderal index	NS (p=0.60)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lee et al. 2016 General population (n=85 infants)	0.14 ng/mL (cord blood mean PFDoDA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.13, 0.04, and 0.05 ng/mL (maternal median PFDoDA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.440)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDoDA)	Birth weight	β -46.86 (-122.0–28.4) β 18.4 (-86.8–123.5), boys only **β -130.4 (-239.1 to -21.7)*, girls only**
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.37 and 0.37 ng/mL (median maternal PFDoDA for boys and girls)	Birth weight	**Inverse association (p<0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	**Inverse association (p<0.05)*, girls only**
		Small for gestational age	NS (p>0.05)
FOSA			
Bae et al. 2015 General population (233 couples)	0.11 and 0.10 ng/mL and 0.10 and 0.12 ng/mL (geometric mean FOSA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.07 (0.81–1.41), maternal FOSA OR 1.14 (0.86–1.51), paternal FOSA

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Robledo et al. 2015a, 2015b General population (n=234 couples)	0.112 and 0.114 ng/mL (maternal and paternal geometric mean FOSA)	Birth weight	
		Boys	**Inverse association (p<0.05)*, maternal only**
		Girls	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BMI = body mass index; FSH = follicle stimulating hormone; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; LH = luteinizing hormone; NS = not significant; NR = not reported; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR = relative risk; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Stein et al. 2013 Community (C8) (n=320 children 6–12 years old)	115.9 ng/mL (estimated *in utero* mean PFOA)	Full scale IQ	**β 4.61 (0.68–8.54)*, 4th quartile**
		Reading and math skills	NS
		Scores on tests of ADHD (improvement)	**β -8.49 (-16.14 to -0.84)*, 4th quartile**
Stein et al. 2014a, 2014b Community (C8) (n=321 children 6–12 years old)	94.1–838.6 ng/mL (4th PFOA quartile measured 3–4 years prior to behavioral assessment)	Executive function scores (mother completed survey)	**β -6.39 (-11.43 to -1.35)*, 4th quartile boys** β -6.39 (-0.03–8.87), 4th quartile girls
		Executive function scores (teacher completed survey)	β -6.42 (-13.29–0.45), 4th quartile boys β -1.92 (-10.39–6.55), 4th quartile girls
		ADHD-like behaviors (mother completed survey)	β -3.82 (-8.96–1.31), 4th quartile boys **β 6.99 (2.47–11.51)*, 4th quartile girls** β 2.30 (-1.18–5.77), 4th quartile boys and girls
		ADHD-like behaviors (teacher completed survey)	β -9.25 (-18.78–0.27), 4th quartile boys β -3.65 (-10.85–3.51) 4th quartile girls **β -6.03 (-11.40 to -0.66)*, 4th quartile boys and girls**
		Behavioral problems and emotional disturbances (mother completed survey)	β -1.55 (-5.91–2.82), 4th quartile boys **β 4.63 (0.72–8.53)*, 4th quartile girls**
		Behavioral problems and emotional disturbances (teacher completed survey)	β -2.47 (-8.24–3.30), 4th quartile boys β -0.91 (-6.19–4.37), 4th quartile girls
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 year)	65.3–2,070.6 ng/mL (4th PFOA quartile)	ADHD	**OR 0.76 (0.64–0.90)*, 4th quartile**
		Learning problems	
		12–15 years old	OR 0.96 (0.73–1.26), 4th quartile
		5–18 years old	OR 0.90 (0.76–1.06), 4th quartile

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	5.5 ng/mL (maternal median PFOA)	Social responsiveness scale score (measure of autistic behaviors)	β -2.0 (-4.4–0.4)
Chen et al. 2013 General population (239 children 2 years of age)	2.5 ng/mL (mean cord PFOA)	Poor performance on tests	NS OR 0.6 (0.08–4.8), whole test OR1.3 (0.3–6.2), cognitive tests OR 0.5 (0.06–4.0), language tests OR 0.8 (0.1–4.7), gross motor tests OR 2.8 (0.6–13.5), fine motor tests OR 0.3 (0.02–2.7), social tests OR 3.2 (0.7–14.3), self-help tests
Donauer et al. 2015 General population (n=349 infants at 5 weeks of age)	5.49 ng/mL (maternal geometric mean PFOA)	Social/easy going	NS (p>0.05)
		Hypotonic	**OR 3.79 (1.1–12.8)* per 10-fold increase in PFOA**
		High arousal/difficult	NS (p=0.3533)
Fei et al. 2008b General population (n=1,400 infants)	5.6 ng/mL (maternal median PFOA)	Apgar scores <10	OR 1.14 (0.57–2.25)
		Motor and mental development at 6 months	NS (p>0.05)
		Neurobehavioral milestones at 18 months	NS (p>0.05)
Fei and Olsen 2011 General population (n=526–787 7-year-old children)	5.4 ng/mL (maternal median PFOA)	Behavioral problems	NS (p>0.15 for trend)
		Motor coordination	NS (p=0.89 for trend)
Forns et al. 2015 General population (n=843 infants)	40 ng/L (median PFOA breast milk level)	Risk of an abnormal score on neurobehavioral assessment questionnaire	OR 1.05 (0.77–1.44) at 6 months of age OR 1.0 (0.78–1.28) at 24 months of age
Goudarzi et al. 2016b General population (n=173 infants at 6 months and 133 at 18 months)	1.2 ng/mL (maternal median PFOA)	MDI/PDI at 6 months of age	NS (p>0.05), boys and girls **Inverse association (p<0.05)*, females**
		MDI/PDI at 18 months of age	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gump et al. 2011 General population (n=83 children aged 9–11 years)	3.23 ng/mL (mean PFOA)	Performance on task requiring behavioral inhibition	NS (p>0.05)
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	4.4 ng/mL (median PFOA)	ADHD (parent reported)	**OR 1.12 (1.01–1.23)*, per 1 ng/mL PFOA**
Høyer et al. 2015a General population (n=1,106 children aged 5–9 years)	1.4 and 1.9–9.8 ng/mL (maternal median and 3rd tertile PFOA)	Motor skills	β -0.2 (-1.2–0.9)
		Abnormal behavior	**OR 2.7 (1.2–6.3)*, 3rd tertile**
		Hyperactivity	**OR 3.1 (1.3–7.2)*, 3rd tertile**
Lien et al. 2016 General population (n=282 children aged 7 years)	1.55 ng/mL (cord blood weighted average PFOA)	Inattention	NS (p=0.7758)
		Hyperactivity/impulsivity	NS (p=0.2997)
		Emotional symptoms	NS (p=0.691)
		Conduct problems	NS (p=0.2664)
		Hyperactivity/inattention	NS (p=0.774)
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	3.7 ng/mL (maternal median serum PFOA)	Verbal comprehension (15-month-olds)	NS (p>0.05)
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	**β -0.04 (-0.08 to -0.01)***
		Language scores (38-month-olds)	NS (p>0.05)
		Communicative scores (38-month-olds)	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	4.06, 3.88, and 4.00 ng/mL (maternal median PFOA for ADHD, autism, and controls)	ADHD	RR 0.98 (0.82–1.16)
		Autism	RR 0.98 (0.73–1.31)
Ode et al. 2014 General population (n=206 children with ADHD and 206 controls; children were 5–17 years old at time of diagnosis)	1.80 and 1.83 ng/mL (cord blood median PFOA in cases and controls)	ADHD	OR 0.98 (0.91–1.02), per 1 ng/mL increase in PFOA
Oulhote et al. 2016 General population (n=567 7-year-old children)	3.19 ng/mL (maternal geometric mean PFOA)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	4.09 ng/mL (geometric mean PFOA in 5-year-old children)	Total behavioral scores and higher internalizing problems, peer relationship, and autism screening scores	Associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	4.51 ng/mL (geometric mean PFOA in 7-year-old children)	Behavioral development scores	No associations
Quaak et al. 2016 General population (n=76 infants 18 months of age)	0.9056 ng/mL (cord mean PFOA)	Score on test evaluating ADHD	NS (p=0.72), 3rd tertile
		Males	NS (p=0.22), 3rd tertile
		Females	NS (p=0.31), 3rd tertile
		Scores on test evaluating externalizing problem	NS (p=0.31), 3rd tertile
		Males	**Association (p=0.05 and 0.09)*, 2nd and 3rd tertiles**
		Females	NS (p=0.74), 3rd tertile
Strøm et al. 2014 General population (n=876 adults age 20 years)	3.7 ng/mL (median maternal PFOA)	ADHD	NS (p=0.45 for trend of 3rd tertile)
		Depression	NS (p=0.28 for trend of 3rd tertile)
		Scholastic achievement	NS (p=0.21 for trend of 3rd tertile)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Vuong et al. 2016 General population (n=256 children aged 5 or 8 years)	5.4 ng/mL (maternal median PFOA)	Behavioral regulation	β 1.11(-1.22–3.44)
		Metacognition	β 0.58 (-1.77–2.93)
		Global executive functioning	β 1.06 (-1.33–3.45)
Vuong et al. 2018 General population (n=208 8-year-old children)	2.4 ng/mL (mean serum PFOA)	Metacognition index score	NS (p>0.05)
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	NS (p>0.05)
		At risk metacognition score	**OR 3.18 (1.17–8.60)***
		At risk behavior regulation score	OR 1.56 (0.49–4.92)
		At risk global executive score	OR 2.69 (0.92–7.90)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	2.50 and 2.50 ng/mL (maternal median PFOA for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS NS
Wu et al. 2012 General population (n=167 pregnant women at 2 hospitals)	18.32 and 9.76 ng/mL (mean maternal serum PFOA at each hospital)	5-minute Apgar score	**β -1.37 (-2.42 to -0.32, p<0.05)***
Zhang et al. 2018 General population (n=167 mother-child pairs)	5.4 ng/mL (median maternal PFOA)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	5.5 ng/mL (median PFOA in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 **Association (p<0.05)*** NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	2.4 ng/mL (median PFOA in 5-year-old children)	Reading scores At 8 years of age	 **Association (p<0.05)***

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 years)	14.8–<20.2, 20.22–<27.9, and 27.9–202.1 ng/mL (2nd, 3rd, and 4th PFOS quartiles)	ADHD	OR 0.99 (0.76–1.30), 4th quartile
		Learning problems 5–18 years old 12–15 years old	 **OR 0.83 (0.70–0.98)*, 2nd quartile** **OR 0.68 (0.52–0.89)*, 3rd quartile**
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	13 ng/mL (maternal PFOS median)	Social responsiveness scale score (test of autism)	No association
Donauer et al. 2015 General population (n=349 infants)	13.25 ng/mL (maternal geometric mean PFOS)	Neurobehavioral outcomes	NS (p>0.05)
		Hypotonic	NS (p=0.3996)
		High arousal/difficult	NS (p=0.4678)
Fei et al. 2008b General population (n=1,400 infants)	35.3 ng/mL (maternal median PFOS)	Apgar scores <10	OR 1.20 (0.67–2.14)
		Neurobehavioral milestones Delay in age of sitting Earlier use of word-like sounds Delays in using 2-word sentences Other milestones	 **Association (p=0.041 for trend)*** **Association (p=0.039 for trend)*** **Association (p=0.050 for trend)*** NS (p>0.05)
		Motor and mental development at 6 months	NS (p>0.05)
Fei and Olsen 2011 General population (n=526–787 children)	34.4 ng/mL (maternal median PFOS)	Behavioral health	NS (p>0.39 for trend)
		Motor coordination	NS (p=0.41 for trend)
Forns et al. 2015 General population (n=843 infants)	110 ng/L (median PFOS breast milk level)	Risk of an abnormal score on neurobehavioral assessment questionnaire	OR 0.96 (0.76–1.20) at 6 months of age OR 0.93 (0.74–1.17) at 24 months of age
Goudarzi et al. 2016b General population (n=173 at 6 months and 133 at 18 months)	5.7 ng/mL (maternal median PFOS)	MDI/PDI at 6 months of age	NS (p>0.05)
		MDI/PDI at 18 months of age	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gump et al. 2011 General population (n=83 children aged 9–11 years)	9.90 ng/mL (mean PFOS)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	22.6 ng/mL (median PFOS)	ADHD (parent reported)	**OR 1.03 (1.01–1.05)*, per 1 ng/mL PFOS**
Høyer et al. 2015a General population (n=1,106 children)	10.0 and 16.6–87.3 ng/mL (maternal median and 3rd tertile PFOS)	Motor skills	β -0.1 (-1.2–1.1)
		Abnormal behavior	OR 1.5 (0.5–4.8), 3rd tertile
		Hyperactivity	OR 1.4 (0.4–4.9), 3rd tertile
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	19.8 ng/mL (maternal median serum PFOS)	Verbal comprehension (15-month-olds)	**β 0.03 (0.01–0.05)***
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	**β -0.01 (-0.01–0.00)***
		Language scores (38-month-olds)	NS (p>0.05)
		Communicative scores (38-month-olds)	NS (p>0.05)
Lien et al. 2016 General population (n=282 children aged 7 years)	4.79 ng/mL (cord blood weighted average PFOS)	Inattention	NS (p=0.8508)
		Hyperactivity/impulsivity	NS (p=0.6857)
		Emotional symptoms	NS (p=0.9431)
		Conduct problems	NS (p=0.4938)
		Hyperactivity/inattention	NS (p=0.5226)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	26.80, 25.40, and 27.40 ng/mL (maternal median PFOS for ADHD, autism, and controls)	ADHD	RR 0.87 (0.74–1.02) **RR 0.79 (0.64–0.98)*, 4th quartile**
		Autism	RR 0.92 (0.69–1.22)
Ode et al. 2014 General population (n=206 children with ADHD and 206 controls)	6.92 and 6.77 ng/mL (cord blood median PFOS in cases and controls)	ADHD	OR 0.98 (0.92–1.04), per 1 ng/mL increase in PFOS
Oulhote et al. 2016 General population (n=567 7-year-old children)	27.42 ng/mL (maternal geometric mean PFOS)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	16.75 ng/mL (geometric mean PFOS in 5-year-old children)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	15.27 ng/mL (geometric mean PFOS in 7-year-old children)	Behavioral development scores	No associations
Quaak et al. 2016 General population (n=76 infants 18 months of age)	1.5836 ng/mL (cord mean PFOS)	Score on test evaluating ADHD	NS (p=0.19), 3rd tertile
		Males	NS (p=0.35), 3rd tertile
		Females	NS (p=0.43), 3rd tertile
		Scores on test evaluating externalizing problem	NS (p=0.31), 3rd tertile
		Males	NS (p=0.74), 3rd tertile
		Females	NS (p=0.31), 3rd tertile
Strøm et al. 2014 General population (n=876 adults age 20 years)	21.4 ng/mL (median maternal PFOS)	ADHD	NS (p=0.38 for trend of 3rd tertile)
		Depression	NS (p=0.14 for trend of 3rd tertile)
		Scholastic achievement	NS (p=0.59 for trend of 3rd tertile)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	13.2 ng/mL (maternal median PFOS)	Behavioral regulation	**β 3.14 (0.68–5.61)***
		Metacognition	**β 3.10 (0.62–5.58)***
		Global executive functioning	**β 3.38 (0.86–5.90)***
		Global executive functioning composite score >60	**OR 2.19 (1.03–4.66)***
Vuong et al. 2018 General population (n=208 8-year-old children)	3.9 ng/mL (mean serum PFOS)	Metacognition index score	NS (p>0.05)
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	NS (p>0.05)
		At risk metacognition score	OR 1.53 (0.67–3.52)
		At risk behavior regulation score	OR 0.40 (0.14–1.14)
		At risk global executive score	OR 1.04 (0.41–2.68)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	13.25 and 12.28 ng/mL (maternal median PFOS for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	13.0 ng/mL (median maternal PFOS)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	6.6 ng/mL (median PFOS in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 **Association (p<0.05)*** NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	3.6 ng/mL (median PFOS in 5-year-old children)	Reading scores At 8 years of age	 **Association (p<0.05)***

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 years)	2.9–<5.2 and 10.1–276.4 ng/mL (2nd and 4th PFHxS quartiles)	ADHD 5–18 years 12–15 years	 **OR 1.27 (1.06–1.52)*, 2nd quartile** **OR 1.46 (1.10–1.93)*, 2nd quartile**
		Learning problems 5–18 years old 12–15 years old	 OR 1.19 (1.00–1.41), 4th quartile OR 1.05 (0.79–1.40), 4th quartile
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	1.6 ng/mL (maternal PFHxS median)	Social responsiveness scale score (test for autism)	No association
Gump et al. 2011 General population (n=83 children aged 9–11 years)	6.06 ng/mL (mean PFHxS)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.01)***
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	2.2 ng/mL (median PFHxS)	ADHD (parent reported)	**OR 1.06 (1.02–1.11)*, per 1 ng/mL PFOS**
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	1.6 ng/mL (maternal median serum PFHxS)	Verbal comprehension (15-month-olds)	NS (p>0.05)
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	NS (p>0.05)
		Language scores (38-month-olds)	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Communicative scores (38-month-olds)	NS (p>0.05)
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	0.84. 0.92, and 0.92 ng/mL (maternal median PFHxS for ADHD, autism, and controls)	ADHD	**RR 0.67 (0.54–0.83)*, 4th quartile**
		Autism	RR 1.10 (0.92–1.33)
Oulhote et al. 2016 General population (n=567 7-year-old children)	4.43 ng/mL (maternal geometric mean PFHxS)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.54 ng/mL (geometric mean PFHxS in 5-year-old children)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.53 ng/mL (geometric mean PFHxS in 7-year-old children)	Behavioral development scores	No associations
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	1.5 ng/mL (maternal median PFHxS)	Behavioral regulation	β 1.19 (-0.54–5.40)
		Metacognition	β 1.31 (-0.43–3.04)
		Global executive functioning	β 1.36 (-0.41–3.12)
		Global executive functioning composite score >60	**OR 1.71 (1.05–2.77)***
Vuong et al. 2018 General population (n=208 8-year-old children)	1.4 ng/mL (mean serum PFHxS)	Metacognition index score	NS (p>0.05)
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	NS (p>0.05)
		At risk metacognition score	OR 1.10 (0.58–2.09)
		At risk behavior regulation score	OR 0.54 (0.22–1.32)
		At risk global executive score	OR 0.65 (0.32–1.32)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	0.69 and 0.69 ng/mL (maternal median PFHxS for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.5 ng/mL (median maternal PFHxS)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.9 ng/mL (median PFHxS in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.2 ng/mL (median PFHxS in 5-year-old children)	Reading scores At 8 years of age	 NS (p>0.05)
PFNA			
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 years)	1.2–<1.5, 1.5–<2.0, and 2.0–24.1 ng/mL (2nd, 3rd, and 4th PFNA quartiles)	ADHD 5–18 years 12–15 years	 OR 0.99 (0.84–1.18), 4th quartile OR 1.00 (0.75–1.32), 4th quartile
		Learning problems 5–18 years old 12–15 years old	 **OR 0.81 (0.69–0.95)*, 3rd quartile** **OR 0.73 (0.55–0.98)*, 4th quartile**
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	0.9 ng/mL (maternal PFNA median)	Social responsiveness scale score (tests for autism)	No association
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.82 ng/mL (mean PFNA)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	0.6 ng/mL (median PFNA)	ADHD (parent reported)	OR 1.32 (0.86–2.02), per 1 ng/mL PFNA

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	0.5 ng/mL (maternal median serum PFNA)	Verbal comprehension (15-month-olds)	NS (p>0.05)
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	NS (p>0.05)
		Language scores (38-month-olds)	NS (p>0.05)
		Communicative scores (38-month-olds)	NS (p>0.05)
Lien et al. 2016 General population (n=282 children aged 7 years)	4.49 ng/mL (cord blood weighted average PFNA)	Inattention	**Inverse association (p=0.0129)***
		Hyperactivity/impulsivity	NS (p=0.0588)
		Emotional symptoms	NS (p=0.1902)
		Conduct problems	NS (p=0.6931)
		Hyperactivity/inattention	**Inverse association (p=0.0484)***
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	0.42, 0.41, and 0.43 ng/mL (maternal median PFNA for ADHD, autism, and controls)	ADHD	RR 0.80 (0.62–1.03)
		Autism	RR 0.80 (0.58–1.11)
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.61 ng/mL (maternal geometric mean PFNA)	Behavioral development scores	No association
Oulhote et al. 2016 General population (n=567 7-year-old children)	1.01 ng/mL (geometric mean PFNA in 5-year-old children)	Total behavioral development score and higher externalizing problems score	**Association***

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Oulhote et al. 2016 General population (n=567 7-year-old children)	1.2 ng/mL (geometric mean PFNA in 7-year-old children)	Behavioral development scores	No association
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	0.9 ng/mL (maternal median PFNA)	Behavioral regulation	β 2.57 (-0.26–5.40)
		Metacognition	β 1.37 (-1.49–4.23)
		Global executive functioning	β 2.01 (-0.89–4.92)
Vuong et al. 2018 General population (n=208 8-year-old children)	0.8 ng/mL (mean serum PFNA)	Metacognition index score	**β 3.4 (0.4–6.3, p<0.05)***
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	**Association (p<0.05)***
		At risk metacognition score	**OR 2.94 (1.52–5.69)***
		At risk behavior regulation score	**OR 2.75 (1.30–5.79)***
		At risk global executive score	**OR 3.07 (1.60–5.90)***
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	1.59 and 1.44 ng/mL (maternal median PFNA for 5- and 8-year-old children)	IQ score	NS (p>0.05)
		IQ scores, age 8 years	
		Full scale IQ	NS (p>0.05)
		Visual IQ	**Association (p<0.05)***
		Performance IQ	NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	0.9 ng/mL (median maternal PFNA)	Reading scores	
		At 5 years of age	NS (p>0.05)
		At 8 years of age	NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.2 ng/mL (median PFNA in 3-year-old children)	Reading scores	
		At 5 years of age	**Association (p<0.05)***
		At 8 years of age	NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	0.7 ng/mL (median PFNA in 5-year-old children)	Reading scores	
		At 8 years of age	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.26 ng/mL (mean PFDA)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	0.15, 0.15, and 0.17 ng/mL (maternal median PFDA for ADHD, autism, and controls)	ADHD	**RR 0.76 (0.64–0.91)*** **RR 0.53 (0.43–0.66)*, 4th quartile**
		Autism	RR 0.79 (0.63–1.01) **RR 0.52 (0.35–0.77)*, 4th quartile**
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.28 ng/mL (maternal geometric mean PFDA)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.28 ng/mL (geometric mean PFDA in 5-year-old children)	Total behavioral development score and higher externalizing problems and hyperactivity/ inattention scores	**Associations***
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.36 ng/mL (geometric mean PFDA in 7-year-old children)	Behavioral development scores	No associations
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	0.2 ng/mL (maternal median PFDA)	Behavioral regulation	β 0.70 (-3.31–1.92)
		Metacognition	β 1.24 (-3.87–1.39)
		Global executive functioning	β -1.13 (-3.79–1.54)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	0.44 and 0.44 ng/mL (maternal median PFDA for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Lien et al. 2016 General population (n=282 children aged 7 years)	7.96 ng/mL (cord blood weighted average PFUnA)	Inattention	NS (p=0.6177)
		Hyperactivity/impulsivity	NS (p=0.3642)
		Emotional symptoms	NS (p=0.0517)
		Conduct problems	NS (p=0.1207)
		Hyperactivity/inattention	NS (p=0.9991)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	3.42 and 3.13 ng/mL (maternal median PFUnA for 5- and 8-year-old children)	IQ score	NS (p>0.05)
		IQ scores, age 8 years	
		Full scale IQ	NS (p>0.05)
		Visual IQ	NS (p>0.05)
		Performance IQ	**Inverse association (p<0.05)***
PFDoDA			
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	0.38 and 0.37 ng/mL (maternal median PFDoDA for 5- and 8-year-old children)	IQ score	
		Age 5 years	NS (p>0.05)
		Age 8 years	NS (p>0.05)
FOSA			
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.75 ng/mL (mean FOSA)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

ADHD = attention deficit hyperactivity disorder; FOSA = perfluorooctane sulfonamide; MDI/PDI = mental and psychomotor development indices; NHANES = National Health and Nutrition Examination Survey; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Lopez-Espinosa et al. 2011 Community (C8) (n=3,076 boys and 2,931 girls aged 8–18 years)	26 and 20 ng/mL (median PFOA in boys and girls)	Age of puberty Boys Girls	 OR 0.95 (0.84–0.07) **OR 0.54 (0.35–0.84)*, 2nd quartile**
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	34.8 and 30.1 ng/mL (median PFOA in boys and girls)	Estradiol	NS (interquartile difference of 4.3, 95% CI -0.4–9.1), boys NS (4.2, 95% CI -0.7–9.4), girls
		Total testosterone	**Inverse association (-4.9, 95% CI -8.7 to -0.8)*, boys** NS (-2.5, 95% CI -6.7–1.8), girls
		Insulin-like growth factor-1	NS (-0.4, 95% CI -3.4–2.7), boys **Inverse association (-3.6, 95% CI -6.6 to -0.5)*, girls**
Christensen et al. 2011 General population (n=448 girls)	3.7 ng/mL (maternal median PFOA)	Earlier age of menarche	OR 1.01 (0.61–1.68)
Itoh et al. 2016 General population (n=189 infants)	1.4 ng/mL (maternal median PFOA)	Cord estradiol	NS (p>0.05)
		Cord testosterone	NS (p>0.05)
		Cord testosterone: estradiol ratio	NS (p>0.05)
		Cord progesterone	NS (p>0.05)
		Cord prolactin	NS (p>0.05)
		Cord LH	NS (p>0.05)
		Cord FSH	NS (p>0.05)
		Cord SHBG	NS (p>0.05)
		Cord insulin-like factor 3	NS (p>0.05)
		Cord inhibin Males Females	 **Association (p=0.040)*** NS (p>0.05)

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kristensen et al. 2013 General population (n=343 females approximately 20 years of age)	3.6 and 4.4–19.8 ng/mL (maternal median PFOA and 3rd PFOA tertile)	Age of menarche	**Association (p=0.01)***
		Menstrual cycle length	NS (p>0.05)
		Total testosterone	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		Dehydroepiandrosterone sulphate	NS (p>0.05)
		Anti-Müllerian hormone	NS (p>0.05)
		Number of follicles/ovary	NS (p>0.05)
Lind et al. 2017a General population (n=649 pregnant women)	1.7 ng/mL (median maternal serum PFOA)	Anogenital distance	NS (p=0.71), boys NS (p=0.71), girls
Maisonet et al. 2015 General population (n=72 girls aged 15 years)	>4.1 ng/mL (maternal 3rd tertile PFOA)	Testosterone	**β 0.24 (0.05–0.43)*, 3rd tertile**
		SHBG	β 5.02 (-13.07–11.00), 3rd tertile
Vesterholm Jensen et al. 2014 General population (n=107 cases cryptorchidism [29 from Denmark and 78 from Finland] and 108 matched controls from Denmark and Finland)	2.6 and 2.1 ng/mL (median cord blood PFOA Denmark and Finland cohorts)	Cryptorchidism	OR 0.51 (0.21–1.20), whole cohort **OR 0.35 (0.12–0.99, p=0.04 for trend)*, Finland cohort 3rd tertile**
PFOS			
Lopez-Espinosa et al. 2011 Community (C8) (n=3,076 boys and 2,931 girls aged 8–18)	20 and 18 ng/mL (PFOS median in boys and girls)	Age of puberty Boys Girls	 **OR 0.58 (0.37–0.90)*, 3rd quartile** **OR 0.55 (0.35–0.86)*, 3rd quartile**

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	22.4 and 20.9 ng/mL (PFOS median in boys and girls)	Estradiol	**Inverse association (interquartile difference of -4.0, 95% CI -7.7 to -0.1)*, boys** NS (-0.3, 95% CI -4.6–4.2), girls
		Total testosterone	**Inverse association (-5.8, 95% CI -9.4 to -2.0)*, boys** **Inverse association (-6.6, 95% CI -10.1 to -2.8)*, girls**
		Insulin-like growth factor-1	**Inverse association (-5.9, 95% CI -8.3 to -3.3)*, boys** **Inverse association (-5.6, 95% CI -8.2 to -2.9)*, girls**
Christensen et al. 2011 General population (n=448 girls)	19.8 ng/mL (maternal median PFOS)	Earlier age of menarche	OR 0.68 (0.40–1.13)
Itoh et al. 2016 General population (n=189 infants)	5.2 ng/mL (maternal median PFOS)	Cord estradiol Males Females	 **Association (p=0.021)*** NS (p>0.05)
		Cord testosterone	NS (p>0.05)
		Cord testosterone: estradiol ratio Males Females	 **Inverse association (p=0.008)*** NS (p>0.05)
		Cord progesterone Males Females	 **Association (p=0.043)*** **Association (p=0.002)***
		Cord prolactin Males Females	 NS (p>0.05) **Association (p=0.001)***
		Cord LH	NS (p>0.05)
		Cord FSH	NS (p>0.05)
		Cord SHBG	NS (p>0.05)

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Cord insulin-like factor 3	NS (p>0.05)
		Cord inhibin Males Females	 **Association (p<0.001)*** NS (p>0.05)
Kristensen et al. 2013 General population (n=343 young women approximately 20 years of age)	21.1 ng/mL (maternal median PFOS)	Age of menarche	NS (p=0.28)
		Menstrual cycle length	NS (p>0.05)
		Total testosterone	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		Dehydroepiandrosterone sulphate	NS (p>0.05)
		Anti-Müllerian hormone	NS (p>0.05)
		Number of follicles/ovary	NS (p>0.05)
Lind et al. 2017a General population (n=649 pregnant women)	8.1 ng/mL (median maternal serum PFOS)	Anogenital distance	β 0.5 (-1.2–2.2, p=0.55), boys **β -0.4(-3.8 to -0.7, p<0.01)*, girls**
Toft et al. 2016 General population (270 cases cryptorchidism, 75 cases hypospadias, and 300 controls)	>1.4 ng/mL (amniotic fluid 3rd tertile PFOS)	Cryptorchidism	OR 1.01 (0.66–1.53), 3rd tertile
		Hypospadias	OR 0.69 (0.35–1.38), 3rd tertile
		Testosterone	**Association (p=0.002)***
		Androstenedione	**Association (p=0.001)***
		Progesterone	**Association (p=0.001)***
		Cortisol	**Association (p<0.001)***
		DHEAS	NS (p=0.93)
		Insulin-like factor 3	**Inverse association (p<0.001)***
Vesterholm Jensen et al. 2014 General population (n=107 cases cryptorchidism [29 from Denmark and 78 from Finland] and 108 matched controls from Denmark and Finland)	9.1 and 5.2 ng/mL (median cord blood PFOS Denmark and Finland cohorts)	Cryptorchidism	OR 0.83 (0.44–1.58), whole cohort

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	8.1 and 7.0 ng/mL (PFHxS median in boys and girls)	Estradiol	NS (interquartile difference of -1.3, 95% CI -5.5–3.1), boys NS (2.1, 95% CI -2.2–6.5), girls
		Total testosterone	NS (-2.7, 95% CI -6.4–1.2), boys NS (0.2, 95% CI -3.5–4.0), girls
		Insulin-like growth factor-1	NS (-2.5, 95% CI -5.2–0.3), boys NS (-2.1, 95% CI -4.8–0.7), girls
Christensen et al. 2011 General population (n=448 girls)	1.6 ng/mL (maternal median PFHxS)	Earlier age of menarche	OR 0.89 (0.65–1.22)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFHxS)	Anogenital distance	NS (p=0.56), boys NS (p=0.10), girls
Maisonet et al. 2015 General population (n=72 girls aged 15 years)	>1.9 ng/mL (maternal 3rd tertile PFHxS)	Testosterone	β 0.18 (0.00–0.35), 3rd tertile
		SHBG	β 5.31 (-21.61–11.00), 3rd tertile
PFNA			
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	1.7 and 1.7 ng/mL (PFNA median in boys and girls)	Estradiol	NS (interquartile difference of -2.5, 95% CI -6.2–1.4), boys NS (-2.4, 95% CI -6.3–1.7), girls
		Total testosterone	NS (-2.1, 95% CI -5.5–1.3), boys NS (-1.9, 95% CI -5.5–1.9), girls
		Insulin-like growth factor-1	**Inverse association (-3.5, 95% CI -6.0 to -1.0)*, boys** **Inverse association (-3.8, 95% CI -6.4 to -1.2)*, girls**
Lind et al. 2017a General population (n=649 pregnant women)	0.7 ng/mL (median maternal serum PFNA)	Anogenital distance	β -0.5 (-2.1–1.1, p=0.63), boys **β -1.8 (-3.5 to -0.1, p=0.05)*, girls**

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Maisonet et al. 2015 General population (n=72 girls aged 15 years)	>0.6 ng/mL (maternal 3rd tertile PFNA)	Testosterone	β 0.05 (-0.14–0.24), 3rd tertile
		SHBG	β 7.91 (-8.69–24.52), 3rd tertile
PFDA			
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFDA)	Anogenital distance	β -0.6 (-2.0–0.9, p=0.97), boys **β -1.3 (-2.8–0.2, p=0.04)*, girls**
FOSA			
Christensen et al. 2011 General population (n=448 girls)	0.2 ng/mL (maternal median FOSA)	Earlier age of menarche	OR 0.91 (0.67–1.24)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; DHEAS = dihydroepiandrosterone sulfate; FOSA = perfluorooctane sulfonamide; FSH = follicle stimulating hormone; LH = luteinizing hormone; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SHBG = sex hormone binding globulin

alterations in birth weight were found for other perfluoroalkyls (PFHxS, PFNA, PFDA, PFUnA, PFDoDA). Overall, no associations were found between serum PFOA, PFOS, PFHxS, PFNA, or PFUnA and increases in the risk of low birth weight or small for gestational age infants. The small number of studies (2 or less) examining potential developmental effects of PFHpA, PFBA, and FOSA do not allow for assessing possible associations with pregnancy outcomes or birth outcomes.

No consistent results for risks of birth defects have been found; these potential endpoints were only examined for a few perfluoroalkyls. The available epidemiological data do not suggest associations between perfluoroalkyls and IQ or scholastic achievement for PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, or PFDoDA. Similarly, no associations were found between PFOA, PFOS, PFHxS, PFNA, or PFDA and increased risk of ADHD; several studies found decreased risk of ADHD. Inconsistent results have been found between PFOA and PFOS and delays in puberty or age of puberty, especially in girls.

Summaries of laboratory animal studies are presented in Tables 2-1, 2-3, 2-4, and 2-5 and the NOAEL and LOAEL values are presented in Figures 2-6, 2-8, 2-9, and 2-10; no data were available for PFHpA or FOSA. Laboratory animal studies provide strong evidence of the developmental toxicity of a number of perfluoroalkyls. Prenatal losses and decreases in pup survival were observed following exposure to PFOA, PFOS, PFNA, PFDA, PFDoDA and PFHxA; no deaths were observed in a single study of PFBS. Decreases in fetal weights, birth weight, and pup weight were observed in studies of PFOA, PFOS, PFNA, PFDA, PFUnA, PFBS, and PFHxA; no effects on weight were observed in studies on PFHxS or PFDoDA. In PFOA studies, delays in mammary gland development were observed at fairly low doses. Several studies have demonstrated biphasic alterations in motor activity in rodents exposed to PFOA, PFOS, and PFHxS; no effects on locomotor activity were observed in a study of PFDA. Studies in laboratory animals have examined a number of developmental endpoints, including pup survival, malformations, birth weight, mammary gland development, and neurodevelopment.

PFOA

Epidemiological Studies—Pregnancy Outcomes. The results of available epidemiological studies of women living near a PFOA facility and the general population do not suggest an association between serum PFOA levels and adverse pregnancy outcomes. No increases in risk of miscarriage (Darrow et al. 2014; Jensen et al. 2015; Savitz et al. 2012b; Stein et al. 2009), stillbirths (Savitz et al. 2012b), pregnancy loss (Buck Louis et al. 2016), or pre-term birth (Chen et al. 2012a; Darrow et al. 2013; Hamm et al. 2010; Manzano-Salgado et al. 2017a; Sagiv et al. 2018; Stein et al. 2009; Whitworth et al. 2012a) were found.

The Whitworth et al. (2012a) general population study reported a decrease in the risk of preterm births among women with serum PFOA levels in the 4th quartile. Most studies did not find an association between maternal PFOA levels and gestational age (Apelberg et al. 2007b; Chen et al. 2012a; Lauritzen et al. 2017; Li et al. 2017; Manzano-Salgado et al. 2017a) or gestational length (Lind et al. 2017; Sagiv et al. 2018). The exception is a study by Wu et al. (2012) of pregnant women with higher serum PFOA levels which found an inverse association between maternal serum PFOA levels and gestational age.

Epidemiological Studies—Birth Outcomes. Community and general population exposure studies have evaluated a number of birth outcomes including birth weight; risk of low birth weight; risk of small for gestational age; birth length; head, chest, and abdominal circumferences; ponderal index; sex ratio; and birth defects. In highly exposed populations, no association between maternal serum PFOA levels and birth weight were found (Darrow et al. 2013; Nolan et al. 2009; Savitz et al. 2012b). Several general population studies have found associations between maternal serum PFOA and birth weight. Fei et al. (2007, 2008a), Lauritzen et al. (2017), Lenters et al. (2016a), Maisonet et al. (2012), Minatoya et al. (2017), Starling et al. (2017), and Wu et al. (2012) found inverse associations between maternal serum PFOA and birth weight. However, 23 other general population studies did not find associations (Alkhalawi et al. 2016; Ashley-Martin et al. 2017; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Govarts et al. 2016; Hamm et al. 2010; Kim et al. 2011; Kobayashi et al. 2017; Lauritzen et al. 2017; Lee et al. 2013, 2016; Li et al. 2017; Lind et al. 2017; Manzano-Salgado et al. 2017a; Monroy et al. 2008; Robledo et al. 2015a; Sagiv et al. 2018; Shi et al. 2017; Wang et al. 2016; Washino et al. 2009; Whitworth et al. 2012a). As illustrated in Figure 2-35, most studies found no association between maternal serum PFOA levels and the risk of low birth weight infants (typically defined as <2,500 g) (Chen et al. 2012a; Darrow et al. 2013; Fei et al. 2007, 2008a; Manzano-Salgado et al. 2017a; Savitz et al. 2012b; Stein et al. 2009) or found a decreased risk of low birth weight infants (Nolan et al. 2009; Savitz et al. 2012a). Similarly, most studies found no increases in the risk for small for gestational age (Chen et al. 2012a; Fei et al. 2007, 2008a; Hamm et al. 2010; Lauritzen et al. 2017; Manzano-Salgado et al. 2017a; Savitz et al. 2012b; Wang et al. 2016; Whitworth et al. 2012a); these data are presented in Figure 2-36. One study (Savitz et al. 2012b) of C8 participants did find an increase in the risk of small for gestational age; however, when the maternal serum PFOA levels were categorized into percentiles, the risk was not increased in infants whose maternal serum PFOA levels were ≥80th percentile (21.0–717.6 ng/mL). A general population study (Lauritzen et al. 2017) also found an increased risk of small for gestational age (Lauritzen et al. 2017). Using data compiled from four European birth cohort studies in which cord serum PFOA was measured or estimated from breast milk levels, Govarts et al. (2018) did not find an association between cord PFOA and the risk of small for gestational age.

Figure 2-35. Risk of Low Birth Weight Infant Relative to PFOA Levels (Presented as Adjusted Odds Ratios)

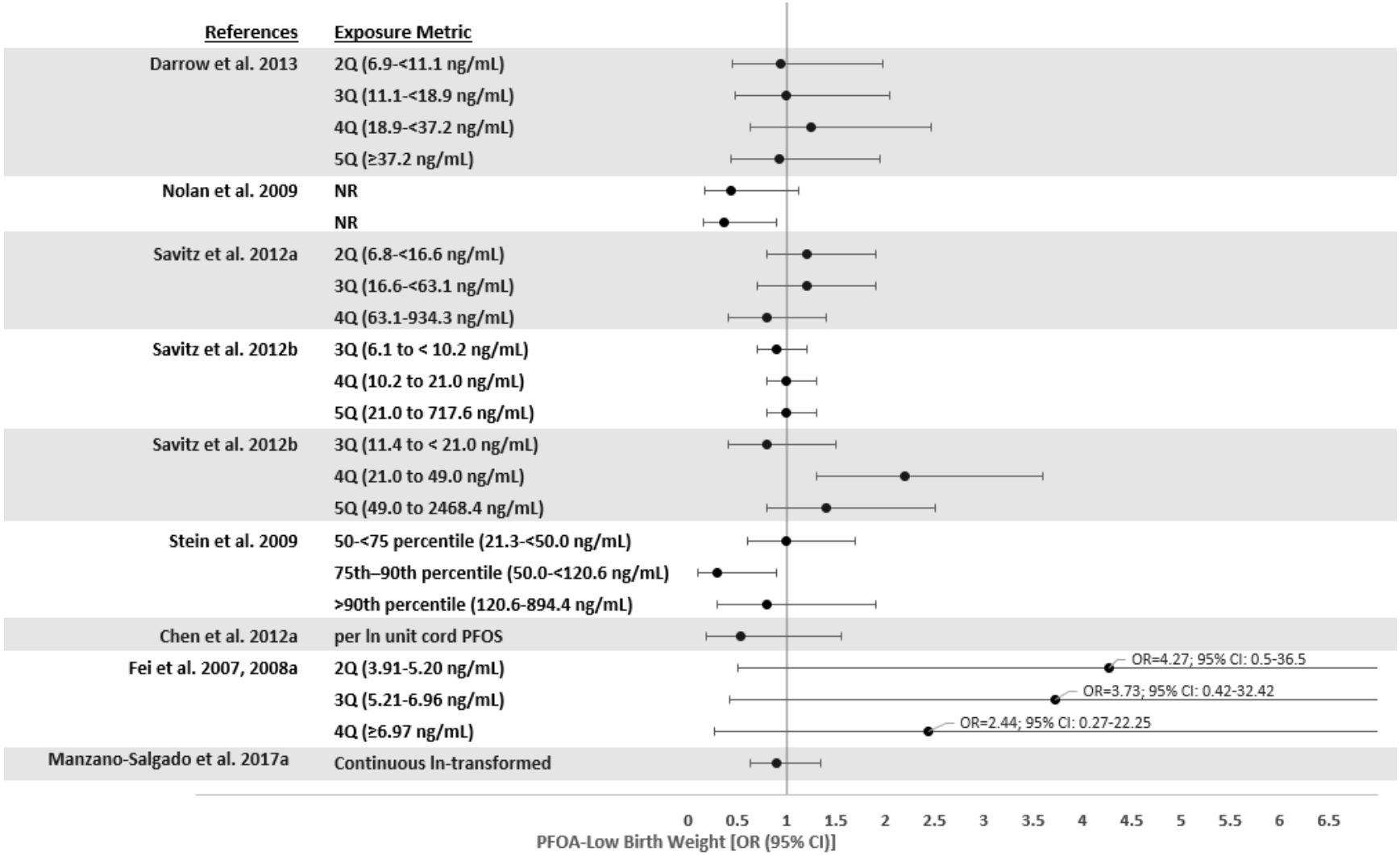
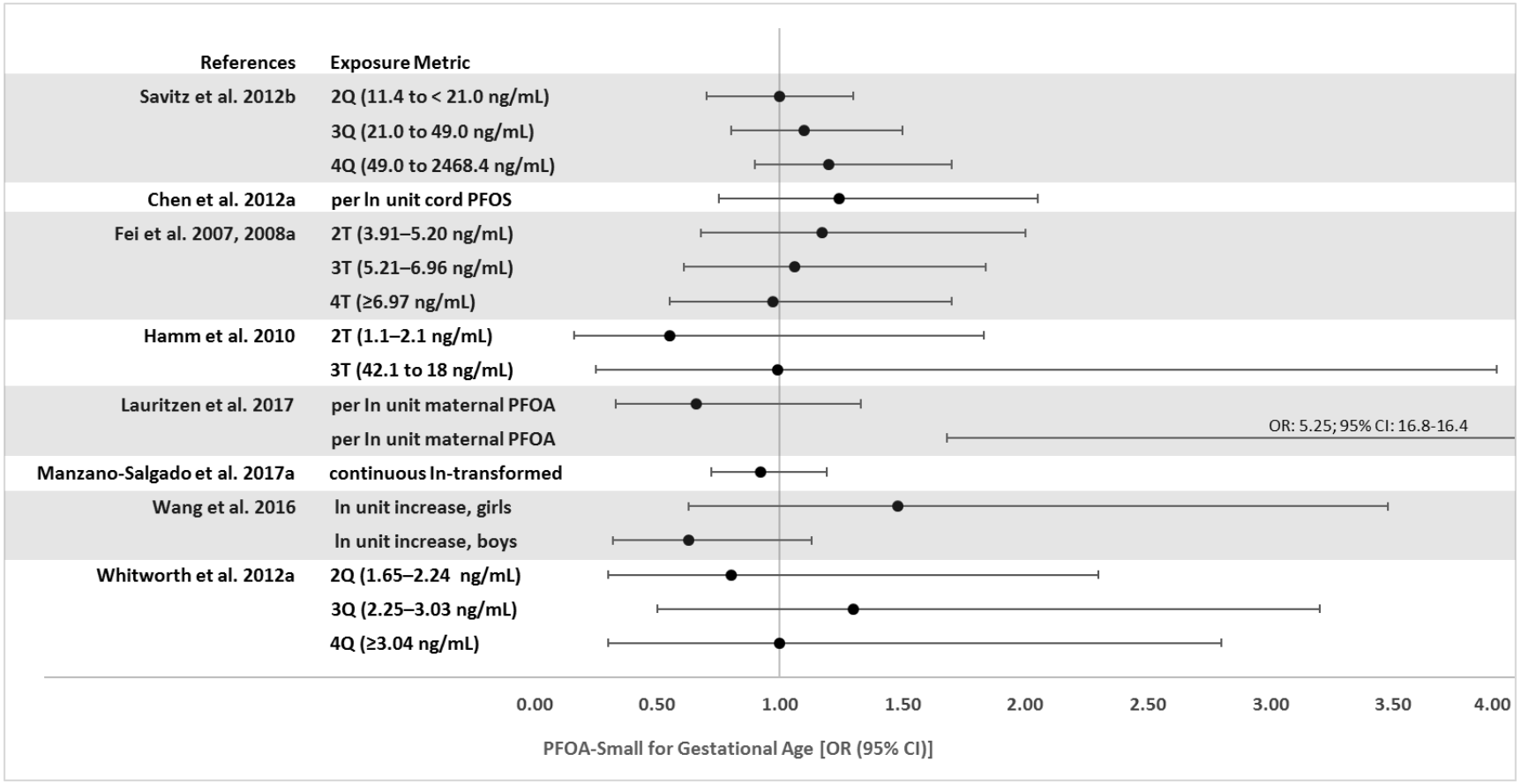


Figure 2-36. Risk of Small for Gestational Age Infant Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



However, among women who smoked during pregnancy, cord serum PFOA was associated with an increased risk of small for gestational age infants (OR 2.177, 95% CI 1.022–4.643); no association was found among nonsmoking women (OR 0.511, 95% CI 0.869–2.632). Six general population studies found inverse associations between maternal serum PFOA levels and birth length, abdominal circumference, and/or ponderal index (ratio of birth weight to birth length) (Alkhalawi et al. 2016; Apelberg et al. 2007b; Cao et al. 2018; Fei et al. 2007, 2008a; Lauritzen et al. 2017; Wu et al. 2012). However, most studies did not find associations between maternal serum PFOA levels and birth length; head, chest, or abdominal circumference; or ponderal index (Alkhalawi et al. 2016; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Kobayashi et al. 2017; Lauritzen et al. 2017; Lee et al. 2013; Maisonet et al. 2012; Manzano-Salgado et al. 2017a; Minatoya et al. 2017; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016). Studies examining newborn leptin and adiponectin levels (Ashley-Martin et al. 2017; Minatoya et al. 2017) and adiposity (Starling et al. 2017) have not found associations with maternal PFOA levels.

In a systematic review of 19 epidemiological studies discussed above, Johnson et al. (2014) evaluated the possible association between PFOA exposure and fetal growth and concluded that there was sufficient evidence that PFOA reduces fetal growth based on a moderate rating of the human evidence. A meta-analysis of the Apelberg et al. (2007b), Chen et al. (2012a), Fei et al. (2007, 2008a), Fromme et al. (2010), Hamm et al. (2009), Kim et al. (2011), Maisonet et al. (2012), Washino et al. (2009), and Whitworth et al. (2012) studies showed an association between PFOA and birth weight; a 1 ng/mL increase in serum or plasma PFOA was associated with a -18.9 g (95% CI -29.8 to -7.9) change in birth weight. The results of this meta-analysis are also reported in Lam et al. (2014). Johnson et al. (2014) and Lam et al. (2014) discuss whether glomerular filtration rate was a possible confounder in evaluating the association between serum PFOA and birth weight. They concluded that there was insufficient evidence of an association between glomerular filtration rate and birth weight.

A second meta-analysis (Verner et al. 2015) of the Apelberg et al. (2007b), Chen et al. (2012a), Fei et al. (2007), Hamm et al. (2010), Maisonet et al. (2012), Washino et al. (2009), and Whitworth et al. (2012a) studies found a similar result, a 1 ng/mL increase in PFOA levels was associated with a 14.72 g (95% CI -21.66 to -7.78) decrease in birth weight. Verner et al. (2015) also utilized a PBPK model to simulate maternal PFOA levels at delivery and evaluate the influence of glomerular filtration rate on the association between maternal PFOA and birth weight. In contrast to the conclusions of Johnson et al. (2014) and Lam et al. (2014), Verner et al. (2015) found that a 1 ng/mL increase in PFOA was associated

with a 7.13 g (95% CI -8.46 to -5.80) decrease in birth weight; suggesting that glomerular filtration rate may be a confounding factor.

A third meta-analysis conducted by Negri et al. (2017) of the Apelberg et al. (2007b), Bach et al. (2016), Chen et al. (2012a), Darrow et al. (2013), Fei et al. (2007), Fromme et al. (2010), Hamm et al. 2009), Kim et al. (2011), Maisonet et al. (2012), Monroy et al. (2008), Washino et al. (2009), and Whitworth et al. (2012a) studies reported a -12.80 g (95% CI -23.21 to -2.38) change in birth weight associated with a 1 ng/mL increase in serum PFOA.

A fourth meta-analysis conducted by Steenland et al. (2018) included 24 studies; 11 of the 12 studies included by Negri et al. (2017) (the Monroy et al. 2008 study was excluded) plus studies by Wu et al. (2012), Robledo et al. (2015a), Callan et al. (2016), Lee et al. (2016), Wang et al. (2016), Lenters et al. (2016a); Minatoya et al. (2017), Shi et al. (2017), Li et al. (2017), Manzano-Selgado et al. (2017); Starling et al. (2017), and Sagiv et al. (2018). The study found a that a 1 ng/mL increase in serum PFOA was associated with a -10.5 g (95% CI -16.7 to -4.4) change in birth weight. In sensitivity analysis, inclusion of the Savitz et al. (2012b) study, which used predicted maternal serum concentrations based on estimated environmental exposure, resulted in a birth weight change of -1.0 g (95% CI -2.4–0.4) per 1 ng/mL increase in serum PFOA. Categorizing studies based on when maternal serum PFOA levels were sampled resulted in differences in birth weight change; -3.3 g (95% CI -9.6–3.0) when sampled early in pregnancy or shortly after conception and -17.8 g (-25.0 to -10.6) when sampled late in pregnancy. The investigators suggested that this may be indicative of reverse causality or confounding.

A small number of studies have examined the potential associations between PFOA exposure and risks of birth defects. In a study of C8 Health Study participants, no increases in the risk of brain, gastrointestinal, kidney, craniofacial, eye, limb, genitourinary, or heart defects were found (Stein et al. 2014c).

Epidemiological Studies—Neurodevelopmental Outcomes. A number of epidemiological studies have evaluated neurodevelopment at various ages using maternal serum PFOA or cord blood PFOA as a biometric of exposure. Fei et al. (2008b) did not find an increased risk of Apgar scores of <10 in newborns. Another study found an inverse association between maternal serum PFOA and the 5-minute Apgar score (Wu et al. 2012). Utilizing the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) in 5-week-old infants, Donauer et al. (2015) found an increased risk of reduced muscle tone (hypotonia), which was associated with maternal serum PFOA levels, but found no associations on tests of social/easy going or high arousal/difficult. Goudarzi et al. (2016b) reported lower scores on tests of

mental and psychomotor development in female 6-month-old infants; no association was found when male and female infants were grouped together. When the infants were tested at 18 months of age, no association between maternal PFOA levels and mental and psychomotor indices were found. Fei et al. (2008b) did not find associations between maternal PFOA levels and the risk of delays in motor, cognitive, or language development in 6- and 18-month-old infants. It is noted that in the Fei et al. (2008b) study, the mothers were asked to recall at what age the infants reached a developmental milestone, whereas standardized tests of development were used in the other two studies. Although the Donauer et al. (2015) and Goudarzi et al. (2016b) studies suggest some delays in neurodevelopment in young infants, more research is needed before establishing a possible relationship with PFOA.

Studies in children have examined possible associations between PFOA and IQ, motor skills, behavior, and ADHD. An association between estimated *in utero* PFOA levels and IQ was found in 6–12-year-old children participating in the C8 Health Studies (Stein et al. 2013); higher IQ scores were found in children with the highest estimated PFOA exposure levels. The study did not find an association with reading or math skills. A general population study (Wang et al. 2015b) did not find an association between maternal serum PFOA levels and IQ scores in children 5 or 8 years of age. Jeddy et al. (2017) did not find an association between maternal PFOA levels and early communication development in 15-month-olds; among 38-month-olds, an inverse association was found for intelligibility scores, but there were no associations with other scores of communication development. In a prospective study, maternal PFOA levels were not associated with reading scores in 5- or 8-year-old children (Zhang et al. 2018). Reading scores at age 5 years were associated with serum PFOA levels when the children were 3 years of age and serum PFOA levels in 5-year-olds were not associated with reading scores at 8 years of age (Zhang et al. 2018). In a study of adults (20 years of age), Strøm et al. (2014) did not find an association between maternal PFOA levels and scholastic achievement. A community study of children and adolescents did not find an association between serum PFOA levels and learning problems in 12–15- or 5–18-year-olds (Stein and Savitz 2011). Two studies (Fei and Olsen 2011; Høyer et al. 2015a) did not find associations between maternal PFOA levels and motor coordination in 7-year-old children or motor skills in 5–9-year-old children.

Several studies have examined possible associations between maternal or child PFOA levels and scores on tests/surveys that assess behavioral problems. No associations were found between maternal PFOA levels and behavioral problems in 7-year-old children (Fei and Olsen 2011) or behavioral regulation problems in 5- or 8-year-old children (Vuong et al. 2016) or 7-year-old children (Oulhote et al. 2016). Similarly, no associations between serum PFOA levels and scores on behavioral tests were observed in 7-

year-old children (Oulhote et al. 2016) or 9–11-year-old children (Gump et al. 2011). No associations between breast milk PFOA levels and behavioral development in 6- and 24-month-old infants were observed (Forns et al. 2015). In contrast, Høyer et al. (2015a) found an association between maternal PFOA levels and behavioral problems in 5–9-year-old children; the risk was increased in children with maternal PFOA levels in the 3rd tertile. Stein et al. (2014a) found an association between the children's serum PFOA levels and survey results on behavioral problems and emotional disturbances in girls aged 6–12 years of age; this association was not found in boys or in boys and girls combined. Additionally, the association was only found when the survey was completed by mothers, but not when completed by the child's teacher. Oulhote et al. (2016) found associations between serum PFOA in 5-year-old children and behavioral survey scores, particularly for internalizing problems, peer relationships, and autism screening scores. In a study of 8-year-old children, Vuong et al. (2018) found an association between PFOA and at risk metacognition scores, but no associations with at risk behavior regulation or global executive scores.

Ten studies have looked for a possible association between PFOA and ADHD in children. Two studies of participants of the C8 Health Study found lower scores on tests for ADHD (Stein et al. 2013) or lower risks of ADHD (Stein and Savitz 2011) associated with estimated *in utero* PFOA or child PFOA levels, respectively. In a third community study in which parents and teachers completed surveys regarding ADHD-like behaviors (Stein et al. 2014a), no association between the child's serum PFOA (measured 3–4 years before the surveys were completed) and ADHD-like behaviors were found when the mothers completed the survey and an inverse association was found when the teachers completed the survey. Segregating the children by sex resulted in an association in girls (mother-completed survey only) and no associations in boys. Two general population studies have found associations between the risk of ADHD or increases in ADHD behavior in children. An increase in the risk of parent-reported ADHD diagnosis was observed in a study of 12–15-year-old NHANES participants (Hoffman et al. 2010). The second study (Høyer et al. 2015a) found increases in hyperactivity among 5–9-year-old children with maternal serum PFOA levels in the 3rd tertile. When this multinational cohort was segregated by country, the association was only found in the group of children from Greenland, but not in the Ukrainian cohort. Median serum PFOA levels were slightly higher in the Greenland cohort; it is also noted that the median maternal PFOS levels were 4 times higher in the Greenland cohort than in the Ukraine cohort. Other general population studies have not found associations. Two case-control studies of children did not find increased risks of being diagnosed with ADHD associated with maternal PFOA levels (Liew et al. 2015) or cord blood PFOA levels (Ode et al. 2014). Two studies did not find associations between cord blood PFOA levels and performance on tests evaluating for ADHD symptoms in 7-year-old children (Lien et al. 2016) or 18-month-old infants (Quaak et al. 2016). A third study found no association between maternal

PFOA levels and ADHD in 20-year-olds (Strøm et al. 2014). In addition to looking at possible relationships between PFOA and ADHD, two studies did not find associations between maternal PFOA levels and autism behaviors (Braun et al. 2014) or the risk of autism diagnosis (Liew et al. 2015).

Epidemiological Studies—Development of the Reproductive System. Studies exploring possible associations between PFOA and alterations in the development of the reproductive system have examined several outcomes including hormone levels in cord blood, hormone levels in children and adolescents, anogenital distance, congenital malformations of reproductive organs, and age of puberty in boys and girls.

A multinational case-control general population study (Vesterholm Jensen et al. 2014) found a decrease in the risk of cryptorchidism in the Finnish cohort, but not in the Danish cohort or in the combined cohort. With the exception of inhibin levels, no associations between maternal serum PFOA levels and cord blood levels of reproductive hormones were found (Itoh et al. 2016). Cord inhibin was associated with maternal serum PFOA levels in male infants, but not in female infants (Itoh et al. 2016). Some alterations in reproductive hormone levels were found in 6–9-year-old boys and girls participating in the C8 Health Study (Lopez-Espinosa et al. 2016). In boys, an inverse association between serum PFOA levels and total testosterone levels were observed; no associations were found for estradiol levels or insulin-like growth factor 1. In girls, an inverse association was found for insulin-like growth factor 1 levels and no associations were found for estradiol or testosterone levels. In adolescent girls, an association between maternal PFOA levels and testosterone levels was found (Maisonet et al. 2015a). This association was not found in young adult females (Kristensen et al. 2013). Other reproductive hormones were not shown to be associated with maternal PFOA levels (Kristensen et al. 2013; Maisonet et al. 2015a). Lind et al. (2017a) found no association between maternal PFOA levels and anogenital distance in boys or girls.

In a community exposure study (Lopez-Espinosa et al. 2011), increasing levels of serum PFOA were associated with delays in menarche in girls aged 8–18 years. Serum PFOA levels in the 2nd, 3rd, and 4th quartiles were associated with 142-, 163-, and 130-day delays in the onset of menarche, respectively. Using PBPK modeling, Wu et al. (2015) examined whether the association between serum PFOA and delays in the onset of menarche observed in the Lopez-Espinosa et al. (2011) study were due to reverse causality using a Monte Carlo PBPK model. They found that rapid growth around the time of menstruation onset may contribute to the apparent association between PFOA and delay of menarche. In the PBPK simulated study, the delay in the onset of menarche was 48 days for the 4th quartile (OR 0.82, 95% CI 0.76–0.88). A delay in menarche was also observed in a general population study; a 162-day

delay was estimated in the daughters of women with maternal serum PFOA levels in the 3rd tertile (Kristensen et al. 2013). A second general population study did not find an association between maternal serum PFOA levels and an earlier age of menarche (Christensen et al. 2011).

The only study available on age of puberty in males (Lopez-Espinosa et al. 2011) did not find an association with serum PFOA levels.

Laboratory Animal Exposure Studies. Exposure of pregnant Sprague-Dawley rats to 25 mg/m^3 APFO on GDs 6–15 resulted in a statistically significant reduction (10.3%) in neonatal body weight on PND 1, but the difference over controls was no longer significant on PND 4 (Staples et al. 1984). Exposure concentrations ≤10 mg/m^3 did not affect neonatal body weight. The incidence of malformations and variations among the exposed groups and controls was comparable.

In utero exposure to PFOA resulted in prenatal losses and decreases in pup survival. An increase in resorbed embryos were observed in mice administered 10 mg/kg on GD 13 (Chen et al. 2017b). An increase in resorptions was observed in mice administered ≥5 mg/kg/day throughout gestation (Lau et al. 2006) or 2 mg/kg/day on GDs 11–16 (Suh et al. 2011). Prenatal losses were also observed in PFOA mouse studies administering ≥6 mg/kg/day (Abbott et al. 2007), 5 mg/kg/day (White et al. 2011), or 20 mg/kg/day (Lau et al. 2006) throughout gestation; an increase in the percentage of dams with total litter loss was also observed at 5 mg/kg/day (Wolf et al. 2007). Administration of 20 mg/kg/day PFOA on GDs 7–17 or 10–17 did not result in litter loss (Wolf et al. 2007); no effect on litter size was observed as a result of administration of 5 mg/kg/day on GDs 8–17 (White et al. 2009). Gestational exposure (GDs 1–17) to PFOA also resulted in perinatal losses in mice administered 3 mg/kg/day PFOA (Ngo et al. 2014) and decreases in pup survival in mice exposed to ≥0.6 mg/kg/day (Abbott et al. 2007), 3 mg/kg/day (Albrecht et al. 2013), or 5 mg/kg/day (Lau et al. 2006; Yahia et al. 2010; White et al. 2011, Wolf et al. 2007); 100% pup mortality was observed in the offspring of mice exposed to 10 mg/kg/day throughout gestation (Yahia et al. 2010). Decreased pup survival was also observed in mice exposed to 5 mg/kg/day PFOA on GDs 15–17 (Wolf et al. 2007). No alterations in fetuses/litter or survival were observed at 1 mg/kg/day PFOA (Lau et al. 2006; White et al. 2011). Butenhoff et al. (2004b) also reported increases in pup mortality on PNDs 6–8 in the offspring of rats administered 30 mg/kg/day PFOA throughout gestation and during lactation.

Decreases in birth weight have not been consistently found in mouse studies with PFOA. No significant alterations in birth weight were observed in mice exposed to 3 mg/kg/day (Albrecht et al. 2013), 5 or

10 mg/kg/day (Lau et al. 2006), or 20 mg/kg/day (Abbott et al. 2007); decreases in birth or fetal weight were observed at 5 mg/kg/day (Hines et al. 2009; Yahia et al. 2010), 10 mg/kg/day (Suh et al. 2011), and 20 mg/kg/day (Lau et al. 2006). A decrease in mean litter weight on PNDs 2–14 was observed in mice administered ≥0.5 mg/kg/day PFOA on GDs 6–17 (Hu et al. 2010) and a decrease in pup body weight on PND 20 was observed in mice exposed to 5 mg/kg/day on GDs 8–17 or 12–17 (White et al. 2007). *In utero* exposure of mice to PFOA throughout gestation resulted in decreases in pup body weight in mice exposed to 1 mg/kg/day (Abbott et al. 2007; Hines et al. 2009), ≥3 mg/kg/day (Lau et al. 2006; Wolf et al. 2007), and 5 mg/kg/day (Yahia et al. 2010; White et al. 2007, 2011). In a cross-fostering study, lactation-only exposure (maternal dose of 5 mg/kg/day PFOA) resulted in decreased body weight in female pups on some PNDs (2, 3, 4, and 22, but not on PNDs 7, 10, 15, or 17) (Wolf et al. 2007). Hines et al. (2009) monitored body weights from birth to 18 months of age in female mice exposed *in utero* to PFOA on GDs 1–17. At weaning, decreases in body weight were observed at 1 and 5 mg/kg/day; by 10 weeks of age, there were no differences in body weight between the controls and mice exposed to ≥1 mg/kg/day. Significant increases in body weight were observed in mice exposed to 0.1 and 0.3 mg/kg/day, and by 20–29 weeks of age, the increases in body weight were observed in mice exposed to 0.01, 0.1, or 0.3 mg/kg/day. The largest increase in body weight gain (9.6%) was observed at 0.1 mg/kg/day; because the weight increase was less than 10%, the 0.1 mg/kg/day was considered a NOAEL. At 40 weeks of age, the increased body weight was observed in the 0.1 and 0.3 mg/kg/day groups. At termination (18 months of age), there were no differences in body weight between the controls and mice exposed to 0.01–3 mg/kg/day; a decrease in body weight was observed at 5 mg/kg/day. During the period of increased body weight in the lower-dose animals, there were no changes in serum glucose levels or the response to a glucose challenge, but there were significant increases in insulin and leptin levels at 0.01 and 0.1 mg/kg/day. Although there were no changes in the percentage of body fat to body weight measurements in mice at 42 weeks of age, at 18 months of age, significant decreases in abdominal body fat and increases in intrascapular brown fat was observed at ≥1 mg/kg/day PFOA (Hines et al. 2009). Based on systematic review of pup body weight data from the Abbott et al. (2007), Hines et al. (2009), Lau et al. (2006), White et al. (2007, 2009, 2011), and Wolf et al. (2007) mouse studies, Koustas et al. (2014) concluded that there was sufficient evidence that exposure to PFOA adversely affected fetal growth in animals. A meta-analysis estimate was a decrease of 0.023 g pup body weight per 1 mg/kg/day increase in PFOA dose.

A few studies have examined the potential of PFOA to induce malformations/variations. Lau et al. (2006) reported reductions in ossification of the proximal phalanges at ≥1 mg/kg/day and supraoccipital at 10 or 20 mg/kg/day. This study also reported enlarged fontanels in pups exposed to ≥1 mg/kg/day and tail and

limb defects at ≥5 mg/kg/day; however, there was no clear dose-response for these effects. Koskela et al. (2016) found altered femur and tibial bone morphology and decreased tibial mineral density in the offspring of mice exposed to 0.3 mg/kg/day in the diet on GDs 1–21. An increased percentage of litters with microcardia was also observed in the offspring of mice exposed to 10 or 20 mg/kg/day (Lau et al. 2006). No increases in the occurrence of malformations/variations were observed in the offspring of rats administered 100 mg/kg/day on GDs 6–15 (Staples et al. 1984) or in a 2-generation study at doses as high as 30 mg/kg/day (Butenhoff et al. 2004b).

Delayed eye opening was observed in the offspring of mice administered ≥1 mg/kg/day PFOA on GDs 1–17 (Abbott et al. 2007) and in mice administered 5 mg/kg/day throughout gestation (Lau et al. 2006; Wolf et al. 2007). Neither Albrecht et al. (2013) nor Lau et al. (2006) found alterations in eye opening in mice exposed to 3 mg/kg/day PFOA on GDs 1–17. Lau et al. (2006) also reported advanced (earlier than controls) preputial separation at ≥1 mg/kg/day and delayed vaginal opening at 20 mg/kg/day. The effect in the male offspring is in contrast to the Butenhoff et al. (2004b) study, which found delays in preputial separation in rats exposed to 30 mg/kg/day PFOA; a delay in vaginal patency was also observed at this dose.

A series of studies conducted by White and associates found significant delays in mammary gland development in the offspring of mice administered 1 mg/kg/day PFOA via gavage on GDs 8–17 (White et al. 2011) or 5 mg/kg/day PFOA on GDs 1–17, 8–17, 12–17, 10–17, 13–17, or 15–17 (White et al. 2007, 2009, 2011). The delay was characterized as reduced ductal elongation and branching and delays in timing and density of terminal end buds and was observed at all observational periods (PNDs 10, 20, 22, and 42, and 63 and 18 months of age). Decreases in mammary epithelial growth, as assessed by developmental scoring, were observed in the offspring of mice exposed to 0.01 mg/kg/day on GDs 1–17 (Tucker et al. 2015), 0.3 mg/kg/day on GDs 1–17 (Macon et al. 2011), or 0.01 mg/kg/day on GDs 10–17 (Macon et al. 2011). Tucker et al. (2015) noted that the delays in mammary gland development began at puberty and continued during young adulthood. Albrecht et al. (2013) did not find any alterations in mammary gland development on PND 20 in mouse offspring following *in utero* exposure to PFOA on GDs 1–17. Delayed mammary gland development was also observed in offspring only exposed via lactation (maternal dose of 3 mg/kg/day PFOA on GDs 1–17); the effects were observed on PNDs 42 and 63, but not on PND 22 (White et al. 2009). In a multigeneration study conducted by White et al. (2011), delays in mammary gland development were not consistently observed in the F2 offspring of F1 females that were exposed *in utero* to 1 or 5 mg/kg/day PFOA. However, delays in mammary gland development were observed in the F1 and F2 offspring exposed to 0.001 mg/kg/day *in utero* (GDs 7–17) and

postnatally. The investigators (White et al. 2011) noted that the delay in mammary gland development did not appear to affect lactational support based on normal survival and growth of the F2 pups. Tucker et al. (2015) noted dose-related strain differences on the effect of PFOA on mammary gland differences; effects were observed in CD-1 mice at ≥0.01 mg/kg/day and in C57BL/6 mice at ≥0.3 mg/kg/day (the highest NOAEL for this strain was 0.1 mg/kg/day); it is noted that the serum PFOA concentrations at a given dose were lower in the C57Bl/6 mice than in the CD-1 mice. Yang et al. (2009) reported strain differences in mammary gland effects in peripubertal mice administered PFOA for 4 weeks beginning on PND 21. In BALB/c mice, reductions in ductal length and decreased numbers of terminal end buds and stimulated terminal ducts were observed at 5 and 10 mg/kg. In contrast, 5 mg/kg resulted in mammary gland growth stimulation in C57BL/6 mice, as evidenced by increased number of terminal end buds with no alterations in ductal length. Mammary gland inhibition was observed in the C57BL/6 mice administered 10 mg/kg. Stimulation of mammary gland growth was also observed in PPARα knockout mice similarly administered 5 mg/kg (Zhao et al. 2010). In a series of experiments to evaluate the mechanism of PFOA-induced alterations in mammary gland development, Zhao et al. (2010) found that PFOA did not result in alterations in ovariectomized C57BL/6 mice administered 5 mg/kg 5 days/week for 4 weeks. In ovary-intact mice, PFOA enhanced mammary gland responses to exogenous estradiol and progesterone. Increased levels of epidermal growth factor receptor, hepatocyte growth factor, cyclin D1, and proliferating cell nuclear antigen levels were also found in PFOA-exposed C57BL/6 and PPARα-knockout mice (Zhao et al. 2010).

A consistent finding in the five mouse studies evaluating the neurodevelopmental toxicity of PFOA is an increase in motor activity. Increases in horizontal and ambulatory locomotor activity (tested on PND 60) were observed in the offspring of mice exposed to 0.1 mg/kg/day in the diet on GD 7 through PND 21 (Sobolewski et al. 2014); a decrease in resting time was also observed in the males. Increased ambulatory activity was observed on PND 18 in the offspring of mice administered 1 mg/kg/day on GDs 1–17 (Goulding et al. 2017). Significant increases in open field activity were observed at PND 36 in the offspring of mice exposed to 1.6 mg/kg/day throughout gestation and lactation (Cheng et al. 2013). Johansson et al. (2008) and Onishchenko et al. (2011) demonstrated a biphasic alteration in motor activity: an initial period of decreased activity followed by increased activity. Johansson et al. (2008) administered a single dose of 8.7 mg/kg/day PFOA to mice on PND 10 and monitored spontaneous activity for a 1-hour period when the mice were 2 or 4 months of age. In the first 20-minute period, there was a decrease in spontaneous activity, followed by a 20-minute period with an activity level similar to controls, and a 20-minute period with significantly increased spontaneous activity. Similarly, Onishchenko et al. (2011) reported an increase in activity in a 48-hour period in the adult offspring of

mice exposed to 0.3 mg/kg/day PFOA throughout gestation; however, there was a decrease in activity during the initial 3 hours of testing. Johansson et al. (2008) also found an increased susceptibility of the cholinergic system in mice exposed to 0.58 or 8.7 mg/kg/day PFOA on PND 10. In control mice, an injection of nicotine resulted in increases in activity; mice exposed to 0.58 mg/kg/day also responded with an increase in activity, although the increase was less than that observed in the controls. In contrast, nicotine resulted in a decrease in activity in mice exposed to 8.7 mg/kg/day. Exposure to PFOA did not alter learning or memory, as evidenced by the lack of effect on maze tests (Cheng et al. 2013; Johansson et al. 2008). Tests of neurobehavioral development found altered motor coordination and impaired negative geotaxis reflex, but no effect on righting reflex or cliff avoidance, in the offspring of mice exposed to 1.6 mg/kg/day throughout gestation and lactation (Cheng et al. 2013). Decreases in initial novel object exploratory behavior were also observed at 0.1 mg/kg/day, but there were no alterations in recognition time for novel objects (Sobolewski et al. 2014).

Support for the heart effects observed in the mouse study conducted by Lau et al. (2006) comes from a series of studies in chicken embryos and hatchlings that demonstrate the developmental cardiotoxicity of PFOA (Jiang et al. 2012, 2013, 2016). The avian model was selected due to the similarity between avian and mammalian cardiovascular development and the lack of direct maternal influence (Jiang et al. 2012). The effects following *in ovo* exposure include thinning of the right ventricular wall in chick embryos and alterations in left ventricular posterior wall dimension, volume, heart rate, stroke volume, and ejection fraction in the hatchlings (Jiang et al. 2012). Tests with WY 14,643, a PPARα agonist, and PFOA provide evidence that the cardiotoxicity involves both PPARα and bone morphorgenic protein 2 (BMP2) pathways (Jiang et al. 2013). Comparisons of results following *in ovo* exposure and *in vitro* exposure suggest that the cardiotoxicity was not likely due to cytotoxicity, but rather an alteration in early cardio morphology and function processes (Jiang et al. 2016).

Summary. Epidemiological studies have examined a number of potential developmental outcomes in communities living near a PFOA facility and in general populations. Although not consistently reported, the available general population studies suggest an inverse association between maternal serum PFOA levels and birth weight; a number of studies have not found this association. Several systematic reviews of these data have concluded that there was sufficient evidence that maternal PFOA levels are associated with reductions in fetal growth. After correcting for glomerular filtration rate, a small decrease in birth weight was associated with increases in maternal serum PFOA. Two of the three studies evaluating possible effects of sexual maturation found small delays in the start of menarche associated with maternal serum PFOA levels. Overall, the data do not suggest associations between serum PFOA levels and

adverse pregnancy outcomes such as miscarriages or stillbirths, most birth outcomes (e.g., risk of low birth weight, risk of small for gestational age, birth length, ponderal index, sex ratio, or birth defects), or neurodevelopmental outcomes (IQ or scholastic achievement, motor skills, and risk of ADHD). Animal studies provide strong evidence that developmental toxicity is a sensitive target of PFOA toxicity. Observed effects include prenatal losses and decreases in pup survival, decreases in birth weight, developmental delays such as delayed eye opening, delays in mammary gland development, and increased motor activity.

PFOS

Epidemiological Studies—Pregnancy Outcomes. No associations between maternal PFOS levels and the risk of miscarriages were observed in several studies (Darrow et al. 2014; Jensen et al. 2015; Stein et al. 2009). Three studies reported increases in the risk of preterm birth associated with maternal serum PFOS levels in the >90th percentile (>23.2 ng/mL) (Stein et al. 2009), maternal serum levels in the 2nd, 3rd, or 4th quartiles (≥18.9 ng/mL) (Sagiv et al. 2018), or cord blood PFOS levels in the 3rd and 4th quartiles (≥5.68 ng/mL) (Chen et al. 2012a), and one study reported a decrease risk in preterm birth (Whitworth et al. 2012a). Three other studies did not find associations for preterm birth (Fei et al. 2007, 2008a; Hamm et al. 2010; Manzano-Salgado et al. 2017a), one study found no association between serum PFOS and pregnancy loss (Buck Louis et al. 2016), and five studies found no associations between maternal PFOS levels and gestational age or length (Lauritzen et al. 2017; Li et al. 2017; Lind et al. 2017a; Manzano-Salgado et al. 2017a).

Epidemiological Studies—Birth Outcomes. Occupational, community, and general population exposure studies have examined the possible associations between maternal PFOS levels and a number of birth outcomes including birth weight; risk of low birth weight; risk of small for gestational age; birth length; head, chest, and abdominal circumferences; ponderal index; sex ratio; and birth defects. Most studies did not find associations between maternal serum PFOS levels and birth weight (Alkhalawi et al. 2016; Apelberg et al. 2007b; Ashley-Martin et al. 2016, 2017; Callan et al. 2016; Cao et al. 2018; Darrow et al. 2013; Bach et al. 2016; Fei et al. 2007, 2008a; Govarts et al. 2016; Hamm et al. 2010; Kim et al. 2011; Kobayashi et al. 2017; Lauritzen et al. 2017; Lee et al. 2013, 2016; Lenters et al. 2016a; Lind et al. 2017a; Maisonet et al. 2012; Manzano-Salgado et al. 2017a; Minatoya et al. 2017; Monroy et al. 2008; Robledo et al. 2015a; Sagiv et al. 2018; Shi et al. 2017; Starling et al. 2017; Whitworth et al. 2012a), including an occupational exposure study (Grice et al. 2007) in which female workers were exposed to very high levels of PFOS (serum levels ranged from 1,300 to 1,970 ng/mL). Five studies did find inverse associations

between birth weight and maternal serum PFOS levels. In the Washino et al. (2009) study, an inverse association was found between maternal serum PFOS levels and birth weight; segregating by sex resulted in an inverse association in girls, but not in boys. The magnitude of the change was small, 148.8 g decrease in birth weight per log unit increase in maternal PFOS for combined. Maisonet et al. (2012) also reported small decreases in birth weight (140.1 g) in infants whose mother's serum PFOS levels were in the 3rd tertile. Lauritzen et al. (2017) also reported an inverse association between birth weight and maternal serum PFOS levels (292 g per ln unit increase in PFOS). Similarly, Chen et al. (2012a) reported an inverse association between cord blood PFOS and birth weight, but the magnitude was small (110.2 g decrease per ln unit increase in cord PFOS levels). Li et al. (2017) also reported a small decrease in birth weight associated with cord PFOS levels (95 g decrease per ln increase in cord PFOS levels); when infants were categorized by sex, the association was only found among boys. Although these studies found decreases in birth weight associated with PFOS levels, no studies found increases in the risk of low birth weight infants (Chen et al. 2012a; Darrow et al. 2013; Fei et al. 2007, 2008a; Manzano-Salgado et al. 2017a; Stein et al. 2009) or small for gestational age infants (Chen et al. 2012a; Fei et al. 2007, 2008a; Hamm et al. 2010; Lauritzen et al. 2017; Manzano-Salgado et al. 2017a; Whitworth et al. 2012a). The ORs for low birth weight and small for gestational age risks are presented in Figures 2-37 and 2-38. Analysis of data compiled from four European birth cohort studies found an inverse association between cord PFOS levels (measured levels and levels estimated from breast milk PFOS levels) and small for gestational age (OR 0.823, 95% CI 0.741–0.913) (Govarts et al. 2018). When subjects were segregated based on whether they smoked during pregnancy, a positive association was found among smokers (OR 1.627, 95% CI 1.024–2.588) and an inverse association was found among nonsmokers (OR 0.661, 95% CI 0.644–0.717). Three studies have evaluated leptin and adiponectin hormone levels or adiposity in newborns. Maternal PFOS levels were not associated with alterations in leptin levels (Ashley-Martin et al. 2017; Minatoya et al. 2017). Mixed results were found for adiponectin levels with one study finding no alterations (Ashley-Martin et al. 2017) and another finding an association (Minatoya et al. 2017). No association was found between maternal PFOS levels and adiposity at birth (Starling et al. 2017).

Verner et al. (2015) conducted a meta-analysis of the Apelberg et al. (2007b), Chen et al. (2012a), Fei et al. (2007), Hamm et al. (2010), Maisonet et al. (2012), Washino et al. (2009), and Whitworth et al. (2012a) studies and found that a 1 ng/mL increase in maternal PFOS levels was associated with a 5.00 g (95% CI -8.92 to -1.09) decrease in birth weight. When the data were re-analyzed utilizing a PBPK model to account for glomerular filtration rate, the magnitude of the effect of PFOS on birth weight decreased (Verner et al. 2015). A 1 ng/mL increase in PFOS was associated with a 2.72 g (95% CI -3.40 to -2.04) decrease in birth weight. A second meta-analysis conducted by Negir et al. (2017)

Figure 2-37. Risk of Low Birth Weight Infant Relative to PFOS Levels (Presented as Adjusted Odds Ratios)

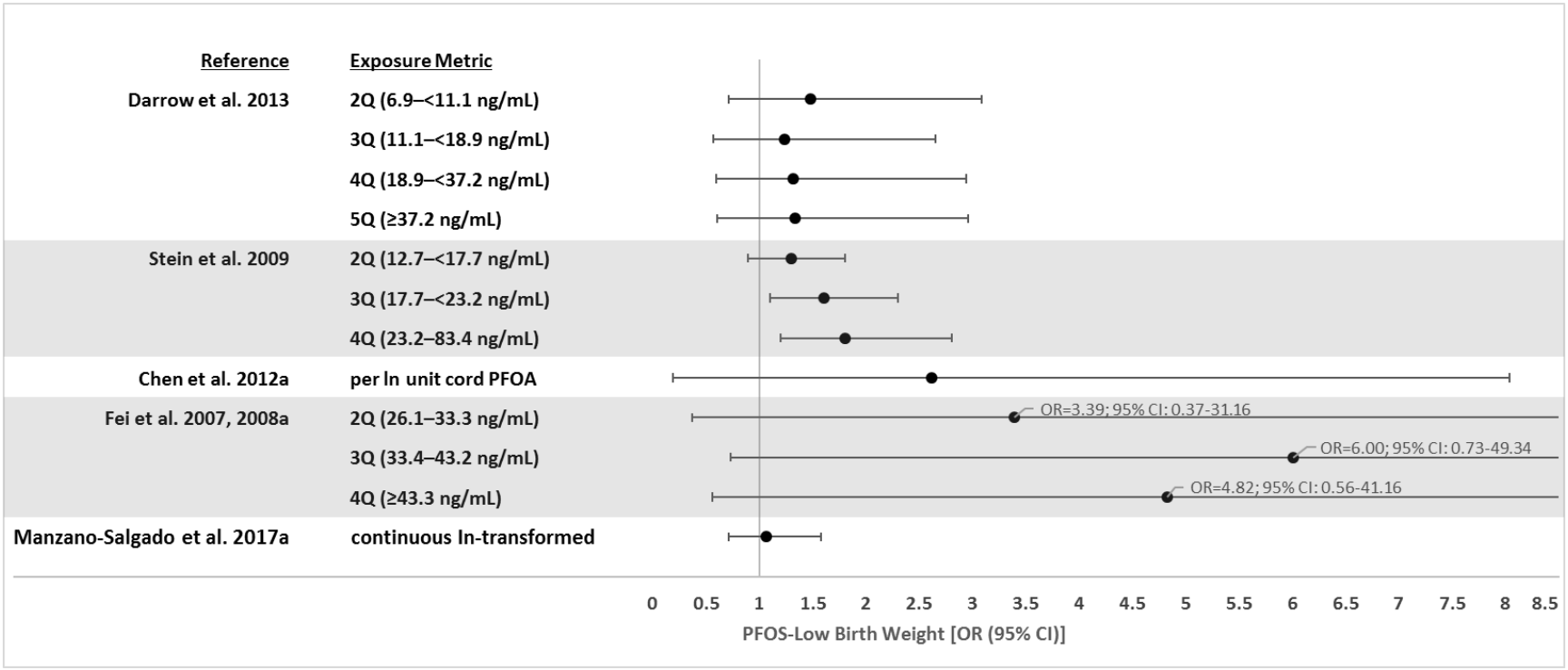
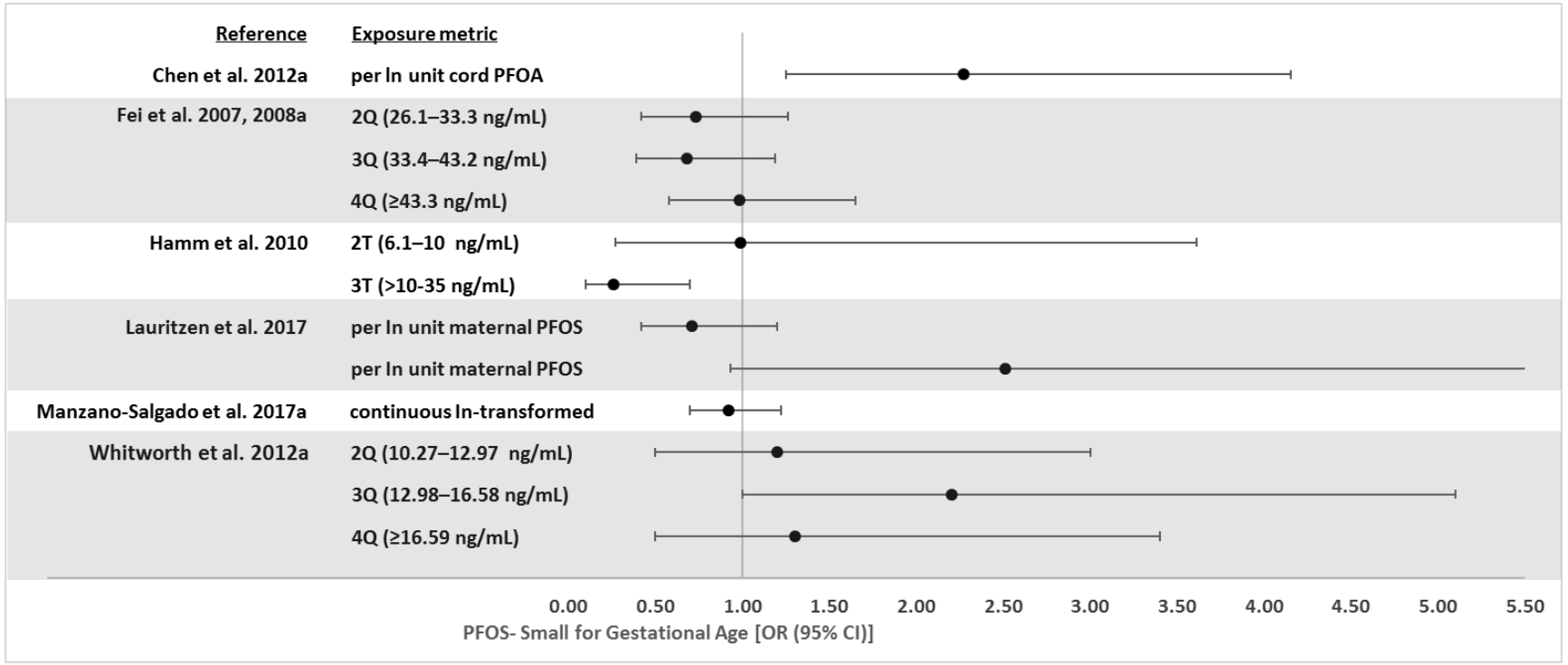


Figure 2-38. Risk of Small for Gestational Age Infant Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



utilized data from the Fei et al. (2007), Monroy et al. (2008), Washino et al. (2009), Hamm et al. (2010), Chen et al. (2012a), Maisonet et al. (2012), Whitworth et al. (2012a), and Bach et al. (2016) studies. The investigators found a -0.92 g (95% CI -3.43–1.60) change in birth weight per 1 ng/mL increase in serum PFOS.

Maternal PFOS was not associated with birth length (Alkhalawi et al. 2016; Apelberg et al. 2007b; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Kobayashi et al. 2017; Laurizten et al. 2017; Lee et al. 2013; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Shi et al. 2017; Washino et al. 2009) with the exception of the finding of small decreases in birth length (≤1.2 cm) that was associated with serum PFOS levels (Fei et al. 2007, 2008a; Lauritzen et al. 2017). Four studies reported inverse associations between ponderal index and cord blood PFOS levels (Apelberg et al. 2007b) or maternal serum PFOS levels (Alkhalawi et al. 2016; Lee et al. 2013; Minatoya et al. 2017); other studies did not find this effect (Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Maisonet et al. 2012; Robledo et al. 2015a; Shi et al. 2017). Two studies reported small decreases in head circumference, which were associated with maternal serum PFOS levels (Apelberg et al. 2007b) and cord blood PFOS (Chen et al. 2012a); other studies have not found associations (Bach et al. 2016; Callan et al. 2016; de Cock et al. 2014; Fei et al. 2007, 2008a; Lauritzen et al. 2017; Lee et al. 2013; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Washino et al. 2009).

One study reported no increases in the risk of birth defects associated with maternal serum PFOS levels (Stein et al. 2009); a second study found an increased risk of congenital cerebral palsy in girls, but not in boys (Liew et al. 2014). Bae et al. (2015) did not find associations between the odds of having a boy and paternal or maternal serum PFOS levels.

Epidemiological Studies—Neurodevelopmental Outcomes. Epidemiological studies examined several aspects of neurodevelopment, including age of reaching neurobehavioral milestones, IQ, motor development, behavior, ADHD, and autism. Fei et al. (2008b) did not find associations between maternal PFOS levels and the risk of having an Apgar score of <10 or in motor and mental development at 6 months. However, the study did find that some neurobehavioral milestones (delay in sitting, early use of word-like sounds, and delays in using two-word sentences) were associated with maternal PFOS levels. Goudarzi et al. (2016b) did not find alterations on mental and psychomotor development in 6- and 18-month-old infants that were associated with maternal serum PFOS levels. A third study of infants did not find alterations in neurobehavioral or muscle coordination tests (Donauer et al. 2015).

In the only study evaluating IQ, Wang et al. (2015b) did not find associations between maternal PFOS levels and IQ score in children 5 or 8 years of age. Zhang et al. (2018) did not find associations between maternal PFOS levels and reading scores in 5- or 8-year-old children. However, associations were found between the child's serum PFOS levels at age 3 years and reading scores at 5 years of age and serum PFOS levels at 5 years of age and reading scores at 8 years of age. Strøm et al. (2014) found no associations between scholastic achievement in 20-year-olds and maternal PFOS levels. In a study of children living in a community with high PFOA contamination, Stein and Savitz (2011) found decreases in the risk of learning problems in children 5–18 or 12–15 years of age. In contrast, Vuong et al. (2016) found increased risks of global executive functioning and metacognition problems that were associated with maternal PFOS levels. Another study found an association between maternal PFOS levels and verbal comprehension in 15-month-olds, but an inverse association with intelligibility scores in 38-month-olds (Jeddy et al. 2017). A subsequent study by Vuong et al. (2018) did not find associations between serum PFOS levels in 8-year-old children and metacognition or global executive functioning scores. Four studies have not found associations between maternal PFOS levels and behavioral health and motor coordination/skills in children (Fei and Olsen 2011; Høyer et al. 2015a; Oulhote et al. 2016), between breast milk PFOS levels and behavioral development in 6- and 24-month-old infants (Forns et al. 2015), or between serum PFOS levels age 5 or 7 years and behavioral development in 7-year-old children (Oulhote et al. 2016). A fifth study (Vuong et al. 2016) found an increased risk for problems with behavioral regulation. The available data do not suggest an association between maternal PFOS levels or cord blood PFOS levels and the risk of ADHD or ADHD behaviors (Hoffman et al. 2010; Liew et al. 2015; Ode et al. 2014; Quaak et al. 2016; Stein and Savitz 2011; Strøm et al. 2014), although Liew et al. (2015) found a decreased risk of ADHD diagnosis in children whose mothers had serum PFOS levels in the 4th quartile. Similarly, Høyer et al. (2015a) did not find increases in the risk of hyperactivity in children and Gump et al. (2011) found a decrease in impulsivity. Braun et al. (2014) and Liew et al. (2015) did not find associations between maternal PFOS and autism risk.

Epidemiological Studies—Development of Reproductive System. Several epidemiological studies have examined the possible associations between PFOS and the development of the reproductive system, including the risk of congenital defects to reproductive organs, alterations in reproductive hormone levels, and age of puberty; the results of these studies are summarized in Table 2-25. No alterations in the risk of cryptorchidism (Toft et al. 2016; Vesterholm Jensen et al. 2014) or hypospadias (Toft et al. 2016) were found in two studies. No association between maternal PFOS levels and anogenital distance was found in boys and an inverse association was found in girls (Lind et al. 2017a). Itoh et al. (2016) reported associations between maternal PFOS levels and alterations in cord blood hormone levels, in particular

estradiol in males, testosterone:estradiol ratio in males (inverse association), progesterone levels in males and females, prolactin levels in females, and inhibin levels in males. Similarly, Toft et al. (2016) found associations between amniotic fluid PFOS levels and levels of testosterone, androstenedione, progesterone, and insulin-like factor 3 (inverse association) in amniotic fluid. Lopez-Espinosa et al. (2016) also found a number of alterations in reproductive hormone levels in 6–9-year-old boys and girls. In the boys, inverse associations between serum PFOS levels and estradiol, total testosterone, and insulin-like growth factor 1 were observed. Inverse associations between total testosterone and insulin-like growth factor 1 and serum PFOS levels were also observed in the girls. A study of young adult women found no associations between reproductive hormone levels and maternal PFOS levels (Kristensen et al. 2013).

A study of 8–18-year-old children found delays in the age of puberty in boys and girls (Lopez-Espinosa et al. 2011) that were associated with serum PFOS levels. In the children with serum PFOS levels in the 3rd and 4th quartiles, the respectively delays were 131 and 190 days in boys and 141 and 138 days in girls. In contrast, two other studies have not found alterations in either the age of menarche or an earlier age of menarche that were associated with maternal PFOS levels (Christensen et al. 2011; Kristensen et al. 2013). The differences in the biomarker of exposure and the potential exposure to high levels of PFOA in the Lopez-Espinosa et al. (2011) community study make it difficult to compare the results of these three studies. As discussed in the PFOA section, Wu et al. (2009) reanalyzed the Lopez-Espinosa et al. (2011) data using a Monte Carlo PBPK model, which accounted for rapid growth occurring around puberty, and found much shorter delays in the age of menarche than found in the Lopez-Espinosa et al. (2011) study. In the girls with simulated serum PPFOS levels in the 4th quartile, the delay was 72 days (OR 0.75, 95% CI 0.70–0.81).

Laboratory Animal Exposure Studies. Increases in fetal mortality and decreases in pup survival have also been observed in rats and mice exposed to PFOS *in utero* (Abbott et al. 2009; Chen et al. 2012b; Fuentes et al. 2006; Grasty et al. 2003, 2005; Lau et al. 2003; Lee et al. 2015a; Luebker et al. 2005a, 2005b; Ngo et al. 2014; Thibodeaux et al. 2003; Xia et al. 2011; Yahia et al. 2008). Increases in the number of resorptions and dead fetuses were observed in mice administered ≥0.5 mg/kg/day (Lee et al. 2015a); increases in abortions between GD 22 and 28 were observed in rabbits treated with 3.75 mg/kg/day PFOS by gavage on GDs 6–20 (Case et al. 2001). Decreases in the number of live fetuses were observed in mice exposed to ≥2.0 mg/kg/day on GDs 11–16 and 20 mg/kg/day on GDs 1–17 (Thibodeaux et al. 2003) or GDs 0–17 (Yahia et al. 2008). Increases in perinatal losses were observed in the litters of mice administered ≥0.1 mg/kg/day PFOS on GDs 1–17 (Ngo et al. 2014). Pup survival is

affected at lower maternal doses. Significant decreases in pup survival were observed in rats at 1.6 mg/kg/day (dams were exposed for 6 weeks prior to mating and during gestation through lactation days 4 or 21) (Luebker et al. 2005a, 2005b) and in mice exposed to 4.5 mg/kg/day on GDs 15–18 (Abbott et al. 2009); no alterations in pup survival were observed in rats or mice exposed to 1 mg/kg/day (Luebker et al. 2005b; Yahia et al. 2008). A series of studies by Grasty et al. (2003) in rats that were exposed for 4 days during different gestational periods showed that the pup was more susceptible if exposure occurred later in gestation. On PND 4, pup survival was 70, 50, 60, 20, or 5% for exposures on GDs 2–5, 6–9, 10–13, 14–17, or 17–20, respectively. Grasty et al. (2003) and others (Abbott et al. 2009; Chen et al. 2012b; Lau et al. 2003) also noted that most deaths occurred within the first 4 PNDs, with the highest rates occurring on PND 1. Lau et al. (2003) and Luebker et al. (2005a) found that cross fostering did not significantly improve pup survival; deaths were observed in the *in utero* only exposure group. However, Luebker et al. (2005a) showed that rats exposed *in utero* and during lactation had the highest pup mortality, as compared to other cross-fostered groups. The mechanism involved in the early pup mortality has not been identified, but there is some indication that pulmonary deficits may be a contributing factor. At high doses (50 mg/kg/day administered on GDs 19–20), pups demonstrated difficulty breathing within minutes of birth (Grasty et al. 2003). Histological examination of the lungs of pups exposed to 25 or 50 mg/kg/day on GDs 19–20 showed evidence of delayed lung maturation (Grasty et al. 2003, 2005), specifically, an increase in the proportion of solid lung tissue and a decrease in the proportion of small airway tissue. A comparison of the lungs of PFOS-exposed neonates to control fetuses (GD 21) showed that 17 and 50% of the lung tissue in the neonates exposed to 25 or 50 mg/kg/day, respectively, on GDs 19–20 was not histologically different from the control fetuses (Grasty et al. 2005). Administration of therapeutic agents known to enhance terminal lung maturation and accelerate surfactant production did not improve pup survival (Grasty et al. 2005). Histological damage has also been reported in pups exposed to lower PFOS levels. Lung atelectasis was observed in pups exposed to 10 mg/kg/day on GDs 0–18 (Yahia et al. 2008). No lung effects were observed in pups exposed to 1 mg/kg/day or in fetuses exposed to 20 mg/kg/day on GDs 0–17 (Yahia et al. 2008). Alveolar hemorrhage, thickened epithelial walls of the pulmonary alveolus, focal lung consolidation, and focal infiltration of inflammation cells were observed in pups exposed to 2 mg/kg/day on GDs 0–21; no lung effects were observed at 0.1 mg/kg/day (Chen et al. 2012b).

Decreases in fetal body weight, birth weight, and pup body weight have been observed in rats, mice, and rabbits exposed to PFOS (Case et al. 2001; Chen et al. 2012b; Era et al. 2009; Fuentes et al. 2006, 2007b; Grasty et al. 2003; Lau et al. 2003; Lee et al. 2015a; Li et al. 2016; Luebker et al. 2005a, 2005b; Rogers et al. 2014; Xia et al. 2011; Yahia et al. 2008). In rats, the lowest-adverse-effect level for decrease in fetal

body weight was 10 mg/kg/day following administration on GDs 2–20 (Thibodeaux et al. 2003) and the highest no-effect level was 5 mg/kg/day, also identified in the Thibodeaux et al. (2003) study. Decreases in rat pup birth weight and body weight on PND 4 were observed in the offspring of rats exposed to 0.4 mg/kg/day for 42 days prior to mating and gestation through lactation day 4 (Luebker et al. 2005b). Mice appear to be less sensitive to the effect of PFOS on pup body weight than rats (Lau et al. 2003). Exposure of rats to 2 mg/kg/day PFOS on GDs 2–21 resulted in significant decreases in birth weight and pup body weight on PNDs 1–3; exposure to 5 mg/kg/day resulted in decreases in pup body weight through PND 19. In contrast, no alterations in birth weight or pup body weight were observed in mice exposed to doses as high as 5 mg/kg/day on GDs 1–18. Fuentes et al. (2007b) reported the lowest LOAEL of 6 mg/kg/day for decreases in pup weight in mice exposed on GDs 12–18. Decreases in fetal body weight were observed in mice exposed to 10 mg/kg/day on GDs 0–17 (Yahia et al. 2008). Fuentes et al. (2006) did not find decreases in fetal body weight following exposure to 6 mg/kg/day on GDs 6–18. In rabbits, a decrease in fetal body weight was observed following exposure to 2.5 mg/kg/day on GDs 6–20, but not at 1 mg/kg/day (Case et al. 2001).

Several studies also reported delays in developmental milestones. Delays in eye opening were observed in rats exposed to 2 mg/kg/day on GDs 2–21 (Lau et al. 2003) or 0.4 mg/kg/day for 42 days prior to mating and throughout the gestation and lactation periods (Luebker et al. 2005a) and in mice exposed to 8.5 mg/kg/day on GDs 15–18 (Abbott et al. 2009). Fuentes et al. (2007b) did not find a delay in eye opening in mouse pups exposed to 6 mg/kg/day on GDs 12–18, but did find a delay in pinna detachment at this dose level. A decrease in neuromuscular development, as evidenced by a delay in tail pull reflex, climbing ability, and forelimb grip strength, was observed in mice exposed to 6 mg/kg/day on GDs 12–18 (Fuentes et al. 2007b).

Prenatal exposure to PFOS has resulted in malformations/anomalies/variations in rats, mice, and rabbits (Case et al. 2001; Era et al. 2009; Thibodeaux et al. 2003; Yahia et al. 2008). An increased incidence of cleft palate was observed in rats exposed to 10 mg/kg/day on GDs 2–20 (Thibodeaux et al. 2003) and in mice exposed to 10 mg/kg/day on GDs 0–17 (Yahia et al. 2008), 15 mg/kg/day on GDs 1–17 (Thibodeaux et al. 2003), 20 mg/kg/day on GDs 1–17 (Era et al. 2009), and 50 mg/kg/day on GDs 11–15 (Era et al. 2009). Other skeletal and external alterations included sternal defects in rats exposed to 10 mg/kg/day on GDs 2–20 (Thibodeaux et al. 2003) and mice exposed to 1 mg/kg/day on GDs 0–17 (Yahia et al. 2008), delayed skeletal ossification in rabbits exposed to 2.5 mg/kg/day on GDs 6–20 (Case et al. 2001), wavy ribs and spina bifida occulta in mice exposed to 10 mg/kg/day on GDs 1–17 (Yahia et al. 2008), and tail abnormalities and delayed ossification of phalanges at 20 mg/kg/day (Yahia et al.

2008). Visceral abnormalities, consisting of enlarged right atrium at 10 mg/kg/day, and ventricular septal defects at 20 mg/kg/day were observed in mice exposed on GDs 1–17 (Thibodeaux et al. 2003). No malformations/anomalies/variations were found by Thibodeaux et al. (2003) in mice exposed to 1 mg/kg/day on GDs 1–17 or by Fuentes et al. (2006) in mice exposed to 6 mg/kg/day on GDs 6–18. In addition to the previously discussed histological alterations observed in the pups exposed to lethal doses, mild to severe intracranial dilatation of blood vessels was observed in fetuses exposed to 20 mg/kg/day on GDs 0–17 and in pups exposed to 10 mg/kg/day on GDs 0–18 (Yahia et al. 2008). No histological alterations were observed in the heart of rat pups exposed to 2 mg/kg/day on GDs 2–21 (Xia et al. 2011); the study also found no alterations in heart rate or blood pressure. Lee et al. (2015b) found increases in cholesterol levels in fetal livers of mice exposed to PFOA on GDs 1–17 and Wan et al. (2014b) found increases in relative liver weights in pups on PND 21.

A study with wild-type mice (129S1/Svlm) and PPARα-null mice evaluated the influence of PPARα on developmental toxicity of PFOS (Abbott et al. 2009). Decreases in pup survival and delays in eye opening were observed in both strains, although lower LOAELs were identified in the wild-type mice. The investigators concluded that neonatal lethality and delayed eye opening was not dependent on PPARα activation.

Neurodevelopmental studies have shown that prenatal and/or postnatal exposure to PFOS can affect motor activity, but does not appear to affect learning or memory. A significant decrease in locomotion was observed in male mice aged 5–8 weeks exposed to 0.3 mg/kg/day on GDs 1–17 when they were placed in a novel environment (Onishchenko et al. 2011). Hallgren et al. (2015) reported biphasic alterations in spontaneous activity in 2-month-old mice administered a single dose of 11.3 mg/kg on PND 10; locomotor activity was reduced during the first 20-minute period, was unchanged in the second period, and increased during the third period. Decreases in circadian activity were noted in males and increases in the number of inactive periods were noted in males and females when they were observed over a 48-hour period. The study also found increased inactivity in an elevated plus maze test. In an open field test of 70-day-old mice exposed to 6 mg/kg/day on GDs 12–18, an increase in the amount of time spent in the center of the field was found; no changes in vertical movement were found (Ribes et al. 2010). In 3-month-old mice exposed to 6 mg/kg/day on GDs 12–18, a decrease in the distance traveled was observed after 20–25 minutes in an open field apparatus; activity was not affected during the first 5 minutes of the test (Fuentes et al. 2007a). In a 15-minute open field test, prenatal exposure to 6 mg/kg/day PFOS on GDs 12–18 did not alter motor activity in 3-month-old mice (Fuentes et al. 2007b). In contrast, Butenhoff et al. (2009b) found a significant increase in locomotion in male rats exposed to

0.3 or 1.0 mg/kg/day PFOS throughout gestation and lactation. However, this effect was only observed in male rats on PND 17; no significant alterations were observed on PNDs 13, 21, or 61. An increase in locomotion was observed in female rats on PND 21 exposed to 1.0 mg/kg/day, but not at other time points. To evaluate the biological relevance of the increased activity, activity was analyzed by 1-minute sequential time periods. The investigators concluded that the increased activity observed in the 0.3 mg/kg/day males at PND 17 and 1.0 mg/kg/day females at PND 21 was not treatment-related due to the lack of significant changes in total or ambulatory activity and the similarity in habituation pattern between the treated groups and controls. In the 1.0 mg/kg/day PND 17 males, the pattern of habituation differed from controls and there was an increase in ambulatory activity; this increase in locomotor activity was considered to be related to PFOS exposure. The increased activity was observed in the last three time periods. Postnatal exposure (PND 10) to 11.3 mg/kg/day resulted in an initial decrease in motor activity followed by an increase in activity in 2- and 4-month-old mice (Johansson et al. 2008). In 2-month-old mice exposed to 0.75 mg/kg/day, there was a decrease in total activity during the first 20 minutes of testing, but not during the remaining 40 minutes of the test; no changes in activity were observed in the 4-month-old mice exposed to 0.75 mg/kg/day. Johansson et al. (2009) also found an altered response to nicotine exposure. Exposure to 11.3 mg/kg/day PFOS resulted in a decrease in motor activity in response to nicotine exposure, as compared to the increased activity observed in controls; no significant alteration was observed at 0.75 mg/kg/day. Two studies testing muscle coordination did not find alterations in the offspring of rats exposed to 3.2 mg/kg/day for 6 weeks prior to mating and throughout gestation and lactation (Luebker et al. 2005a) or mice exposed to 6 mg/kg/day on GDs 12–18 (Fuentes et al. 2007b). A decrease in muscle coordination was observed in mice exposed to 0.3 mg/kg/day on GDs 1–17 (Onishchenko et al. 2011). Perinatal exposure to PFOS did not significantly alter learning or memory in rats exposed to 2 mg/kg/day on GDs 2–21 and tested on PND 21 (Lau et al. 2003), the offspring of rats exposed to 3.2 mg/kg/day for 6 weeks prior to mating and throughout gestation and lactation and tested on PNDs 21 and 70 (Luebker et al. 2005a), or mice exposed to 6 mg/kg/day on GDs 12–18 and tested at 3 months of age (Fuentes et al. 2007a). In contrast, decreases in spatial learning ability were observed in the offspring of mice exposed to 0.8 mg/kg/day on GD 1 through PND 1 or on PNDs 1–7 (Wang et al. 2015b).

The effect of pre- and/or postnatal exposure to PFOS on serum lipid levels, thyroid function, and immune function has also been evaluated by a small number of studies. In the offspring of rats exposed to 1.6 mg/kg/day for 6 weeks prior to mating through GD 20, a significant decrease in fetal serum cholesterol levels and an increase in LDL cholesterol levels were observed (Luebker et al. 2005b). In rats exposed through PND 4, there was a decrease in serum triglyceride levels in the pups exposed to

1 mg/kg/day (Luebker et al. 2005b). No alterations in thyroid histology or follicular morphology were observed in rats exposed to 1 mg/kg/day on GD 0–PND 20 (Chang et al. 2009), and no alterations in TSH levels were observed in the Chang et al. (2009) study or in rats exposed to 2 mg/kg/day on GDs 2–21 (Lau et al. 2003). Decreases in total and free T4 levels were observed in rats exposed to 1 mg/kg/day on GDs 2–21 (Lau et al. 2003); free T4 levels remained low through PND 35. Similarly, a cross-fostering study found decreases in T4 levels in rats exposed to 3.2 mg/kg/day *in utero*, during lactation only, and throughout gestation and lactation (Yu et al. 2009b). Altered immune function was observed in mice exposed to PFOS on GDs 1–17 (Keil et al. 2008). At 5 mg/kg/day, an altered IgM antibody response to sRBCs was observed in 8-week-old males; decreases in CD3+ and CD4+ lymphocytes were also observed. At 1 mg/kg/kg/day, there was decreased in NK cell activity in males; no effects were observed at 0.1 mg/kg/day.

Summary. A number of epidemiological studies have evaluated developmental outcomes in occupational, community (living near a PFOA facility), and general exposure populations. Overall, these studies have not found associations between serum PFOS and adverse pregnancy outcomes (miscarriage, preterm birth), most birth outcomes (risks of low birth weight or small for gestational age, birth length, head, chest or abdominal circumferences, ponderal index, sex ratio, or birth defects), or neurodevelopmental outcomes (IQ, motor development, behavior, ADHD, or autism). It is noted that some studies have found associations for these effects and for some effects, only a couple of studies examined the endpoint. Although most studies did not find associations between maternal PFOS and birth weight, a meta-analysis did find a small decrease in birth weight was associated with increasing maternal PFOS levels, after adjustment for glomerular filtration rate. There is also some suggestive evidence that PFOS levels may be associated with small delays in the age of puberty in boys and girls. Studies in laboratory animals clearly indicate that developmental toxicity is a sensitive outcome of PFOS exposure. Oral exposure studies have reported increases in fetal mortality and decreases in pup survival; decreases in fetal body weight, birth weight, and pup body weight; delays in developmental milestones such as eye opening; increases in skeletal malformations/anomalies/variations such as cleft palate and delayed skeletal ossification; and decreases in offspring motor activity.

PFHxS

Epidemiological Studies—Pregnancy Outcomes. Six studies, summarized in Table 2-22 have evaluated possible associations between pregnancy outcomes and maternal PFHxS levels. Jensen et al. (2015) did not find an association between maternal PFHxS levels and the risk of miscarriage. Hamm et al. (2010)

found a decreased risk of preterm births among women with serum PFHxS levels in the 3rd tertile and Manzano-Salgado et al. (2017a) and Sagiv et al. (2018) found no associations with risk of preterm birth. Other studies have found no associations with gestational age (Li et al. 2017; Manzano-Salgado et al. 2017a) or length (Lind et al. 2017a; Sagiv et al. 2018).

Epidemiological Studies—Birth Outcomes. General population studies have evaluated possible associations between maternal PFHxS levels and birth outcomes including birth weight, length, small for gestation age, and birth defects; studies are summarized in Table 2-23. Bach et al. (2016) and Maisonet et al. (2012) reported inverse associations between maternal PFHxS levels and birth weight; however, other studies have not found associations (Alkhalawi et al. 2016; Ashley-Martin et al. 2017; Callan et al. 2016; Cao et al. 2018; Hamm et al. 2010; Kim et al. 2011; Li et al. 2017; Lee et al. 2013, 2016; Lenters et al. 2016a; Lind et al. 2017a; Manzano-Salgado et al. 2017a; Monroy et al. 2008; Sagiv et al. 2018; Shi et al. 2017; Starling et al. 2017). Manzano-Salgado et al. (2017a) did not find an association between maternal PFHxS levels and the risk of low birth weight infants. Hamm et al. (2010) and Manzano-Salgado et al. (2017a) did not find an association between maternal PFHxS level and the relative risk of small for gestational age. Ashley-Martin et al. (2017) did not find an association between maternal PFHxS levels and infant leptin or adiponectin levels, but Starling et al. (2017) found an inverse association between maternal PFHxS levels and adiposity at birth. Several studies did not find associations between maternal PFHxS levels and birth length, head circumference, or ponderal index (Alkhalawi et al. 2016; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Lee et al. 2013; Manzano-Salgado et al. 2017a; Shi et al. 2017). Maisonet et al. (2012) found an inverse association for birth length, but no association for ponderal index. Cao et al. (2018) found an association between head circumference and cord PFHxS levels. Only one study examined possible birth defects; Liew et al. (2014) did not find an association between maternal PFHxS levels and the risk of congenital cerebral palsy in a case-control study.

Epidemiological Studies—Neurodevelopmental Outcomes. Epidemiological studies, summarized in Table 2-24, have examined PFHxS-related alterations in risks of ADHD, autism, intelligence, and behavior. Wang et al. (2015b) did not find associations between maternal PFHxS levels and IQ in 5- or 8-year-old children and Jeddy et al. (2017) did not find associations between maternal PFHxS levels and verbal comprehension or vocabulary comprehension production in 15-month-old infants or intelligibility, language, or communication scores in 38-month-old children. Zhang et al. (2018) did not find associations between reading scores at 5 or 8 years of age and maternal PFHxS levels or PFHxS levels at age 3 or 5 years. Vuong et al. (2016) found a higher risk of performing poorly on tests of global executive function with increasing maternal PFHxS levels. However, no association was found between

serum PFHxS levels and metacognition or global executive function in 8-year-old children (Vuong et al. 2018). No association between serum PFHxS levels and the risk of learning problems was found in children living in a community with high PFOA levels (Stein and Savitz 2011). Gump et al. (2011) found an inverse association between serum PFHxS levels and performance on tasks requiring behavioral inhibition; Vuong et al. (2016, 2018) did not find alterations in behavioral regulation associated with maternal PFHxS levels or 8-year-old's PFHxS levels and Oulhote et al. (2016) did not find associations between behavioral development scores in 7-year-old children and maternal PFHxS levels or PFHxS levels at 5 or 7 years of age. Two studies evaluated the risk of ADHD and reported conflicting findings. Stein and Savitz (2011) reported increases in risk of ADHD in 5–18- and 12–15-year-olds with serum PFHxS levels in the 2nd, 3rd, or 4th quartile, whereas Liew et al. (2015) reported an inverse association between maternal PFHxS levels and risk of ADHD. This study also did not find an increase in the risk of autism; Braun et al. (2014) also found no association between maternal PFHxS levels and performance on tests assessing autism.

Epidemiological Studies—Development of the Reproductive System. No associations between reproductive hormone levels and serum PFHxS levels (Lopez-Espinosa et al. 2016) or maternal serum PFHxS levels (Maisonet et al. 2015a) were found in boys and girls 6–9 years of age or in girls 15 years of age. Lind et al. (2017a) did not find an association between maternal PFHxS levels and anogenital distance in boys or girls. Christensen et al. (2011) did not find an association between maternal PFHxS levels and risk of an earlier menarche. Summaries of these epidemiological studies are presented in Table 2-25.

Laboratory Animal Studies. Administration of 9.2 mg/kg/day PFHxS on PND 10 resulted in a decrease in spontaneous motor activity during the first 20 minutes of the test and an increase in activity in the last 20 minutes of the test (Viberg et al. 2013). The study also assessed the influence of PFHxS on nicotine-induced behavior. In the 9.2 mg/kg/day PFHxS group, exposure to nicotine did not significantly affect spontaneous motor activity, which was in contrast to the nicotine-induced increases in spontaneous motor activity observed in the controls and lower PFHxS groups. Studies evaluating the developmental toxicity of PFHxS did not find alterations in litter size, pup survival, or pup body weight in rats exposed to 10 mg/kg/day PFHxS or mice exposed to 3 mg/kg/day for 14 days prior to mating and throughout gestation and lactation (Butenhoff et al. 2009a; Chang et al. 2018). Although the rat study did not find alterations in litter size (Butenhoff et al. 2009a), the mouse study found a decrease in the number of pups per litter, without a change in the pup to implantation site ratio at ≥1 mg/kg/day (Chang et al. 2018). Similarly, no alterations in litter size, perinatal loss, or sex ratio were observed in the offspring of rats

administered up to 25 mg/kg/day PFHxS on GDs 7–22 (Ramhøj et al. 2018). The study did find decreases in male birth weights (3.5%) at ≥5 mg/kg/day and 30 and 45% decreases in pup serum thyroxine levels at 5 and 25 mg/kg/day (Ramhøj et al. 2018).

PFNA

Epidemiological Studies—Pregnancy Outcomes. Seven studies (summarized in Table 2-22) have examined pregnancy outcomes. Jensen et al. (2015) found an increase in the risk of having a miscarriage before gestation week 12, which was associated with maternal serum PFNA levels. Another study found no alteration in the risk of pregnancy loss (Buck Louis et al. 2016). No alterations in the risk of preterm birth was found in studies conducted by Chen et al. (2012a), Manzano-Salgado et al. (2017a), and Sagiv et al. (2018). Other studies found no association between PFNA and gestational age (Li et al. 2017; Manzano-Salgado et al. 2017a) or length (Lind et al. 2017a; Sagiv et al. 2018)

Epidemiological Studies—Birth Outcomes. Several studies have examined the possible associations between birth outcomes and maternal PFNA levels, these studies are summarized in Table 2-23. Most studies did not find an association between birth weight and maternal PFNA levels (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Lee et al. 2016; Lenters et al. 2016a; Li et al. 2017; Lind et al. 2017a; Manzano-Saldago et al. 2017a; Monroy et al. 2008; Robledo et al. 2015a; Shi et al. 2017). No alterations in the risk of low birth weight or small for gestational age were found in studies conducted by Chen et al. (2012a) and Manzano-Salgado et al. (2017a). Wang et al. (2016) did find an inverse association between maternal PFNA levels and birth weight in girls only and Starling et al. (2017) and Sagiv et al. (2018) found inverse associations in boys and girls combined; Starling et al. (2017) also found an inverse association between maternal PFNA levels and adiposity. Chen et al. (2012a) found an association between maternal PFNA levels and birth length, but other studies have not found alterations (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016). Most studies did not find alterations in ponderal index or head circumference (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016); Chen et al. (2012a) reported an inverse association between cord PFNA levels on ponderal index. No associations between maternal PFNA or paternal PFNA levels and the odds of a male birth were observed in a general population study (Bae et al. 2015). Liew et al. (2014) did not find alterations in the risk of congenital cerebral palsy that were associated with maternal PFNA levels.

Epidemiological Studies—Neurodevelopmental Outcomes. Several potential neurodevelopmental outcomes have been examined in epidemiological studies; these studies are summarized in Table 2-24. No association between maternal PFNA levels and full-scale IQ scores were observed in 8-year-old children (Wang et al. 2015b); however, an association was found for visual IQ. Maternal PFNA levels were not associated with IQ scores in 5-year-old children (Wang et al. 2015b). Stein and Savitz (2011) found a decrease in the risk of learning problems in 5–18- or 12–15-year-olds with serum PFNA levels in the two highest quartiles or in the 4th quartile, respectively. No associations were found between maternal PFNA levels and verbal and vocabulary comprehension in 15-month-olds or language skills and intelligence scores in 38-month-olds (Jeddy et al. 2017). Vuong et al. (2016) did not find an association between maternal PFNA levels and metacognition or global executive functioning in 5- or 8-year-old children. In a subsequent study (Vuong et al. 2018), associations were found between serum PFNA levels at age 8 years and metacognition and global executive function scores, which were indicative of poorer performance; When the children were categorized by sex, the associations were only found in boys. The study also found associations between PFNA levels and at risk metacognition and global executive functioning scores. Reading scores in 5-year-old children were associated with serum PFNA levels when the children were 3 years of age but were not associated with maternal PFNA levels (Zhang et al. 2018), and reading levels at 8 years of age were not associated with maternal, 3-year-old, or 5-year-old serum PFNA levels (Zhang et al. 2018).

Mixed results have been found in studies on behavior. Gump et al. (2011) found a decrease in behavioral response inhibition that was associated with serum PFNA levels in children aged 9–11 years, and Lien et al. (2016) reported inverse associations between cord blood PFNA levels in inattention and hyperactivity/inattention in 7-year-old children, but no effect on hyperactivity/impulsivity. Vuong et al. (2016) did not find an association between maternal PFNA levels and behavior regulation, but serum PFNA levels in 8-year-old children were associated with higher at risk behavioral regulation scores (Vuong et al. 2018). Three studies have not found associations between PFNA levels and ADHD risk (Hoffman et al. 2010; Liew et al. 2015; Stein and Savitz 2011). Similarly, maternal PFNA levels do not appear to be associated with autism (Braun et al. 2014; Liew et al. 2015).

Epidemiological Studies—Development of the Reproductive System. An inverse association between PFNA levels and insulin-like growth factor 1 was found in boys and girls aged 6–9 years (Lopez-Espinosa et al. 2016). No associations were found between PFNA and estradiol or total testosterone in 6–9 years olds (Lopez-Espinosa et al. 2016) or between maternal PFNA and testosterone or sex hormone binding globulin levels in 15-year-old girls (Maisonet et al. 2015a). Additionally, no association between

maternal serum PFHxS levels and risk of earlier age of menarche were observed in girls (Christensen et al. 2011). Summaries of these three studies are presented in Table 2-25.

Laboratory Animal Studies. Three studies were identified that examined the developmental toxicity of PFNA in laboratory animals. Full litter resorptions were observed in mice administered 10 mg/kg/day on GDs 1–17; maternal weight loss was also observed at this dose level (Das et al. 2015). At ≤1.5 mg/kg/day, decreases in postnatal survival were observed (Das et al. 2015; Wolf et al. 2010). Decreases in birth weight were observed in female offspring of rats administered 5 mg/kg/day PFNA on GDs 1–20 (Rogers et al. 2014). Postnatal growth was decreased on PNDs 1–24 in the offspring of mice administered ≥3 mg/kg/day PFNA on GDs 1–17 (Das et al. 2015); the decreases in body weight persisted in the males through PND 287 and in the females through PND 50. No skeletal or visceral abnormalities were observed in mouse pups (Das et al. 2015). Reductions in nephron endowment (number of functioning nephrons at birth) were observed in male rat pups on PND 22 (Rogers et al. 2014). This study also found increases in systolic blood pressure in pups at 10 weeks of age; no alterations were observed at 26 or 52 weeks of age. Delays in eye opening and decreased in pup body weight gain were observed in offspring of mice administered 2.0 mg/kg/day on GDs 1–18 (Wolf et al. 2010). Studies in PPARα knockout mice did not find alterations in pup survival, birth weight, pup body weight gain, or day of eye opening at maternal doses as high as 2.0 mg/kg/day (Wolf et al. 2010). Comparison between the results in tests using wild-type mice and knockout mice suggests that PPARα plays a role in PFNA developmental toxicity (Wolf et al. 2010).

PFDA

Epidemiological Studies—Pregnancy Outcomes. Four epidemiological studies examined pregnancy outcomes. Jensen et al. (2015) found an increased risk of miscarriage that was associated with maternal PFDA levels. The remaining studies found no associations between maternal PFDA levels and pregnancy loss (Buck Louis et al. 2016), gestational age (Li et al. 2017), or gestational length (Lind et al. 2017a).

Epidemiological Studies—Birth Outcomes. A small number of epidemiological studies examined risks of adverse birth outcomes associated with maternal PFDA exposure; these studies are summarized in Table 2-23. Wang et al. (2016) found an inverse association between maternal PFDA levels and birth weight in female infants only. This study also found an increased risk for small for gestational age among female infants. Other studies have not found associations (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Lee et al. 2016; Lenters et al. 2016a; Li et al. 2017; Lind et al. 2017a; Robledo et al. 2015a; Shi et

al. 2017; Starling et al. 2017). Starling et al. (2017) also found no association with adiposity at birth. Epidemiological studies have not found associations between birth length, ponderal index, and/or head circumference and maternal PFDA levels (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016). Liew et al. (2014) did not find alterations in the risk of congenital cerebral palsy in boys or girls and Bae et al. (2015) did not find alterations in odds of a male birth associated with maternal or paternal PFDA levels. Additionally, Kim et al. (2016b) did not find associations between serum PFDA levels and thyroid parameters.

Epidemiological Studies—Neurodevelopmental Outcomes. Several studies have evaluated the potential of PFDA to adversely affect neurodevelopment; see Table 2-24 for a summary of the studies. Wang et al. (2015b) did not find associations between maternal PFDA levels and IQ in 5- and 8-year-old children. Similarly, Vuong et al. (2016) did not find alterations in scores on tests of global executive functioning and metacognition in 5- or 8-year-old children. This study also found no alteration in behavioral regulation. In contrast, Gump et al. (2011) found increases in impulsivity. Oulhote et al. (2016) found an association between serum PFDA levels in 5-year-old children and total behavioral development score and higher externalizing and hyperactivity/inattention scores in 7-year-old children; the study did not find associations between behavioral development at age 7 years and maternal PFDA levels or 7-year-old PFDA levels. Liew et al. (2015) found decreases in the risk of ADHD and autism in children.

Epidemiological Studies—Developmental of the Reproductive System. In the only study examining reproductive outcomes, Lind et al. (2017a) found an inverse association between maternal PFDA levels and anogenital distance in girls, but not in boys.

Laboratory Animal Studies. An increase in fetal mortality was observed in mice exposed to 12.8 mg/kg/day PFDA on GDs 6–15 (Harris and Birnbaum 1989); this dose level was also associated with a marked decrease in fetal weight/litter (50% lower than controls), 100% incidence of variations in ossification of the braincase, decreases in maternal body weight, and maternal mortality. Decreases in fetal body weight/litter were observed at ≥1 mg/kg/day. The study did not find alterations in the occurrence of cleft palate, soft tissue malformations, or skeletal malformations. In mice exposed to 10.8 mg/kg/day PFDA on PND 10, there was no effect on spontaneous activity, habituation, performance on an elevated maze test, or response to a nicotine injection (Johansson et al. 2008). These results differ from the Johansson et al. (2008) findings when mice were exposed to PFOA or PFOS and the findings of Viberg et al. (2013) in mice exposed to PFHxS.

PFUnA

Epidemiological Studies—Pregnancy Outcomes. A limited number of epidemiological studies evaluated pregnancy outcomes. Jensen et al. (2015) did not find an alteration in the risk of miscarriage before gestation week 12. No association between gestational age and maternal PFUnA levels were found in a study conducted by Li et al. (2017).

Epidemiological Studies—Birth Outcomes. The results from a study conducted by Wang et al. (2016) found an inverse association between maternal PFUnA levels and birth weight and an increased risk of small for gestation age among female infants. Callan et al. (2016) reported an association between maternal PFUnA levels and optimal body weight but did not find an association with birth weight. The remaining epidemiological studies have not found alterations in infant size (birth weight, birth length, ponderal index, head circumference) (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Lee et al. 2016; Lenters et al. 2016a; Li et al. 2017; Shi et al. 2017) or the risks of low birth weight (Chen et al. 2012a) or small for gestational age (Chen et al. 2012a). No association between serum PFUnA levels and thyroid parameters were observed in infants (Kim et al. 2016a). The results of the epidemiological studies examining associations between birth outcome and PFUnA are presented in Table 2-23.

Epidemiological Studies—Neurodevelopmental Outcomes. The results of two studies examining possible associations between neurodevelopmental outcome and PFUnA are summarized in Table 2-24. Wang et al. (2015b) found no association between maternal PFUnA levels and IQ score in 5- and 8-year-old children; the study did find an inverse association with scores on tests assessing performance IQ. Lien et al. (2016) found no associations between cord blood PFUnA levels and performance on behavioral tests.

Laboratory Animal Studies. One study was identified that examined the potential developmental toxicity of PFUnA (Takahashi et al. 2014); the study found decreases in pup body weight at birth and on PND 4 in the offspring of rats administered via gavage 1.0 mg/kg/day PFUnA.

PFHpA

Epidemiological Studies. In the only epidemiological study evaluating developmental outcomes, Li et al. (2017) found no association between cord PFHpA levels and gestational age. The study did find an inverse association for birth weight in boys only, but not in girls and in boys and girls combined.

PFBS

Laboratory Animal Studies. No alterations in pup survival, body weight, or development were observed at doses as high as 1,000 mg/kg/day in a 2-generation rat study of potassium PFBS (Lieder et al. 2009b). In contrast to these findings, Feng et al. (2017) reported decreases in pup body weight, delays in eye opening, vaginal opening, and first estrous in the offspring of mice administered PFBS on GDs 1–20. York (2002) reported decreases in fetal body weight at 1,000 mg/kg/day in a rat study; however, a subsequent study (York 2003a) found decreases in body weights in the fetuses of rats administered 2,000 mg/kg/day, but not 1,000 mg/kg/day.

Reproductive and endocrine effects were also observed in the offspring at 200 and 500 mg/kg/day; these effects consisted of decreases in number of ovarian follicles and corpora lutea at diestrus, decreases in uterine weight and endometrial and myometrial thickness; increases in the average number of days in estrous stage; decreases in estrogen and progesterone levels; increases in luteinizing hormone levels; decreases in total T4, free T4, and total T3; and increases in TSH levels (Feng et al. 2017).

PFBA

Epidemiological Studies. Li et al. (2017) did not find an association between cord PFBA levels and gestational age or birth weight. In a study conducted by Kim et al. (2016a), no associations were found between serum PFBA levels and thyroid parameters in infants.

Laboratory Animal Studies. A delay (approximately 1 day) in eye opening was observed in the offspring of mice administered via gavage 35 mg/kg/day PFBA on GDs 1–17 (Das et al. 2008).

PFDoDA

Epidemiological Studies—Pregnancy Outcomes. In the only study examining pregnancy outcome (Table 2-22), Li et al. (2017) found no association between cord serum PFDoDA levels and gestational age.

Epidemiological Studies—Birth Outcomes. General population studies conducted by Cao et al. (2018), Lee et al. (2016), and Lenters et al. (2016a) did not find associations between cord blood PFDoDA or maternal PFDoDA levels and birth weight, birth length, and/or ponderal index. Wang et al. (2016) found an inverse association between maternal PFDoDA levels and birth weight and head circumference in female infants; no alteration in the risk of small for gestation age was found. Li et al. (2017) also found an association between cord PFDoDA levels and birth weight in girls only; no association was found in boys or in boys and girls combined. The results of these three studies are summarized in Table 2-23.

Epidemiological Studies—Neurodevelopmental Outcomes. As summarized in Table 2-24, only one study examined neurodevelopmental outcomes. In this study, maternal PFDoDA levels were not associated with IQ scores in 5- or 8-year-old children (Wang et al. 2015b).

Laboratory Animal Studies. One study evaluated the developmental toxicity of PFDoDA; no alterations in the number of live pups born, birth weight, growth, or the prevalence of external, visceral, or skeletal anomalies were observed at 0.1 or 0.5 mg/kg/day (Kato et al. 2015). At the next highest dose (2.5 mg/kg/day), only 1 of the 12 dams delivered live pups; 2 of these pups died on PND 0 and decreases in body weight gain were observed in the remaining pups.

PFHxA

Laboratory Animal Studies. Administration of 500 mg/kg/day NaPFHx on GDs 1–20 resulted in 10% decreases in fetal weight in rats (Loveless et al. 2009). Similarly, decreases in pup body weight (17–18% during the lactation period) were observed in the offspring of rats administered 500 mg/kg/day NaPFHx for 70 days prior to mating, during mating, and throughout gestation and lactation (Loveless et al. 2009). This study also found no alterations in pup clinical signs, survival, or developmental landmarks. No alterations in litter size, pup survival, or pup body weight, or occurrence of internal malformations were observed in the offspring of rats administered 315 mg/kg/day PFHxA (TWA dose) prior to mating through lactation day 4 (Kirkpatrick 2005).

FOSA

Epidemiological Studies. Robledo et al. (2015a) found an inverse association between maternal FOSA levels and birth weight in boys, but not in girls; paternal FOSA levels were not associated with birth weight. The study did not find alterations in birth length, head circumference, or ponderal index (see Table 2-23). Bae et al. (2015) did not find alterations in the odds of a male birth that was associated with maternal or paternal FOSA levels. As summarized in Table 2-24, only one study evaluated possible associations between FOSA and neurodevelopmental outcomes. Gump et al. (2011) reported an inverse association between serum FOSA levels and performance on tasks requiring behavioral inhibition. In the only study examining development of the reproductive system, Christensen et al. (2011) did not find an association between maternal serum FOSA levels and the risk of an earlier age of menarche in girls (see Table 2-25).

2.18 OTHER NONCANCER

Overview. A number of epidemiological studies have examined the possible associations between perfluoroalkyls and outcomes related to diabetes; the results of these studies are summarized in Table 2-26, with additional study details presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 14. Overall, the epidemiological studies do not provide support for an association between serum perfluoroalkyl levels and increases in the risk of diabetes or related outcomes (e.g., increases in blood glucose, glucose tolerance) for PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFHpA, or FOSA. Additionally, results of studies on PFOA, PFOS, and PFHxS do not suggest an association between perfluoroalkyls and gestational diabetes. No epidemiological studies examining other noncancer endpoints were identified for PFBS, PFBA, PFDoDA, or PFHxA. Only four laboratory animal studies examined other noncancer endpoints reporting inflammation of the salivary glands in rats exposed to PFOA, pancreatic acinar cell hyperplasia in rats exposed to PFOA, and an increase in serum glucose levels in rats administered PFNA (Table 2-5). The fourth study did not find increases in serum glucose in rats exposed to PFOS (Table 2-4).

PFOA

Epidemiological Studies. A cohort mortality study conducted by Leonard et al. (2008; Leonard 2006) of workers at the Washington Works facility found a significant increase in deaths from diabetes, as

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Leonard 2006 Occupational (n=6,027)	>250–≤750 ng/mL PFOA	Diabetes deaths	**SMR 183 (112–283)* (males only)**
Leonard et al. 2008 Occupational (n=6,027)	NR	Diabetes deaths	**SMR 197 (123–298)***
Lundin et al. 2009 Occupational (n=3,992)	Probable exposure	Diabetes deaths	**SMR 2.0 (1.2–3.2)***
Raleigh et al. 2014 Occupational (n=9,027)	NR	Diabetes deaths	SMR 0.76 (0.50–1.11)
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative	Risk of diabetes	RR 1.10 (0.77–1.57) no lag RR1.12 (0.76–1.66) 10-year lag
Steenland and Woskie 2012 Occupational (n=1,088)	580 ng/mL (median PFOA)	Diabetes deaths	**SMR 1.90 (1.35–2.61)* no lag** SMR 1.90 (0.98–3.33) 10-year lag SMR 1.73 (0.83–3.18) 20-year lag
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Self-reported diabetes	**SPR 1.54 (1.16–2.05)***
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 without diabetes)	68.4 ng/mL, 92.8 ng/mL, 86.5 ng/mL, 82.3 ng/mL (mean and serum PFOA in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all) Adults (>20 years) Youth (≤20 years)	**OR 0.69 (0.65–0.74)*** **OR 0.74 (0.70–0.79)*** **OR 0.72 (0.54–0.97)***
		Type 2 diabetes Adults (>20 years) Youth (≤20 years)	**OR 0.87 (0.89–0.91)*** **OR 0.91 (0.89–0.94)*** **OR 0.92 (0.88–0.96)***
		Uncategorized diabetes Adults (>20 years) Youth (≤20 years)	**OR 0.92 (0.88–0.97)*** OR 1.13 (0.82–1.56) OR 1.18 (0.90–1.55)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karnes et al. 2014 Community (n=32,254 C8 participants)	Estimated cumulative	Self-reported diabetes	HR 1.00 (0.99–1.00, p=0.60), retrospective analysis HR 1.00 (1.00–1.01, p=0.31), prospective analysis
		Fasting blood glucose	NS (p>0.05)
MacNeil et al. 2009 Community (n=13,922 C8 participants)	122.7 ng/mL (mean PFOA)	Validated diabetes	OR 0.72 (0.52–1.00) (10^{th} decile)
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	4.82 ng/mL (geometric mean serum PFOA)	Type 2 diabetes	HR 1.06 (0.89–1.28, p=0.50)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	**Association (p<0.05)***
		HOMA-IR	**Association (p<0.05)***
		HOMA-β	**Association (p<0.05)***
		HbA1c	**Association (p<0.05)***
Domazet et al. 2016 General population (n=501children assessed at ages 9, 15, and 21 years)	9.7 and 9.0 ng/mL (median serum PFOA in males and females at age 9 years)	Glucose At age 15 At age 21	 β 1.87 (-1.19–4.93) β -1.01 (-14.62–30.07)
		Insulin At age 15 At age 21	 β -12.99 (-25.95–2.23) β -13.98 (-36.23–16.00)
		HOMA-IR At age 15 At age 21	 β -12.54 (-25.59–2.77) β -14.16 (-36.60–16.28)
		HOMA-β At age 15 At age 21	 **β -11.10 (-20.28 to -1.01)*** β -7.82 (-22.44–9.66)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Domazet et al. 2016 General population (n=501children assessed at ages 9, 15, and 21 years)	3.7 and 3.4 ng/mL (median serum PFOA in males and females at age 15 years)	Glucose at age 21	β 5.83 (-3.70–16.92)
		Insulin at age 21	β -0.59 (-44.76–79.43)
		HOMA-IR at age 21	β 0.93 (-44.45–83.55)
		HOMA-β at age 21	β -11.70 (-37.16–24.67)
Fisher et al. 2013 General population (n=2,700)	2.46 ng/mL (geometric mean PFOA)	Insulin	NS (p=0.12)
		Blood glucose	NS (p=0.17)
		HOMA-IR	NS (p=0.10)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	5.3 ng/mL (geometric mean maternal PFOA)	HOMA-IR	β -0.7 (-9.8–9.4)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	4.2 ng/mL (geometric mean PFOA in child)	HOMA-IR	**β -10.1 (-17.3 to -2.3)***
He et al. 2018 General population (NHANES) (n=7,904 adults)	2.1–3.34, 3.34–5.1, and >5.1 ng/mL (serum PFOA for 2nd, 3rd, and 4th quartiles)	Diabetes	**OR 2.13 (1.30–3.46)*, 2nd quartile males** OR 1.47 (0.87–2.48), 4th quartile, females
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	1.67 ng/mL (maternal median serum PFOA)	Fasting glucose	β-1.3 (3.0–0.5),
		Fasting insulin	β -4.0 (-12.2–5.0)
		2-hour glucose in oral glucose tolerance test	β-2.6 (-6.9–1.8)
		HOMA-IR	β -5.2 (-14.2–4.7)
		HOMA-β	β -0.4 (-8.0–8.0)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	1.88 ng/mL (median serum PFOA)	Fasting blood glucose	β 1.262 (-1.108–3.633, p=0.294)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group)	HOMA-IR	β -0.05 (-0.21–0.12, p=0.58)
Lin et al. 2009 General population (NHANES) (n=474 adolescents)	1.51 ng/mL (mean log PFOA)	Insulin	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Fasting blood glucose	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
General population (NHANES) (n=969 adults)	1.48 ng/mL (mean log PFOA)	Insulin	Association (p<0.05)
		β-cell function	Association (p<0.05)
		Fasting blood glucose	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
Lind et al. 2014 General population (n=1,016)	3.3 ng/mL (median PFOA)	Diabetes	OR 0.97 (0.61–1.53, p=0.88)
		HOMA-IR	NS (p=0.20)
Liu et al. 2018b General population (NHANES) (n=1,871 adults)	1.86 ng/mL (geometric mean serum PFOA)	Fasting glucose	NS (p>0.05)
		Insulin	NS (p>0.05)
		2-hour glucose in glucose tolerance test	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HbA1C	**Association (p<0.05)***
		β cell function	**Association (p<0.05)***
Melzer et al. 2010 General population (NHANES) (n=3,966)	10.39 ng/mL (M) 9.47 ng/mL (F) (4th PFOA quartile)	Self-reported diabetes	OR 0.69 (0.41–1.16, p=0.158)
Nelson et al. 2010 General population (NHANES) (n=306 adolescent and 524 adults)	4.6 ng/mL (mean PFOA)	HOMA (adolescent)	NS (p=0.16) (M), NS (p=0.11) (F)
		HOMA (adult)	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shapiro et al. 2016 General population (1,274 pregnant women)	1.68 ng/mL (geometric mean PFOA)	Gestational diabetes	NS (p=0.86 for trend)
		Impaired glucose tolerance	NS (p=0.36 for trend)
Starling et al. 2017 General population (n=604 mother-infant pairs)	1.4–17.0 ng/mL (3rd quartile maternal serum PFOA)	Maternal glucose levels	**β -0.025 (-0.046 to -0.004)*, 3rd quartile**
Su et al. 2016 General population (n=571)	5.8–8.0 ng/mL (2nd PFOA quartile)	Diabetes	**OR 0.39 (0.16–0.96)* (inverse association)**
		Fasting blood glucose	**Inverse association (p<0.01 for trend)***
		Glucose tolerance	**Inverse association (p<0.01 for trend)***
		Glycated hemoglobin	**Inverse association (p=0.04 for trend)***
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	5.48–112 ng/mL (3rd tertile serum PFOA)	Type 2 diabetes	**OR 1.54 (1.04–2.28)*, 3rd tertile**
Wang et al. 2018 General population (n=385 pregnant women)	7.3 ng/mL (median maternal serum PFOA); ≥10.1 ng/mL (3rd tertile serum PFOA)	Fasting blood glucose	β -0.005 (-0.018–0.008, p=0.465)
		Fasting insulin	β 0.069 (-0.005–0.143, p=0.068)
		HOMA-IR	β 0.074 (-0.011–0.158, p=0.087)
		Blood glucose in oral glucose tolerance test	β 0.014 (-0.013–0.041, p=0.305)
		Gestational diabetes mellitus	HR 2.11 (0.76–5.86, p=0.151), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	1.90 ng/mL (median serum PFOA)	Fasting blood glucose	β 0.545 (-1.8–2.887)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhang et al. 2015a General population (n=258)	3.07 and 3.94 ng/mL (geometric mean PFOA in women with or without gestational diabetes)	Gestational diabetes	**OR 1.86 (1.14–3.02)***
PFOS			
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 with no diabetes)	21.8, 25.2, 25.1, 23.1 ng/mL (mean and serum PFOS in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all)	**OR 0.65 (0.61–0.70)***
		Type 2 diabetes	**OR 0.86 (0.82–0.90)***
		Uncategorized diabetes	OR 0.93 (0.86–1.03)
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	4.82 ng/mL (geometric mean serum PFOS)	Type 2 diabetes	HR 0.87 (0.74–1.02, p=0.08)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	**Association (p<0.05)***
		HOMA-IR	**Association (p<0.05)***
		HOMA-β	**Association (p<0.05)***
		HbA1c	**Association (p<0.05)***
Domazet et al. 2016 General population (n=501 children assessed at ages 9, 15, and 21 years)	44.5 and 39.9 ng/mL (median serum PFOS in males and females at age 9 years)	Glucose	
		At age 15	**β 0.88 (0.07–1.60)***
		At age 21	β 0.64 (-0.55–1.84)
		Insulin	
		At age 15	β -0.29 (-4.26–3.67)
		At age 21	β -1.01 (-8.00–6.62)
		HOMA-IR	
		At age 15	β -12.54 (-25.59–2.77)
		At age 21	β -0.83 (-7.91–6.71)
		HOMA-β	
		At age 15	β -0.29 (-4.17–3.76)
		At age 21	β -1.66 (-5.70–2.67)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Domazet et al. 2016 General population (n=501children assessed at ages 9, 15, and 21 years)	22.3 and 20.8 ng/mL (median serum PFOS in males and females at age 15 years)	Glucose at age 21	β 0.81 (-1.27–2.72)
		Insulin at age 21	β 4.78 (-6.82–17.77)
		HOMA-IR at age 21	β 4.74 (-6.69–17.91)
		HOMA-β at age 21	β 1.81 (-4.77–9.09)
Fisher et al. 2013 General population (n=2,700)	8.04 ng/mL (geometric mean PFOS)	Insulin	NS (p=0.88)
		Blood glucose	NS (p=0.96)
		HOMA-IR	NS (p=0.25
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	24.4 ng/mL (geometric mean maternal PFOS)	HOMA-IR	β -0.6 (-8.2–7.6)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	6.2 ng/mL (geometric mean PFOS in child)	HOMA-IR	**β -10.1 (-16.4 to -3.3)***
He et al. 2018 General population (NHANES) (n=7,904 adults)	>25.5 ng/mL (4th quartile serum PFOS)	Diabetes	OR 1.75 (1.00–3.04), 4th quartile males OR1.41 (0.82–2.41), 4th quartile, females
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	8.37 ng/mL (maternal median serum PFOS)	Fasting glucose	β -0.1 (-2.3–2.2)
		Fasting insulin	β 2.7 (-8.5–15.2)
		2-hour glucose in oral glucose tolerance test	β 2.9 (-2.8–8.9)
		HOMA-IR	β -2.9 (-7.1–14.1)
		HOMA-β	β -2.6 (-9.7–16.6)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	5.68 ng/mL (median serum PFOS)	Fasting blood glucose	β 0.707 (-1.921–3.336, p=0.595)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group)	HOMA-IR	β -0.06 (-0.18–0.06, p=0.31)
Lin et al. 2009 General population (NHANES) (n=474 adolescents)	3.11 ng/mL (log mean PFOS)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)
General population (NHANES) (969 adults)	3.19 ng/mL (log mean PFOS)	Insulin	**Association (p<0.05)***
		HOMA-IR	**Association (p<0.05)***
		β-cell function	**Association (p<0.05)***
		Blood glucose	NS (p>0.05)
Lind et al. 2014 General population (n=1,016)	13.2 ng/mL (median PFOS)	Diabetes	OR 1.43 (0.94–22.16, p=0.09)
		HOMA	NS (p=0.51)
Liu et al. 2018b General population (NHANES) (n=1,871 adults)	5.28 ng/mL (geometric mean serum PFOS)	Fasting glucose	**Inverse association (p<0.05)***
		Insulin	NS (p>0.05)
		2-hour glucose in glucose tolerance test	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HbA1C	NS (p>0.05)
		β cell function	NS (p>0.05)
Melzer et al. 2010 General population (NHANES) (n=3,966)	57.73 ng/mL (M) 50.96 ng/mL (F) (4th quartile mean PFOS)	Self-reported diabetes	OR 0.87 (0.57–1.31, p=0.491)
Nelson et al. 2010 General population (NHANES) (n=306 adolescent and 524 adults)	25.3 ng/mL (mean PFOS)	HOMA (adolescent)	NS (p=0.18) (M), NS (p=0.22) (F)
		HOMA (adult)	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shapiro et al. 2016 General population (1,274 pregnant women)	4.58 ng/mL (geometric mean PFOS)	Gestational diabetes	NS (p=0.70 for trend)
		Impaired glucose tolerance	NS (p=0.74 for trend)
Starling et al. 2017 General population (n=604 mother-infant pairs)	2.4 ng/mL (maternal median serum PFOS)	Maternal glucose levels	β -0.009 (-0.020–0.003)
Su et al. 2016 General population (n=571)	>4.8 ng/mL (4^{th} PFOS quartile)	Diabetes	**OR 3.37 (1.18–9.65)***
		Fasting blood glucose	**Association (p<0.01 for trend)***
		Glucose tolerance test	**Association (p≤0.01 for trend)***
		Glycated hemoglobin	**Association (p=0.04 for trend)***
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	26.3–41.4 ng/mL (2^{nd} tertile serum PFOS)	Type 2 diabetes	**OR 1.63 (1.25–2.12)*, 2^{nd} tertile**
Wang et al. 2018 General population (n=385 pregnant women)	5.4 ng/mL (median maternal serum PFOS); ≥7.3 ng/mL (3^{rd} tertile serum PFOS)	Fasting blood glucose	β -0.009 (-0.019–0.002, p=0.108)
		Fasting insulin	β 0.013 (-0.048–0.074, p=0.672)
		HOMA-IR	β 0.074 (-0.011–0.158, p=0.087)
		Blood glucose in oral glucose tolerance test	β 0.006 (-0.015–0.028, p=0.562)
		Gestational diabetes mellitus	HR 0.71 (0.29–0.75, p=0.453), 3^{rd} tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	3.00 ng/mL (median serum PFOS)	Fasting blood glucose	β -1.237 (-2.63–1.59)
Zhang et al. 2015a General population (n=258)	13.10 and 12.04 ng/mL (geometric mean PFOS in women with or without gestational diabetes)	Gestational diabetes	OR 1.13 (0.75–1.72)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 with no diabetes)	3.4, 3.8, 4.2, and 5.2 ng/mL (mean and serum PFHxS in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all)	**OR 0.59 (0.54–0.64)***
		Type 2 diabetes	**OR 0.74 (0.71–0.77)***
		Uncategorized diabetes)	**OR 0.84 (0.78–0.90)***
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	4.82 ng/mL (geometric mean serum PFOA)	Type 2 diabetes	HR 0.99 (0.87–1.12, p=0.82)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HOMA-β	NS (p>0.05)
		HbA1c	NS (p>0.05)
Fisher et al. 2013 General population (n=2,700)	2.18 ng/mL (geometric mean PFHxS)	Insulin	NS (p=0.89)
		Blood glucose	NS (p=0.98)
		HOMA-IR	NS (p=0.20)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	2.5 ng/mL (geometric mean maternal PFHxS)	HOMA-IR	β -2.07 (-5.9–2.0)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	2.2 ng/mL (geometric mean PFHxS in child)	HOMA-IR	β -1.7 (-3.8–0.5)
He et al. 2018 General population (NHANES) (n=7,904 adults)	0.9–1.64, 1.64–2.9, and >2.9 (2^{nd}, 3^{rd}, and 4^{th} quartile serum PFHxS)	Diabetes	**OR 1.99 (1.19–3.33)*, 2^{nd} quartile males** OR 1.22 (0.71–2.11), 4^{th} quartile, females

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	0.31 ng/mL (maternal median serum PFHxS)	Fasting glucose	**β 1.7 (0.2–3.2)***
		Fasting insulin	**β 7.7 (0.1–15.9)***
		2-hour glucose in oral glucose tolerance test	β 2.9 (-0.8–6.8)
		HOMA-IR	**β 9.5 (1.0–18.8)***
		HOMA-β	β 2.3 (-4.3–9.4)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.793 ng/mL (median serum PFHxS)	Fasting blood glucose	β 0.925 (-1.779–2.164, p=0.500)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group)	HOMA-IR	β -0.09 (-0.18–0.003, p=0.04)
Lin et al. 2009 General population (NHANES) (n=474 adolescents);	0.95 ng/mL (log mean)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)
General population (NHANES) (n=969 adults)	0.60 ng/mL (log mean PFHxS)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		B-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)
Lind et al. 2014 General population (n=1,016)	2.1 ng/mL (median PFHxS)	Diabetes	OR 1.00 (0.74–1.35, p=0.98)
		HOMA	NS (p=0.29)
Nelson et al. 2010 General population (NHANES) (n=306 adolescent and 524 adults)	2.6 ng/mL (mean PFHxS)	HOMA (adolescent)	NS (p=0.20) (M), **Inverse association (p=0.001)* (F)**
		HOMA (adult)	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shapiro et al. 2016 General population (n=1,274 pregnant women)	1.02 ng/mL (geometric mean PFHxS)	Gestational diabetes	NS (p=0.73 for trend)
		Impaired glucose tolerance	NS (p=0.44 for trend)
Starling et al. 2017 General population (n=604 mother-infant pairs)	1.1–10.9 ng/mL (3rd quartile maternal serum PFHxS)	Maternal glucose levels	**β -0.023 (-0.044 to -0.002)*, 3rd quartile**
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	2.15 and 2.01 ng/mL (serum PFHxS in cases and controls, respectively)	Type 2 diabetes	OR 1.26 (0.86–1.86), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	3.80 ng/mL (median serum PFHxS)	Fasting blood glucose	β -0.29 (-1.9–1.32)
PFNA			
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 with no diabetes)	1.4, 1.5, 1.5, and 1.6 ng/mL (mean and serum PFNA in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all)	**OR 0.65 (0.57–0.74)***
		Type 2 diabetes	**OR 0.94 (0.88–1.00)***
		Uncategorized diabetes)	OR 0.95 (0.85–1.06)
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	0.53 ng/mL (geometric mean serum PFNA)	Type 2 diabetes	HR 0.99 (0.87–1.12, p=0.82)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HOMA-β	NS (p>0.05)
		HbA1c	NS (p>0.05)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	0.6 ng/mL (geometric mean maternal PFNA)	HOMA-IR	β 1.4 (-8–11.7)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	1.7 ng/mL (geometric mean PFNA in child)	HOMA-IR	β -0.6 (-3.2–2.6)
He et al. 2018 General population (NHANES) (n=7,904 adults)	>1.64 (4th quartile serum PFNA)	Diabetes	OR 1.19 (0.73–1.95), 4th quartile males OR 1.01 (0.62–1.65), 4th quartile, females
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	0.65 ng/mL (maternal median serum PFNA)	Fasting glucose	β 0.03 (-2.1–2.2)
		Fasting insulin	**β 12.1 (0.7–24.8)***
		2-hour glucose in oral glucose tolerance test	β 1.3 (-6.5–4.2)
		HOMA-IR	β 12.2 (-0.5–26.4)
		HOMA-β	**β 12.4 (0.2–23.7)***
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.938 ng/mL (median serum PFNA)	Fasting blood glucose	β 0.428 (-1.785–2.641, p=0.703)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group)	HOMA-IR	β 0.01 (-0.13–0.14, p=0.89)
Lin et al. 2009 General population (NHANES) (n=474 adolescents)	0.35 ng/mL (log mean PFNA)	Insulin	**Inverse association (p<0.05)***
		HOMA-IR	NS (p>0.05)
		β-cell function	**Inverse association (p<0.05)***
		Blood glucose	NS (p>0.05)
General population (NHANES) (n=969 adults)	0.21 ng/mL (log mean PFNA)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lind et al. 2014 General population (n=1,016)	0.7 ng/mL (median PFNA)	Diabetes	OR 1.30 (0.85–1.97, p=0.22)
		HOMA	NS (p=0.90)
Nelson et al. 2010 General population (NHANES) (n=306 adolescents and 524 adults)	1.3 ng/mL (mean PFNA)	HOMA (adolescent)	NS (p=0.83) (M), (p=0.20) (F)
		HOMA (adult)	NS (p>0.05)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.5–6.0 ng/mL (2nd half maternal serum PFNA)	Maternal glucose levels	**β -0.025 (-0.042 to -0.009)*, 2nd half**
Su et al. 2016 General population (n=571)	>5.1 ng/mL (4th PFNA quartile)	Diabetes	**OR 0.31 (0.11–0.88)* 4th quartile (inverse association)**
		Fasting blood glucose	NS (p=0.10 for trend)
		Glucose tolerance test	**Inverse association (p<0.01 for trend)***
		Glycated hemoglobin	NS (p=0.11 for trend)
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	0.60 and 0.61 ng/mL (serum PFNA in cases and controls, respectively)	Type 2 diabetes	OR 0.99 (0.67–1.48), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.50 ng/mL (median serum PFNA)	Fasting blood glucose	β -0.627 (-2.54–1.29)
Zhang et al. 2015a General population (n=258)	1.23 and 1.20 ng/mL (geometric mean PFNA in women with or without gestational diabetes)	Gestational diabetes	OR 1.06 (0.70–1.60)
PFDA			
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	0.3 ng/mL (geometric mean PFDA in child)	HOMA-IR	**β -14.7 (-22.1 to -6.5)***

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	0.26 ng/mL (maternal median serum PFDA)	Fasting glucose	β 1.3 (-3.6–1.0)
		Fasting insulin	β -0.2 (-11.2–12.1)
		2-hour glucose in oral glucose tolerance test	β -3.3 (-8.7–2.5)
		HOMA-IR	β -1.5 (-13.5–12.1)
		HOMA-β	β 3.9 (-6.4–15.2)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.0592 ng/mL (median serum PFDA)	Fasting blood glucose	β -0.201 (-1.280–0.878, p=0.713)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group)	HOMA-IR	β -0.04 (-0.11–0.03, p=0.26)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.2–3.5 ng/mL (2nd half maternal serum PFDA)	Maternal glucose levels	**β -0.024 g (-0.041 to -0.007)*, 2nd half**
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	0.13 and 0.16 ng/mL (serum PFDA in cases and controls, respectively)	Type 2 diabetes	OR 0.71 (0.48–1.05), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.40 ng/mL (median serum PFDA)	Fasting blood glucose	β -2.543 (-4.65 to -0.44)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhang et al. 2015a General population (n=258)	0.41 and 0.40 ng/mL ng/mL (geometric mean PFDA in women with or without gestational diabetes)	Gestational diabetes	OR 1.04 (0.70–1.53)
PFUnA			
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.652 ng/mL (median serum PFUnA)	Fasting blood glucose	β 1.350 (-0.020–2.721, p=0.053)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCHR group and comparison group)	HOMA-IR	β -0.04 (-0.10–0.02, p=0.21)
Lind et al. 2014 General population (n=1,016)	0.3 ng/mL (median PFUnA)	Diabetes	OR 0.95 (0.59–1.52; p=0.81)
		HOMA	NS (p=0.32)
Su et al. 2016 General population (n=571)	6.4–9.2 ng/mL (3rd PFUnA quartile)	Diabetes	**OR 0.24 (0.08–0.78)* 3rd quartile (inverse association)**
		Fasting blood glucose	**Inverse association (p<0.01 for trend)***
		Glucose tolerance test	**Inverse association (p<0.01 for trend)***
		Glycated hemoglobin	NS (p=0.17 for trend)
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.30 ng/mL (median serum PFUnA)	Fasting blood glucose	**β -1.821 (-3.45 to -0.189)***

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHpA			
Lind et al. 2014 General population (n=571)	0.05 ng/mL (median PFHpA)	Diabetes	OR 1.02 (0.77–1.34, p=0.90)
		HOMA	NS (p=0.56)
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.20 ng/mL (median serum PFHpA)	Fasting blood glucose	β -1.101 (-5.54–3.34)
FOSA			
Lind et al. 2014 General population (n=571)	0.11 ng/mL (median FOSA)	Diabetes	OR 1.07 (0.75–1.53, p=0.71)
		HOMA	NS (p=0.070)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 14 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

(F) = females; FOSA = perfluorooctane sulfonamide; HOMA = homeostatic model assessment; HR = hazard ratio; IR = insulin resistance; (M) = males; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; SMR = standardized mortality ratio; SPR = standardized prevalence ratio; WTCHR = World Trade Center Health Registry

compared to workers at other DuPont facilities in the region. In an update of the Leonard et al. (2008) study, Steenland and Woskie (2012) found an increased risk of diabetes deaths when compared to other regional DuPont employees, but not when compared to the U.S. population. However, when the workers were categorized by estimated cumulative exposure levels, the exposure-response trend was not statistically significant. Lundin et al. (2009) also found an increase in deaths from diabetes in workers exposed to APFO at the 3M Cottage Grove facility in Minnesota, as compared to Minnesota death rates. The increase was only found in workers with probable exposure to APFO, but not with definite exposure; no deaths from diabetes were observed in the workers with definite exposure to APFO. As noted by Steenland and Woskie (2012), diabetes mortality may not be a good surrogate for the underlying diabetes incidence data. Raleigh et al. (2014) did not find an increase in diabetes deaths at the Cottage Grove facility and Steenland et al. (2015) did not find an increased risk of diabetes associated with estimated cumulative PFOA exposure at the Washington Works facility.

In community exposure studies, Anderson-Mahoney et al. (2008) found an increased prevalence of self-reported diabetes in residents living near the Washington Works facility, as compared to expected rates taken from NHANES. Conway et al. (2016) found increases in the prevalence of type 1 diabetes, type 2 diabetes, and uncategorized diabetes in C8 Health Study participants. When the participants were categorized by age, the increases in type 1 diabetes and type 2 diabetes prevalences were found in adults and children; uncategorized diabetes was not increased in either group. In contrast, Karnes et al. (2014) did not find an increased risk of self-reported diabetes associated with estimated cumulative PFOA levels and MacNeil et al. (2009) did not find an increased risk of validated diabetes in C8 Health Study participants.

General population studies found either an inverse association between serum PFOA and risk of diabetes (Su et al. 2016), an association (He et al. 2018; Sun et al. 2018), or no association (Cardenas et al. 2017; Lind et al. 2014; Melzer et al. 2010). Additionally, most general population studies have not found associations between serum PFOA levels and insulin (Fisher et al. 2013; Lin et al. 2009; Liu et al. 2018b), blood glucose levels (Fisher et al. 2013; Lin et al. 2009; Liu et al. 2018b; Su et al. 2016; Yang et al. 2018), homeostatic model assessment for insulin resistance (HOMA-IR) (Fisher et al. 2013; Lin et al. 2009; Lind et al. 2014; Liu et al. 2018b; Nelson et al. 2010), or glucose tolerance (Liu et al. 2018b; Su et al. 2016). Cardenas et al. (2017) did find associations between serum PFOA and glycemic parameters in cross-sectional analyses; however, in longitudinal analyses, no associations were found between serum PFOA and fasting blood glucose, fasting insulin, HOMA-IR, HOMA-β, or HbA1c. Studies in children

have not found associations between serum PFOA and blood glucose, blood insulin, HOMA-IR, and/or HOMA-β (Domazet et al. 2016; Fleisch et al. 2017; Kang et al. 2017; Koshy et al. 2017).

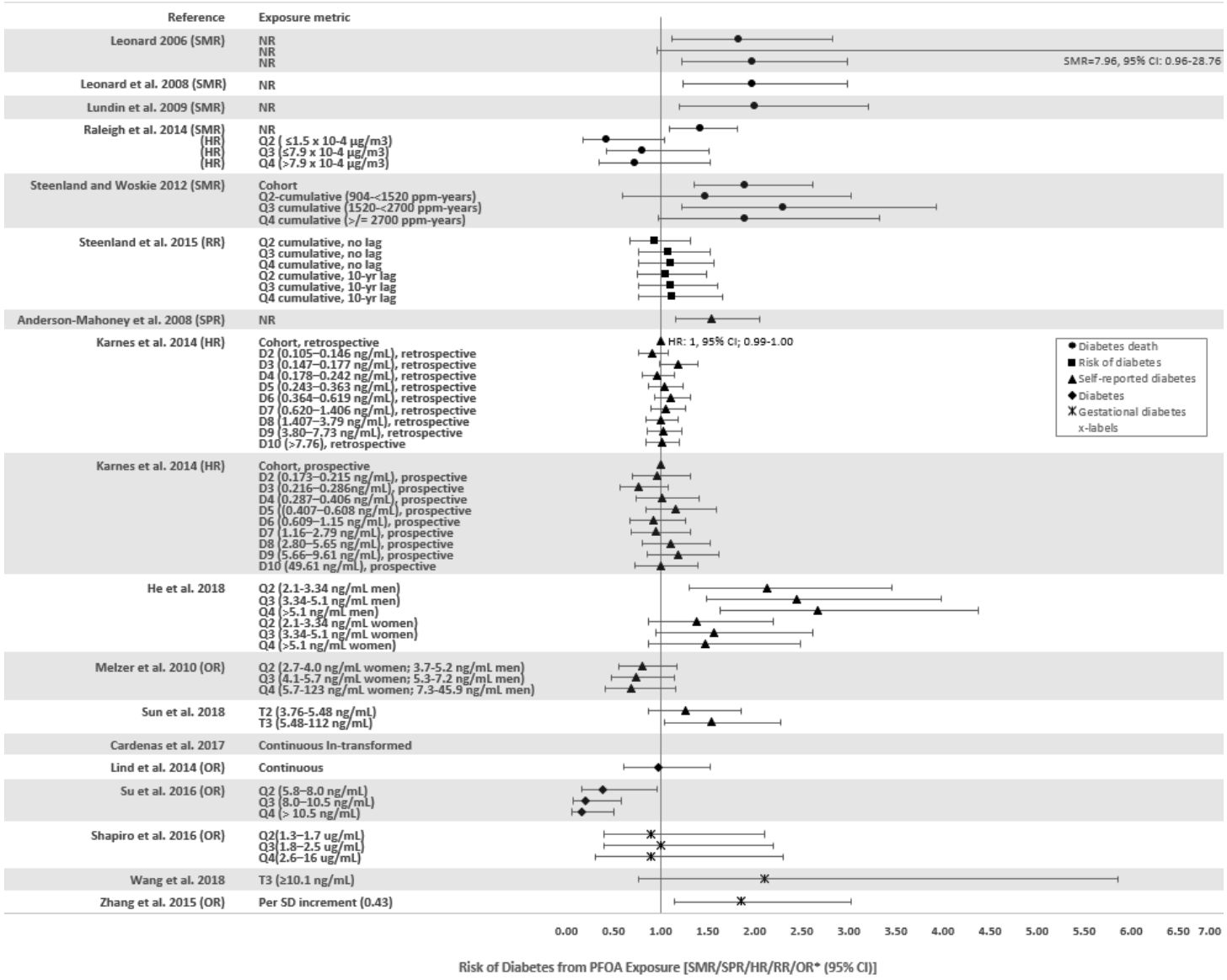
Three studies evaluated the risk of gestational diabetes and found mixed results. In a case-control study, Zhang et al. (2015a) found an increased risk of gestational diabetes associated with serum PFOA, whereas Shapiro et al. (2016) and Wang et al. (2018) did not find associations between serum PFOA and gestational diabetes or impaired glucose tolerance. Additionally, Starling et al. (2017) found an inverse association between blood glucose levels and serum PFOA in pregnant women. In contrast, Jensen et al. (2018) did not find associations between serum PFOA and fasting glucose, fasting insulin, HOMA-IR, HOMA-β, or blood glucose levels in a glucose tolerance test in pregnant women. The ORs for the risk of diabetes and gestational diabetes are graphically presented in Figure 2-39.

Laboratory Animal Studies. Two chronic-duration oral studies examined other noncancer endpoints. Inflammation of the salivary gland was observed in rats exposed to 1.5 mg/kg/day (3M 1983; Butenhoff et al. 2012c) and an increased incidence of acinar cell hyperplasia was observed in rats exposed to 13.6 mg/kg/day (Biegel et al. 2001).

PFOS

Epidemiological Studies. Inverse associations between serum PFOS and the prevalence of type 1 diabetes and type 2 diabetes were observed among participants of the C8 Health Study (Conway et al. 2016). In a general population study conducted by Su et al. (2016), an increased risk of diabetes was noted, as well as associations between serum PFOS levels and fasting blood glucose, response to glucose tolerance test, and glycated hemoglobin levels. Cardenas et al. (2017) also found associations between serum PFOS and fasting blood glucose, fasting insulin, HOMA-IR, and HOMA-β; however, these associations were not found in longitudinal analyses over a 3-year period. In a prospective case-control study, Sun et al. (2018) reported an association between serum PFOS and type 2 diabetes. Four other general population studies did not find increased risks of diabetes (Cardenas et al. 2017; He et al. 2019; Lind et al. 2014; Melzer et al. 2010). Several studies have not found associations between serum PFOS levels and insulin, blood glucose, or HOMA-IR levels (Fisher et al. 2013; Lin et al. 2009; Lind et al. 2014; Liu et al. 2018b; Nelson et al. 2010; Yang et al. 2018). In NHANES adult participants, Lin et al. (2009) found associations between serum PFOS and insulin and HOMA-IR and Liu et al. (2018b) found an inverse association with fasting glucose levels. No associations were found in adolescent participants

Figure 2-39. Diabetes Risk Relative to Serum PFOA Levels (Presented as Adjusted Ratios)



(Lin et al. 2009). Studies in children have not found associations between serum PFOS and fasting blood glucose, fasting blood insulin, HOMA-IR, and/or HOMA-β (Domazet et al. 2016; Fleisch et al. 2017; Kang et al. 2018; Koshy et al. 2017).

No alterations in the risk of gestational diabetes were observed in three general population studies (Shapiro et al. 2016; Wang et al. 2018' Zhang et al. 2015a). Shapiro et al. (2016), Starling et al. (2017), and Wang et al. (2018) studies also found no association between serum PFOS and blood glucose levels, glucose tolerance or other glycemic measurements in pregnant women. The ORs for the risk of diabetes and gestational diabetes are graphically presented in Figure 2-40.

Laboratory Animal Studies. Perinatal exposure to 3 mg/kg/day PFOS did not result in alterations in serum insulin or glucose levels in the offspring on PND 63 (Wan et al. 2014b). However, when the offspring were fed a high fat diet, increases in fasting glucose levels were observed at 0.3 and 3 mg/kg/day and fasting serum insulin levels were increased at 3 mg/kg/day.

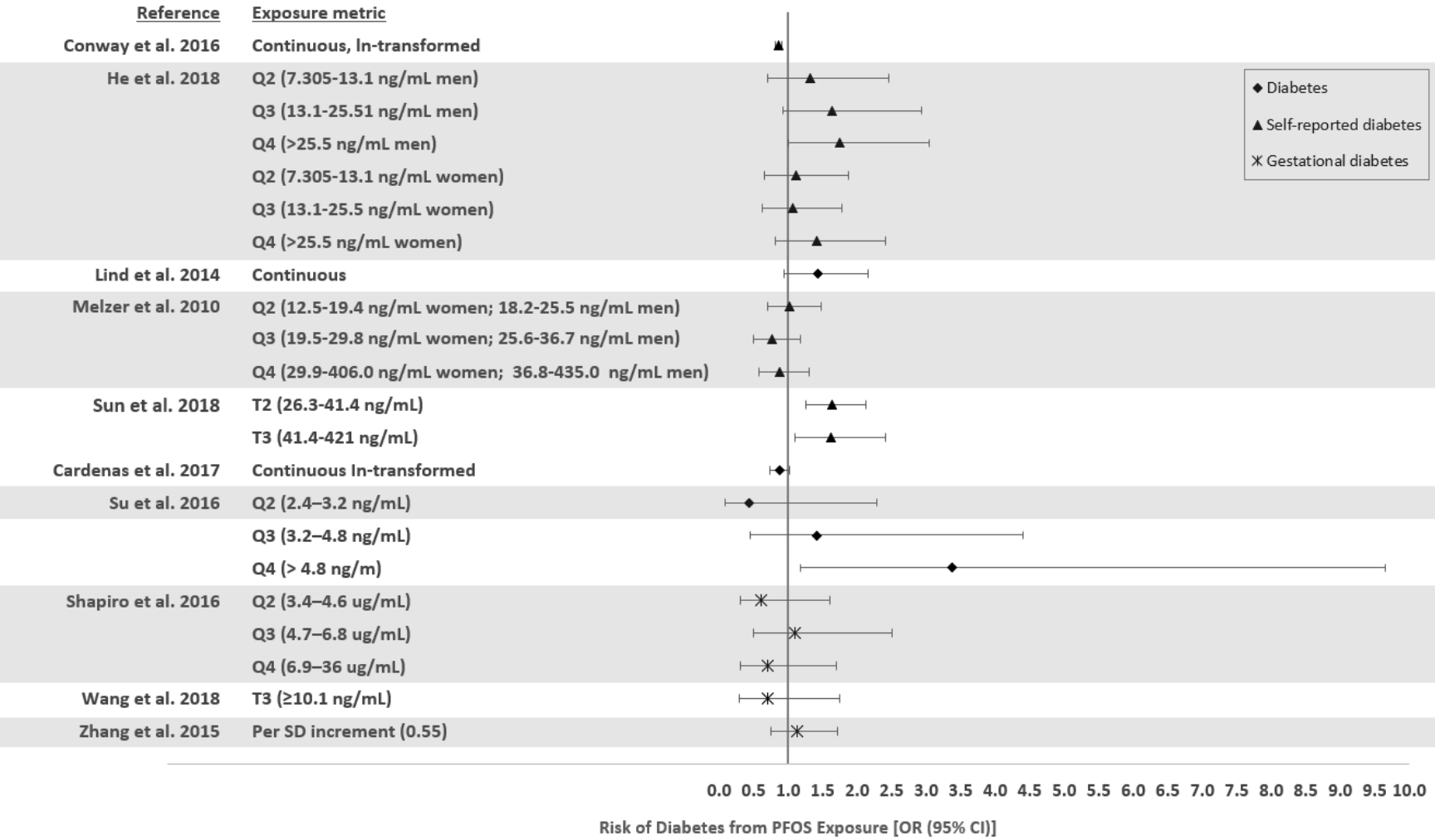
PFHxS

Epidemiological Studies. A study of C8 Health Project participants found inverse associations between serum PFHxS levels and the prevalence of type 1 diabetes, type 2 diabetes, and uncategorized diabetes (Conway et al. 2016). General population studies have examined diabetes-related outcomes and have not found associations between serum PFHxS levels and diabetes risk (Cardenas et al. 2017; He et al. 2018; Lind et al. 2014; Sun et al. 2018), gestational diabetes (Shapiro et al. 2016) or insulin, blood glucose, or HOMA-IR levels (Cardenas et al. 2017; Fisher et al. 2013; Jensen et al. 2018; Lin et al. 2009; Lind et al. 2014; Nelson et al. 2010; Yang et al. 2018). An inverse association between serum PFHxS levels and blood glucose levels was found in pregnant women (Starling et al. 2017). No associations between serum PFHxS and glycemic parameters were found in children (Fleisch et al. 2017; Kang et al. 2018; Koshy et al. 2017).

PFNA

Epidemiological Studies. An inverse association between serum PFNA levels and the risk of diabetes was observed in a general population study (Su et al. 2016) and for type 1 diabetes and type 2 diabetes in C8 Health Study participants (Conway et al. 2016). Four other studies did not find associations for diabetes (He et al. 2018; Lind et al. 2014; Sun et al. 2018) or gestational diabetes (Zhang et al. 2015a).

Figure 2-40. Diabetes Risk Relative to Serum PFOS Levels (Presented as Adjusted Odds Ratios)



The Su et al. (2016) study also reported an inverse association between PFNA levels and response on a glucose tolerance test. A study by Starling et al. (2017) also found an inverse association between PFNA levels and blood glucose levels in pregnant women. A study of adolescent NHANES participants found decreasing levels of insulin with increasing serum PFNA levels (Lin et al. 2009); this association was not found in adult NHANES participants (Lin et al. 2009). Several studies did not find associations between serum PFNA levels and fasting blood glucose, glucose tolerance, HOMA-IR, and/or HOMA-β (Cardenas et al. 2017; Fleisch et al. 2017; Jensen et al. 2018; Kang et al. 2018; Koshy et al. 2017; Lin et al. 2009; Lind et al. 2014; Nelson et al. 2010; Yang et al. 2018).

Laboratory Animal Studies. An increase in serum glucose levels was observed in rats administered via gavage 1 mg/kg/day PFNA for 14 days (Fang et al. 2012a).

PFDA

Epidemiological Studies. Two studies evaluated the potential association between PFDA and diabetes risk. Sun et al. (2018) did not find an association for type 2 diabetes risk and Zhang et al. (2015a) did not find an association between serum PFDA levels and the risk of gestational diabetes. Other studies have examined possible associations between serum PFDA and glycemic measurements. Fleisch et al. (2017) found an inverse association with HOMA-IR in children. Other studies in children (Kang et al. 2018; Koshy et al. 2017), adults (Yang et al. 2018), and pregnant women (Jensen et al. 2018) did not find associations for fasting blood glucose, fasting insulin, glucose tolerance, HOMA-IR, and/or HOMA-β.

PFUnA

Epidemiological Studies. Six epidemiological studies evaluating associations between PFUnA and diabetes-related outcomes have found conflicting results. Su et al. (2016) found inverse associations between serum PFUnA levels and diabetes risk, fasting blood glucose levels, and glucose tolerance test results, Yang et al. (2018) found an inverse association with fasting blood glucose levels, and Starling et al. (2017) found an inverse association with blood glucose levels in pregnant women. Whereas Lind et al. (2014) found no alterations in the risk of diabetes or HOMA, and Kang et al. (2018) and Koshy et al. (2017) found no associations between serum PFUnA levels and fasting blood glucose and HOMA-IR, respectively, in studies in children.

PFHpA

Epidemiological Studies. Lind et al. (2014) did not find associations between serum PFHpA levels and the risk of diabetes or HOMA alterations and Yang et al. (2018) did not find an association with fasting blood glucose levels.

FOSA

Epidemiological Studies. In the one epidemiological study identified, no associations between serum FOSA levels and the risk of diabetes or HOMA were found (Lind et al. 2014).

2.19 CANCER

Overview. A number of occupational exposure, community, and general population studies have examined possible associations between perfluoroalkyls and cancer risk; these studies are summarized in Table 2-27 and the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 15. Occupational and community exposure studies have found increases in the risk of testicular and kidney cancer associated with PFOA. No consistent epidemiologic evidence for other cancer types were found for PFOA. For PFOS, one occupational exposure study reported an increase in bladder cancer, but this was not supported by subsequent occupational studies. General population studies have not consistently reported increases in malignant tumors for PFOS. A small number of epidemiology studies examined possible associations between other perfluoroalkyls and cancer risk. No consistent associations were observed for breast cancer risk for PFHxS, PFNA, PFHpA, or PFDoDA; increased breast cancer risks were observed for PFDA and FOSA, but this was based on a single study. No associations between PFOA, PFOS, PFHxS, PFNA, PFDA, or PFUnA and prostate cancer risk were found. However, among men with a first-degree relative with prostate cancer, associations were found for PFOA, PFOS, PFHxS, PFDA, and PFUnA, but not for PFNA. Epidemiological studies examining potential cancer effects were not identified for PFBS, PFBA, or PFHxA.

Laboratory animal studies have evaluated the carcinogenicity of PFOA and PFOS; the results of these studies are summarized in Tables 2-3 and 2-4. In laboratory animals, there is some evidence for increases in Leydig cell adenomas, pancreatic acinar cell adenomas, and hepatocellular adenomas in male rats exposed to PFOA in the diet. An increase in hepatocellular adenomas was observed in male rats exposed to dietary PFOS for 2 years; thyroid follicular cell adenomas were observed in rats exposed to PFOS for

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gilliland 1992; Gilliland and Mandel 1993 Occupational (n=389 deaths) Reference population: Minnesota general population	NR	All cancer deaths	SMR 0.86 (0.72–1.01), males SMR 0.75 (0.56–0.99), females
		Prostate cancer	SMR 2.03 (0.55–4.59) RR 1.13 (1.01–1.27) for a 1-year increase in employment length **RR 3.3 (1.02–10.6)* for a 10-year employment length**
Leonard 2006; Leonard et al. 2008 Occupational (n=6,027) Reference population: DuPont workers at other regional facilities	5–9,550 ng/mL (estimated range of PFOA)	All cancer deaths	SMR 100 (88–114), males SMR 149 (77–260), females
		Kidney cancer deaths	SMR 185 (95–323), males
		Biliary passages and liver cancer deaths	SMR 133 (53–274)
		Pancreatic cancer deaths	SMR 100 (50–180)
		Bladder or other urinary organ cancer deaths	SMR 131 (53–269)
		Prostate cancer deaths	SMR 65 (34–114)
		Bronchus, trachea, lung cancer deaths	SMR 81 (63–104)
Lundin et al. 2009 Occupational (n=3,993) Reference population: Minnesota general population; for HR analysis comparisons with workers with low exposure or <1 year of exposure	2,600–5,200 and 300–1,500 ng/mL (range of PFOA in subset of current workers with definite exposure jobs and probable exposure jobs)	All cancer deaths	SMR 0.9 (0.5–1.4), definite exposure SMR 0.9 (0.8–1.1), probable exposure
		Pancreas cancer deaths	SMR 0.9 (0.0–4.7), definite exposure SMR 1.0 (0.4–2.1), probable exposure
		Trachea, bronchus, and lung cancer deaths	SMR 1.2 (0.5–2.3), definite exposure SMR 1.0 (0.7–1.4), probable exposure
		Prostate cancer deaths	SMR 2.1 (0.4–6.1), definite exposure SMR 0.9 (0.4–1.8), probable exposure **HR 6.6 (1.1–37.7), high exposure ≥6 months** **HR 3.7 (1.3–10.4), definite exposure for ≥5 years**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Raleigh et al. 2014 Occupational (n=9,207) Reference population: Minnesota general population	>7.9x10^{-4} µg/m^3 (cumulative exposure, 4th PFOA quartile)	All cancer deaths	SMR 0.87 (0.78–0.97)
		Pancreatic cancer deaths	SMR 0.85 (0.50–1.34)
		Prostate cancer deaths	SMR 0.83 (0.53–1.23)
		Kidney cancer deaths	SMR 0.53 (0.20–1.16)
		Liver cancer deaths	SMR 0.81 (0.35–1.59)
		Breast cancer deaths	SMR 0.82 (0.41–1.47)
		Bladder cancer deaths	SMR 0.89 (0.38–1.76)
Raleigh et al. 2014 Occupational (n=9,207) Reference population: non-APFO exposed workers at a St. Paul facility	≤7.9x10^{-4} and >7.9x10^{-4} µg/m^3 (cumulative exposure,3rd and 4th PFOA quartile)	Pancreatic cancer deaths	HR 1.23 (0.50–3.00), 3rd and 4th quartiles combined
		Pancreatic cancer	HR 1.36 (0.59–3.11), 3rd and 4th quartiles combined
		Prostate cancer deaths	HR 1.32 (0.61–2.84), 4th quartile
		Prostate cancer	HR 1.11 (0.82–1.49), 4th quartile
		Kidney cancer deaths	HR 0.39 (0.11–1.32), 3rd and 4th quartiles combined
		Kidney cancer	HR 0.73 (0.21–2.48), 4th quartile
		Liver cancer deaths	HR 0.67 (0.14–3.27), 3rd and 4th quartiles combined
		Breast cancer deaths	HR 0.54 (0.15–1.94), 3rd and 4th quartiles combined
		Breast cancer	HR 1.27 (0.70–2.31), 4th quartile
		Bladder cancer deaths	HR 1.96 (0.63–6.15), 3rd and 4th quartiles combined
		Bladder cancer	HR 1.66 (0.86–3.18), 4th quartile
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative exposure	Bladder cancer	**Inverse association (p=0.04 or p=0.06 for trend) with no lag or 10-year lag** **RR 0.23 (0.05–0.93), 4th quartile with no lag**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Colorectal cancer	NS (p=0.91 and 0.86 for trend), with no lag or 10-year lag
		Prostate cancer	NS (p=0.83 and 0.91 for trend), no lag or 10-year lag
		Melanoma	NS (p=0.16 and 0.55 for trend), no lag or 10-year lag
Steenland and Woskie 2012 Occupational (n=1,084 deceased workers) Reference population: DuPont workers at other regional facilities	≥2,700,000 ng/mL-years (estimated cumulative 4th PFOA quartile)	All cancer deaths	SMR 0.94 (0.76–1.16), 4th quartile
		Pancreatic cancer deaths	SMR 0.92 (0.30–2.16), 4th quartile
		Lung cancer deaths	SMR 0.75 (0.48–1.11), 4th quartile
		Prostate cancer deaths	SMR 0.57 (0.16–1.46), 4th quartile
		Bladder cancer deaths	SMR 0.36 (0.10–2.01), 4th quartile
		Kidney cancer deaths	**SMR 2.66 (1.15–5.24)*, 4th quartile** **SMR 2.82 (1.13–5.81)*, 10-year lag** **SMR 3.67 (1.48–7.57)*, 20-year lag**
Barry et al. 2013 Community and occupational (n=32,254)	Estimated cumulative exposure 24.2 and 112.7 ng/mL (median PFOA)	Testicular cancer	**HR 1.34 (1.00–1.79, p=0.05)* no lag** HR 1.28 (0.95–1.73, p=0.10) 10-year lag **HR 3.17 (0.75–13.45, p=0.04 for trend)*, 4th quartile**
		Kidney cancer	HR 1.10 (0.98–1.24, p=0.10), no lag (continuous) HR 1.58 (0.88–2.84, p=0.18 for trend), 4th quartile
		Breast cancer	**HR 0.94 (0.89–1.00, p=0.05)*, no lag** **HR 0.93 (0.88–0.99, p=0.03)*, 10-year lag**
		Colorectal cancer	HR 0.99 (0.92–1.07, p=0.84), no lag

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	86.6 ng/mL (mean PFOA)	Prostate specific antigen	NS (p<0.05)
Innes et al. 2014 Community (n=208 cases of colorectal cancer and 47,359 cancer-free adults)	13.5–27.8 ng/mL (2nd PFOA quartile)	Colorectal cancer	**OR 0.47 (0.31–0.74)*, 2nd quartile**
Vieira et al. 2013 Community (n=25,107)	30.8–109 and 110–655 ng/mL (estimated PFOA in high and very high exposure groups)	Kidney cancer	**AOR 2.0 (1.3–3.2)*, high exposure group** AOR 2.0 (1.0–3.9), very high exposure group
		Testes cancer	AOR 2.8 (0.8–9.2), very high exposure group
		Prostate cancer	AOR 1.5 (0.9–2.5), very high exposure group
		Breast cancer	AOR 1.4 0.9–2.3) very high exposure group, females only
Bonefeld-Jorgensen et al. 2011 General population (n=31 breast cancer cases and115 matched controls)	2.5 and 1.6 ng/mL (median PFOA in cases and controls)	Breast cancer	AOR 1.20 (0.77–1.88, p=0.43)
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 233 matched controls)	5.2 ng/mL (mean PFOA)	Breast cancer	RR 1.00 (0.90–1.11).
Eriksen et al. 2009 General population (n=713 for prostate cancer, n=332 for bladder cancer, n=128 for pancreatic cancer, n=67 for liver cancer, and n=772 controls)	6.8 and 6.0 ng/mL (median PFOA in male and female cancer patients) 6.9 and 5.4 ng/mL (median PFOA in male and female controls)	Prostate cancer	IRR 1.18 (0.84–1.65)
		Bladder cancer	IRR 0.81 (0.53–1.24)
		Pancreas cancer	IRR 1.55 (0.85–2.80)
		Liver cancer	IRR 0.60 (0.26–1.37)

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Hardell et al. 2014 General population (n=201 cases and 186 controls)	2.3 and 1.9 ng/mL (mean PFOA in cases and controls)	Prostate cancer	OR 1.1 (0.7–1.7) **OR 2.6 (1.2–6.0)*, among subjects with a heredity risk and serum PFOA above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	2.08 and 1.48 ng/mL (median serum PFOA in cases and controls, respectively)	Breast cancer	**OR 1.26 (1.01–1.58, p=0.039)*, continuous** **OR 2.64 (1.17–5.97, p=0.019)*, 3rd tertile**
PFOS			
Alexander et al. 2003 Occupational (n=2,083; 145 deaths) Reference population: Alabama general population	NR	All cancer deaths	SMR 0.84 (0.50–1.32), high potential exposure group
		Bladder and other urinary organs cancer	**SMR 12.77 (2.63–37.35)*, high potential exposure group** **SMR 16.12 (3.32–47.14)*, high exposure group ≥1 year exposure**
Alexander and Olsen 2007 Occupational (n=1,895; 1,488 deaths) Reference population: NIOSH SEER referent data	NR	Bladder cancer	SIR 1.74 (0.64–3.79), high potential exposure group SIR 1.43 (0.16–5.15) ≥10-year exposure group
Grice et al. 2007 Occupational (n=1,400 current, retired, or former workers)	1,300–1,970 ng/mL (PFOS levels in high potential exposure group)	Colon cancer	OR 1.69 (0.68–4.17)
		Melanoma	OR 1.01 (0.25–4.11)
		Prostate cancer	OR 1.08 (0.44–2.69)
Olsen et al. 2004a Occupational (current and retired workers)	NR	Malignant melanoma of the skin	RRE_pC 12 (1.0–>100) RRE_pC 10 (0.7–>100), >10 years employment
		Malignant neoplasm of the colon	RRE_pC 5.4 (0.5–>100) RRE_pC 12 (0.8–>100), >10 years employment
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	22.18 ng/mL (mean PFOS)	Prostate specific antigen	NS ($p<0.05$)

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Innes et al. 2014 Community (n=208 cases of colorectal cancer and 47,359 cancer-free adults)	13.6–20.1 ng/mL (2nd PFOS quartile)	Colorectal cancer	**OR 0.35 (0.24–0.53)***
Bonefeld-Jorgensen et al. 2011 General population (n=31 breast cancer cases and 115 matched controls)	45.6 and 21.9 ng/mL (median PFOS in cases and controls)	Breast cancer	**OR 1.03 (1.001–1.07, p=0.05)***
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	30.6 ng/mL (mean PFOS)	Breast cancer	RR 0.99 (0.98–1.01)
Eriksen et al. 2009 General population (n=713 for prostate cancer, n=332 for bladder cancer, n=128 for pancreatic cancer, n=67 for liver cancer, and n=772 controls)	35.1 and 32.1 ng/mL and 35.0 and 29.3 ng/mL (median PFOS in male and female cancer patients and males and females in the comparison group)	Prostate cancer	IRR 1.05 (0.97–1.14)
		Bladder cancer	IRR 0.93 (0.83–1.03)
		Pancreas cancer	IRR 0.99 (0.86–1.14)
		Liver cancer	IRR 0.59 (0.27–1.27)
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	11 and 10 ng/mL (mean PFOS in cases and controls)	Prostate cancer	OR 1.0 (0.6–1.5) **OR 2.7 (1.04–6.8)*, among subjects with a heredity risk and serum PFOS above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	35.50 and 18.2 ng/mL (median serum PFOS in cases and controls, respectively)	Breast cancer	**OR 1.02 (1.01–1.03, p=0.005)*, continuous** **OR 3.13 (1.20–8.15, p=0.020)*, 2nd tertile**
PFHxS			
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	3.58 ng/mL (mean PFHxS)	Prostate specific antigen	NS (p<0.05)
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	1.2 ng/mL (mean PFHxS)	Breast cancer	**RR 0.66 (0.47–0.94)***

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	1.1 and 0.940 ng/mL (mean PFHxS in cases and controls)	Prostate cancer	OR 1.3 (0.8–1.9) **OR 4.4 (1.7–12)*, among subjects with a heredity risk and serum PFHxS above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	2.52 and 1.14 ng/mL (median serum PFHxS in cases and controls, respectively)	Breast cancer	**OR 1.16 (1.02–1.32, p=0.029)*, continuous** **OR 2.69 (1.23–5.88, p=0.013)*, 3rd tertile**
PFNA			
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	1.47 ng/mL (mean PFNA)	Prostate specific antigen	NS (p<0.05)
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	0.5 ng/mL (mean PFNA)	Breast cancer	RR 0.76 (0.30–1.94)
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.679 and 0.631 ng/mL (mean PFNA in cases and controls)	Prostate cancer	OR 1.2 (0.8–1.8) OR 2.1 (0.9–4.8), among subjects with a heredity risk and serum PFNA above the median
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	3.28 and 1.83 ng/mL (median serum PFNA in cases and controls, respectively)	Breast cancer	OR 1.07 (0.98–1.17, p=0.116), continuous **OR 2.43 (1.07–5.51, p=0.034)*, 2nd tertile** OR 2.07 (0.90–4.76, p=0.056), 3rd tertile
PFDA			
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.338 and 0.291 ng/mL (mean PFDA in cases and controls)	Prostate cancer	OR 1.4 (0.9–2.1) **OR 2.6 (1.1–6.1)*, among subjects with a heredity risk and serum PFDA above the median**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	1.30 n and 1.01 ng/mL (median serum PFDA in cases and controls, respectively)	Breast cancer	OR 1.17 (0.97–1.40, p=0.094), continuous **OR 2.36 (1.04–5.36, p=0.041)*, 3rd tertile**
PFUnA			
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.308 and 0.285 ng/mL (mean PFUnA in cases and controls)	Prostate cancer	OR 1.2 (0.8–1.9) **OR 2.6 (1.1–5.9)*, among subjects with a heredity risk and serum PFUnA above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	2.23 and 2.02 ng/mL (median serum PFUnA in cases and controls, respectively)	Breast cancer	OR 1.06 (0.97–1.15, p=0.207), continuous **OR 2.00 (0.88–4.53, p=0.019)*, 3rd tertile**
PFHpA			
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	0.11 and 0.08 ng/mL (median serum PFHpA in cases and controls, respectively)	Breast cancer	OR 6.98 (0.61–80.0, p=0.119), continuous OR 1.52 (0.54–4.24, p=0.425), 3rd tertile
PFDoDA			
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	0.40 and 0.21 ng/mL (median serum PFDoDA in cases and controls, respectively)	Breast cancer	OR 1.03 (1.01–1.06, p=0.447), continuous OR 0.93 (0.45–1.91, p=0.839), 3rd tertile

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	3.5 ng/mL (mean FOSA)	Breast cancer	**RR 1.89 (1.01–3.54)*, among women with serum FOSA >5.75 ng/mL**

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 15 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

AOR = adjusted odds ratio; CI = confidence interval; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; IRR = incidence rate ratio; NIOSH = National Institute for Occupational Safety and Health; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR = relative risk; RRE_pC = risk ratio episodes of care; SEER = Surveillance Epidemiology and End Results; SIR = standardized incidence ratio; SMR = standardized mortality ratio

1 year and allowed to recover for an additional year. A discussion of the relevance of the rodent carcinogenicity data to humans is included in Section 2.20.6.

EPA (2016e, 2016f) has concluded that there is suggestive evidence of the carcinogenic potential of PFOA and PFOS in humans. IARC (2017) concluded that PFOA is possibly carcinogenic to humans (Group 2B).

PFOA

Epidemiological Studies. Several studies have examined the possible association between occupational exposure to PFOA and increased cancer risk in workers at two U.S. facilities—3M facility in Cottage Grove, Minnesota (Gilliland and Mandel 1993; Lundin et al. 2009; Raleigh et al. 2014) and DuPont Washington Works facility in West Virginia (Leonard 2006; Leonard et al. 2008; Steenland and Woskie 2012; Steenland et al. 2015). In addition, the potential carcinogenicity of PFOA has been assessed in the community near the Washington Works facility (Barry et al. 2013; Innes et al. 2014; Vieira et al. 2013) and in the general population (Bonefeld-Jorgensen 2011, 2014; Eriksen et al. 2009; Hardell et al. 2014).

Occupational exposure studies have not found increases in the risk of all cancer deaths (Gilliland and Mandel 1993; Leonard 2006; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012). The occupational exposure studies have consistently found no increases in the risk of pancreatic, liver, or respiratory tract cancers or deaths from these cancers (Leonard 2006; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012); a general population case-control study also found no associations between serum PFOA and pancreas or liver cancer (Eriksen et al. 2009). Additionally, two case-control studies did not find associations between serum PFOA levels and risk of breast cancer (Bonefeld-Jorgensen et al. 2011, 2014); a third case-control study found an association between serum PFOA and breast cancer (Wielsøe et al. 2017). Steenland et al. (2015) found an inverse association between estimated cumulative PFOA exposure and bladder cancer in workers; other studies have not found associations (Eriksen et al. 2009; Gilliland and Mandel 1993; Leonard 2006; Leonard et al. 2008; Raleigh et al. 2014; Steenland and Woskie 2012).

Some associations between PFOA and cancer effects have been observed, including prostate, kidney, and testicular cancers. Ten years of employment in the Chemical Division of the 3M Cottage Grove facility was associated with a 3.3-fold increase in the relative risk of prostate cancer mortality, as compared to no employment in PFOA production areas (Gilliland and Mandel 1993; data also reported in Gilliland 1992);

no increase in prostate cancer risk was observed when all workers in the Chemical Division were analyzed. The investigators noted that the prostate cancer findings are based on a small number of cases and could have resulted from chance or unrecognized confounding from exposure to other factors. An update of this study conducted by Lundin et al. (2009) did not find an increase in prostate cancer deaths in workers with definite PFOA exposure. When the cohort was divided into the three exposure categories and duration of definite exposure, increased risks for prostate cancer were found in the high-exposure category and in workers with definite exposure for at least 5 years, as compared with workers in the low-exposure category and with the shortest cumulative exposure duration, respectively. Interpretation of the Gilliland and Mandel (1993) and Lundin et al. (2009) studies is limited by the qualitative assessment of potential exposure and the fact that workers in the low exposure categories were likely research-and-development professionals rather than production workers (Raleigh et al. 2014). In the most recent evaluation of the Cottage Grove facility, which involved extensive exposure assessment, Raleigh et al. (2014) did not find increases in prostate cancer deaths when compared to the general population or to workers at another facility and did not find an increase in the incidence of prostate cancer when the workers were categorized by cumulative exposure levels. Studies of the Washington Works facility workers did not find increases in prostate cancer deaths (Leonard et al. 2008; Steenland and Woskie 2012) or incidence (Steenland et al. 2015). A case-control general population study by Hardell et al. (2014) did find an increase in prostate risk only among subjects with a heredity risk (first-degree relative with prostate cancer) and serum PFOA levels above the median. In a study of community members, Ducatman et al. (2015b) did not find an association between prostate-specific antigen (PSA) levels and serum PFOA levels in men 20–49 or 50–69 years of age.

In the earliest cancer assessment of workers at the Washington Works facility (Leonard 2006; Leonard et al. 2008), an increase in the number of deaths from kidney cancer relative to workers at other regional DuPont facilities was observed; however, the CI included unity. In a follow-up study that used serum PFOA levels collected in current workers to assess job title exposure (Steenland and Woskie 2012), an increase in kidney cancer deaths was observed in workers with the highest exposures when analyzed with no lag, a 10-year lag, or a 20-year lag. Steenland and Woskie (2012) also found an increase in deaths from mesothelioma; the investigators noted that this was likely due to asbestos exposure. Steenland and Woskie (2012) noted that tetrafluoroethylene, a rodent kidney carcinogen, is used in the manufacture of a variety of fluoropolymers and noted that the tetrafluoroethylene is well controlled due to its volatile and explosive properties. It is noted that in a multisite study of tetrafluoroethylene workers, which included workers at the Washington Works facility (Consonni et al. 2013), an increased risk of renal cancer (SMR 1.44, 95% CI 0.69–2.65) was found, although the CI included unity. Consonni et al. (2013) noted that

88% of the workers were also exposed to PFOA. When PFOA exposure was used as an exposure variable, the findings were the similar as when tetrafluoroethylene was used as the exposure variable, and thus, it was difficult for the investigators to evaluate separate associations for each compound. It is noted that increases in kidney deaths were not observed in the Cottage Grove facility (Raleigh et al. 2014), which did not use tetrafluoroethylene (Chang et al. 2014).

Three studies have examined the community living near the Washington Works facilities; some of these studies also included workers at the facility. Barry et al. (2013) reported an increased risk of testicular cancer that was associated with estimated cumulative PFOA exposure. Vieira et al. (2013) also reported an increase in testicular cancer, but the CIs of the adjusted odds ratio (AOR) included unity. When the participants were grouped by water district, an increased risk of testicular cancer (AOR 5.1, 95% CI 1.6–15.6) was observed in the Little Hocking water district, which had the highest PFOA levels in the water. The Vieira et al. (2013) study also found increased risks of kidney cancer among participants with high or very high exposure to PFOA; Barry et al. (2013) also concluded that there was an association between estimated cumulative PFOA exposure and kidney cancer, although the CIs for the hazard ratio included unity. The third study of the Washington Works community found an inverse association between serum PFOA and risk of colorectal cancer (Innes et al. 2014).

In their review of the available epidemiological data, IARC (2017) concluded that the evidence for testicular cancer was "considered credible and unlikely to be explained by bias and confounding, however, the estimate was based on small numbers." Similarly, IARC (2017) concluded that the evidence for kidney cancer was also credible but noted that chance, bias, and confounding could not be ruled out with reasonable confidence. They considered that there was limited evidence in humans for the carcinogenicity of PFOA.

Laboratory Animal Exposure Studies. Two studies have examined the carcinogenic potential of PFOA in rats. In the first study of male and female Sprague-Dawley rats exposed to PFOA in the diet for 2 years (3M 1983; Butenhoff et al. 2012c), significant increases in the incidence of fibroadenoma of the mammary gland in females and Leydig cell adenoma were found in males exposed to 15 mg/kg/day. A high incidence of pituitary adenoma occurred among all groups, including controls. The incidence of hepatocellular carcinoma was not significantly increased. The investigators noted that the incidence of fibroadenoma in the mammary gland in the 15 mg/kg/day group was similar to the incidence found in untreated aging rats and that the incidence of Leydig cell adenoma was similar to the spontaneous incidence of this tumor in aged rats. The mammary gland pathology slides from this study (3M 1983;

Butenhoff et al. 2012c) study were re-examined in 2005 by a Pathology Working Group (PWG) using current diagnostic criteria (Hardisty et al. 2010). The incidences of fibroadenoma found by the PWG were 36, 44, and 46% in the 0, 1, and 15 mg/kg/day groups, respectively; there were no statistically significant differences between the groups (Hardisty et al. 2010). Additionally, there were no significant differences in the incidence of adenocarcinoma, total benign neoplasms, or total malignant neoplasms between the groups. In the second study of male Sprague-Dawley rats exposed to PFOA in the diet for 2 years (Biegel et al. 2001), an increase in the incidence of hepatocellular adenomas was found, but there were no hepatocellular carcinomas in the treated group. PFOA also increased the incidence of Leydig cell adenomas. In addition, PFOA increased the incidence of pancreatic acinar cell adenomas; a pancreatic carcinoma was observed in one treated rat. Hepatic peroxisome proliferation was increased significantly at all interim evaluation time points (1, 3, 6, 9, 12, 15, 18, and 21 months), but there was no increase in cell proliferation. In Leydig cells, neither peroxisome proliferation nor cell proliferation were increased.

PFOA was a positive modulator of hepatocarcinogenesis in male Wistar rats in a biphasic (initiation with diethylnitrosamine followed by oral treatment with PFOA) or triphasic (initiation with diethylnitrosamine [DEN] followed by dosing with 2-acetylaminofluorene and then PFOA) promotion protocol (Abdellatif et al. 1991, 2004). PFOA induced a marked increase in acylCoA oxidase activity and only a slight increase in catalase activity (Abdellatif et al. 2004). Since PFOA did not significantly increase 8-hydroxy-deoxyguanosine (a marker of oxidative DNA damage *in vivo*) in isolated liver DNA, it appeared that PFOA did not require extensive DNA damage for its promoting activity (Abdellatif et al. 2004). PFOA was also found to act as a promoter in male Wistar rats in an initiation-selection-promotion protocol (Nilsson et al. 1991).

IARC (2017) concluded that there was limited evidence in experimental animals for the carcinogenicity of PFOA.

PFOS

Epidemiological Studies. Four studies have evaluated the carcinogenic potential in workers at a Decatur, Alabama perfluorooctanesulphonyl fluoride (PFOSF) based fluorochemical production facility. In the earliest study, no increase in all cancer deaths was found, as compared to the Alabama general population (Alexander et al. 2003). An increased risk of bladder cancer was observed in workers with high potential exposure and in workers with a high potential exposure for ≥1 year; the mortality ratio was based on three

cases in the high exposure group. In a reanalysis of workers at this facility conducted by Alexander and Olsen (2007), 11 cases of bladder cancer were identified from worker surveys (n=6) and death certificates (n=5). Only two of the six self-reported bladder cancer diagnosis were confirmed via medical records; the other four subjects declined to give consent for medical verification. When compared to incidence data from the National Institute for Occupational Safety and Health (NIOSH) Surveillance Epidemiology and End Results (SEER) referent data, the standardized incidence ratios for the high potential exposure group were elevated, but the CIs included unity. When compared with workers with <1 year of high exposure, workers with 5–<10 and ≥10 years of high exposure had relative risks of 1.92 (95% CI 0.30–12.06) or 1.52 (95% CI 0.21–10.99). Although the study did not adjust for smoking, the investigators noted that 83% of the living bladder cancer cases (five of the six subjects) reported cigarette use, as compared to 56% reported in the noncases. An additional limitation of the study is inclusion of four cases of bladder cancer that were not verified by medical records. The results of this study do not appear to confirm the findings of increased bladder cancer in the mortality study (Alexander et al. 2003). In a subsequent study of this facility, treatment for bladder cancer was not reported among current workers (Olsen et al. 2004a). The study did find increases in the number of episodes of care for malignant neoplasm of the prostate or malignant neoplasms of the colon, as compared to long-term workers in another division, but the CIs included unity. No increases in the risk ratio episodes of care were found for liver, rectum, or respiratory tract (Olsen et al. 2004a). A fourth study of this facility (Grice et al. 2007) examined possible associations between colon cancer, melanoma, and prostate cancer and PFOS exposure. The risks of these cancers were not associated with any of the PFOS-exposure categories for analyses that included all self-reported or only validated cancers.

General population case-control studies have evaluated several cancer types. Innes et al. (2014) reported an inverse association between PFOS and colorectal cancer. A small-scale study of 31 cases by Bonefeld-Jorgensen et al. (2011) found a slight increase in breast cancer risk, a finding not replicated in another larger study of a different population (Bonefeld-Jorgensen et al. 2014). A third case-control study found associations between serum PFOS and breast cancer in subjects with serum PFOS levels in the second tertile and higher (Wielsøe et al. 2017). Eriksen et al. (2009) and Hardell et al. (2014) did not find increases in the risk of prostate cancer associated with serum PFOS. However, an increased risk of prostate cancer was found among subjects with a first-degree relative with prostate cancer and PFOS levels above the median level (Hardell et al. 2014). Eriksen et al. (2009) also found no associations between serum PFOS and the risk of bladder cancer, pancreatic cancer, or liver cancer. Ducatman et al. (2015b) did not find an association between serum PFOS levels and PSA levels in men participating in the C8 studies.

Laboratory Animal Studies. In a 2-year PFOS dietary exposure study bioassay in male and female Sprague-Dawley rats (Butenhoff et al. 2012b; unpublished study by Thomford 2002b), a significant positive trend of hepatocellular adenoma was observed in males; the incidence was significantly higher than controls at 1.04 mg/kg/day. No hepatocellular adenomas were seen in a group of rats exposed to 1.17 mg/kg/day for 1 year and allowed to recover for the second year. High-dose males from the recovery group showed a significant increase in thyroid follicular cell adenoma relative to controls. No significant increase in this type of tumor was observed in rats exposed for 2 years. In females, there was a significant positive trend for incidences of hepatocellular adenoma, which was associated with a significant increase in the 1.04 mg/kg/day group. In females, there were also significant negative trends for mammary adenoma and fibroadenoma carcinoma combined.

PFHxS

Epidemiological Studies. Three case-control studies have examined the possible association between serum PFHxS and cancer. Bonefeld-Jorgensen et al. (2014) found in inverse association between PFHxS levels and breast cancer risk. In contrast, Wielsøe et al. (2017) found a positive association between serum PFHxS levels and breast cancer risk. No association between PFHxS and prostate cancer was observed (Hardell et al. 2014), with the exception of increased risk in men with a first-degree relative with prostate cancer and above-median serum PFHxS levels. No associations between serum PFHxS and PSA levels were observed in a cross-sectional study of men 20–49 or 50–69 years of age participating in the C8 Health Studies (Ducatman et al. 2015b).

PFNA

Epidemiological Studies. The carcinogenic potential of PFNA has been examined in three case-control studies. No consistent associations between serum PFNA levels and breast cancer (Bonefeld-Jorgensen et al. 2014; Wielsøe et al. 2017) or prostate cancer (Hardell et al. 2014) were found. Serum PSA levels were not associated with serum PFNA levels in men participating in the C8 Health Study (Ducatman et al. 2015b).

PFDA

Epidemiological Studies. Hardell et al. (2014) examined the possible association between the serum PFDA level and risk of prostate cancer and only found an association in men with a heredity risk factor and PFDA levels above the median. In a case-control study of breast cancer, Wielsøe et al. (2017) found an association among women with serum PFDA levels in the third quartile.

PFUnA

Epidemiological Studies. An increased risk of prostate cancer was found in men with first-degree relatives with prostate cancer and serum PFUnA levels above the median (Hardell et al. 2014). An increased breast cancer risk was found in women with serum PFUnA levels in the third quartile (Wielsøe et al. 2017).

PFHpA

Epidemiological Studies. One study evaluated possible associations between serum PFHpA and cancer risk and found no association for breast cancer (Wielsøe et al. 2017).

PFDoDA

Epidemiological Studies. In the only cancer study for PFDoDA, Wielsøe et al. (2017) did not find an increased risk of breast cancer in women associated with serum PFDoDA levels.

FOSA

Epidemiological Studies. Bonefeld-Jorgensen et al. (2014) reported an increased risk of breast cancer among women with serum FOSA levels >5.75 ng/mL.

2.20 MECHANISM OF TOXICITY

The primary effects observed in rodents exposed to perfluoroalkyls are liver toxicity, developmental toxicity, and immune toxicity. The cellular mechanisms by which hepatic effects are induced have been extensively studied, while more limited data are available on mechanisms for other effects. The available

data indicate that perfluoroalkyls produce a number of adverse effects through activation of the PPARα, a member of the nuclear receptor superfamily that mediates a broad range of biological responses (Issemann and Green 1990). However, some adverse effects of perfluoroalkyls occur through PPARα-independent mechanisms, which may include activation of other nuclear receptors, increased oxidative stress, dysregulation of mitochondrial function, and inhibition of gap junction intercellular communication (GJIC). In the sections below, cellular mechanisms of action that are mediated by PPARα and independent of PPARα are discussed, followed by discussions of mechanisms specific to the hepatic, developmental, immunotoxic, and hormone effects of perfluoroalkyls.

2.20.1 Cellular Mechanisms of Toxicity

PPARα-Dependent Mechanisms

Activation of the PPARα receptor in rodents initiates a characteristic sequence of morphological and biochemical events, principally, but not exclusively, in the liver. These events include marked hepatocellular hypertrophy due to an increase in number and size of peroxisomes, a large increase in peroxisomal fatty acid β oxidation, increased cytochrome 450-mediated ω hydroxylation of lauric acid, and alterations in lipid metabolism. Although there is uncertainty regarding the exact and possibly, multiple mechanisms for liver effects of perfluoroalkyls, peroxisome proliferation mediated by PPARα is a contributing mechanism. Proliferation of peroxisomes in laboratory animals exposed to perfluoroalkyls is discussed in Section 2.9 (Hepatic); as discussed in that section, hepatic peroxisome proliferation has been shown in rats exposed to PFOA and in mice exposed to PFDA.

Many, but not all, of the adverse effects induced by perfluoroalkyls in rodents are mediated through activation of the PPARα. Ligands, including perfluoroalkyls, bind to and activate PPARα, causing a conformational change in the receptor that leads to dissociation of co-repressors and enables heterodimerization with the retinoid X receptor (Corton et al. 2014). The activated receptor complex binds to a DNA direct repeat motif (the peroxisome proliferator response element or PPRE) located in the promoters of peroxisome proliferator responsive genes. The binding of the receptor complex leads to recruitment of co-activators, which acetylate histones and remodel chromatin, enabling RNA polymerase to transcribe the target gene(s). PPARα regulates lipid homeostasis by modulating the expression of genes involved in fatty acid uptake, activation, and oxidation. Activation of nuclear receptors including PPARα is a complex, dynamic process that depends on levels of expression of the receptors in different

tissues, competition among receptors for endogenous and exogenous ligands and for binding sites on chromatin, and availability and abundance of co-activators and/or co-repressors (Corton et al. 2014).

PPARα Receptor Activation. Many perfluoroalkyls, including PFOA, PFOS, PFUnA, PFHpA, and PFDoDA have been shown to activate PPARα in mammalian cells *in vitro* (Bjork and Wallace 2009; Bjork et al. 2011; Shipley et al. 2004; Takacs and Abbott 2007; Vanden Heuvel et al. 2006; Wolf et al. 2008b, 2012). Cell systems used in these studies include COS-1 cells expressing mouse, rat, or human PPARα, and cultured rat, mouse, and human hepatocytes. In these studies, perfluoroalkyl sulfonate compounds were less potent than perfluoroalkyl carboxylate compounds in activating PPARα-induced gene expression, and the potency of stimulation within each class increased with carbon chain length (Bjork and Wallace 2009; Wolf et al. 2008b, 2012). In comparison with naturally occurring long-chain fatty acids such as linoleic and α linoleic acids, PFOA and PFOS are relatively weak ligands for PPARα (Vanden Heuvel et al. 2006)

PPARα-Dependent Gene Expression Changes. Perfluoroalkyls have been shown to induce changes in the expression of genes under the regulation of PPARα. The expression of PPARα target genes in the liver involved in fatty acid metabolism, cell cycle control, peroxisome biogenesis, and proteasome structure and organization were upregulated, while inflammatory response genes in the liver were downregulated in wild-type mice exposed orally to PFOA or the PPARα agonist WY-14,643 (Rosen et al. 2008a). Furthermore, PFOA and PFDA have been shown to downregulate, via PPARα activation, genes involved in bile transport in the livers of wild-type mice exposed by intraperitoneal administration (Cheng and Klaassen 2008a). Both compounds decreased expression of organic anion transporting polypeptides [*OATP1a1*, *1a4,* and *1b2*], and PFDA also downregulated sodium-taurocholate cotransporting polypeptide [*Nctp*], via activation of PPARα. Many of these expression changes may play roles in the hepatic effects of perfluoroalkyls.

Gene expression changes induced by perfluoroalkyls have been extensively studied in experiments aimed at determining the extent to which the adverse effects of these compounds are dependent on activation of PPARα or interaction with other nuclear receptors (Foreman et al. 2009; Rosen et al. 2008a, 2008b, 2010, 2017). These studies, comparing gene expression changes in wild-type and PPARα-null mice exposed to perfluoroalkyls, demonstrate the following:

- A majority of the gene expression changes induced in rodents by perfluoroalkyls tested to date, especially PFOA and PFNA, are dependent on activation of PPARα.

- Perfluoroalkyls also induce gene expression changes that are independent of PPARα.
- The extent to which gene expression changes induced by perfluoroalkyls are dependent on activation of PPARα varies by compound.

Species Differences in PPARα Activation. Species differences in response to PPARα activators have been reviewed by Corton et al. (2014). Studies of PPARα activation by structurally diverse ligands in various species have shown that rats and mice are the most sensitive species to PPARα agonists, whereas guinea pigs, hamsters, nonhuman primates, and humans are less responsive (Corton et al. 2014). However, the differences do not appear to result from differences in the PPARα gene itself: PPARα cDNA from humans is indistinguishable from the rodent PPARα. Species differences in response to exogenous PPARα activators may stem from any or all of the following: (1) differences in the expression of PPARα in a given tissue; (2) differences in the gene product structure; and (3) differences in the ligand-mediated transactivation of PPARα. Experiments quantifying mRNA and/or protein levels of PPARα show ~10-fold higher expression of PPARα in the livers of mice and rats compared with humans and guinea pigs, but available data are limited and require further study to validate these differences (Corton et al. 2014). In humans, variants of PPARα that may affect its transactivation potential have been identified. For example, humans produce higher levels of a truncated PPARα (that lacks a ligand binding domain) compared with mice and rats (Corton et al. 2014). The truncated form appears to inhibit the activity of the full-length receptor, possibly via sequestering critical co-activators. Other, non-truncated variants of PPARα have been identified in humans, but the sensitivity of these variants to PPARα activators does not differ markedly from that of the wild-type receptor.

Species and compound-related differences in PPARα transactivation by perfluoroalkyls have been demonstrated *in vitro* (Shipley et al. 2004; Takacs and Abbott 2007; Vanden Heuvel et al. 2006; Wolf et al. 2008b, 2012). In a comparison of human and mouse PPARα transactivation by different perfluoroalkyls in transfected COS-1 cells, Wolf et al. (2008b, 2012; see Table 2-28) found that some perfluoroalkyls exhibited marked species differences in transactivation potency (for example, PFUnA, PFDA, PFDoDA), while other compounds showed similar transactivation potency for both human and mouse PPARα (for example, PFNA, PFOA, perfluoropentanoic acid [PFPeA]).

Table 2-28. Transactivation of Human and Mouse PPARα in Transfected Cos-1 Cells Exposed to Perfluoroalkyls (In Order of Decreasing C_{20max} in the Mouse)

		Human		Mouse		C_{20max} (μM)[a]	
Perfluoroalkyl	Carbon number	NOEC (μM)	LOEC (μM)	NOEC (μM)	LOEC (μM)	Human	Mouse
PFNA	9	1	5	1	5	11	5
PFOA[b]	8	5, 0.5	10,1	0.5, 1	1, 3	7	6
PFUnA	11	50	75	5	10	86	8
PFHpA	7	<0.5	0.5	3	5	15	11
PFDA	10	100	>100[c]	<5	5	–	20
PFDoDA	12	75	90	3	5	NA	33
PFPeA	5	0.5	1	1	5	52	45
PFHxA	6	5	10	10	20	86	45
PFBA	4	30	40	30	40	75	51
PFHxS	6	5	10	10	20	81	76
PFOS	9	20	30	60	90	262	94
PFBS	4	20	30	120	150	206	317

[a]Perfluoroalkyl concentration yielding 20% of maximum response given by the most active compound (PFNA).
[b]Results from two separate experiments.
[c]Slope for human PPARα dose-response line was not significant.

– = not active; LOEC = lowest-observed-effect concentration; NA = not available; NOEC = no-observed-effect concentration; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFPeA = perfluoropentanoic acid; PFUnA = perfluoroundecanoic acid; PPAR = peroxisome proliferator activated receptor

Source: Wolf et al. 2008b, 2012

In addition to differences in transactivation of PPARα, Corton et al. (2014) noted that there are species differences in the transcripts controlled by PPARα. While PPARα activation leads to hypolipidemic changes in both humans and laboratory rodents, the gene sets responsible for these changes may differ. In a comparison between human and mouse hepatocytes exposed to the prototypical PPARα ligand WY-14,643, some genes (ACOX1, ECH1, PEX11A, and ACAA1) were induced in both species, while some (*Ehhadh*, *Pxmp4*, *Acot4*, and *Peci*) were induced only in mouse hepatocytes (Corton et al. 2014). Importantly, PPARα activators induce large increases in the expression of fatty acyl-CoA oxidase (ACO, which is believed to play a role in oxidative stress-induced liver cancer) in rodent hepatocytes, but relatively weak increases in human hepatocytes (Corton et al. 2014). Other hypothesized explanations for the species difference in response to exogenous PPARα ligands include variations in the structure of the

PPRE that alter the response of the human genes compared with rodents; differences between humans and rodents in the functions of genes under the regulation of PPARα; and differences in the ability of ligand-bound human and mouse PPARα receptor complex to recruit or interact with co-activators (Corton et al. 2014).

PPARα-Independent or Associative Mechanisms

Experiments using PPARα-null mice have demonstrated that perfluoroalkyls exert some adverse effects, including developmental and hepatic effects, through mechanisms other than activation of PPARα. These may include activation of other nuclear receptors, increased oxidative stress, dysregulation of mitochondrial function, and inhibition of GJIC. While some of these effects have been seen after exposure to PPARα activators (Corton et al. 2014), these mechanisms may also occur independent of PPARα activation.

Activation of Other Nuclear Receptors. Examination of gene expression changes, as well as studies using other knock-out mice, have shown that some of the PPARα-independent effects induced by perfluoroalkyls may be mediated by activation of other nuclear receptors, especially PPARγ, CAR, and ERα. In a series of experiments, Rosen et al. (2008b, 2010, 2017) compared the gene expression changes induced by perfluoroalkyls in wild-type and PPARα -null mice with gene expression changes induced by known agonists of PPARγ, CAR, and ERα. Using these data, the study authors estimated the percentage of gene expression changes that were independent of activation of PPARα, and identified other nuclear receptors potentially involved in the changes induced by the perfluoroalkyls. The results, summarized in Table 2-29, show that between 10 and 24% of gene expression changes induced by perfluoroalkyls are independent of PPARα. All four compounds tested (PFOA, PFOS, PFNA, and PFHxS) were shown to alter the expression of PPARγ- and CAR-regulated genes in PPARα-null mice, and PFNA and PFHxS also altered the expression of ERα-regulated genes in the knock-out mice. In contrast, none of the compounds altered the expression of genes commonly affected by an agonist of LXR in either wild-type or null mice.

Table 2-29. Gene Expression Changes Induced by Perfluoroalkyls

	Dose (mg/kg/day for 7 days)	% PPARα-independent gene changes	Gene expression changes similar to those induced by prototypical agonist							
			PPARγ		CAR		ERα		LXR	
			WT	PPARα-null	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null
PFOA	3	~14	+	+	+	+	+	–	–	–
PFOS	10	~16	+	+	+	+	+	–	–	–
PFNA	1	~10	+	+/–	+	–	+	–	–	–
	3	~17	+	+	+	+	+	+	–	–
PFHxS	3	24	+	–	+	–	+	+	–	–
	10	22	+	+	+	+	+	+	–	–
WY-14,643[a]	0.1% in diet	2	+	–	–	–	+	–	–	–

[a]WY-14,643 is a PPARα agonist.

+ = significant ($p<0.0001$) similarity to gene expression changes induced by prototypical receptor agonist as assessed by running Fisher test; +/– = equivocal evidence; CAR = constitutive androstane receptor; ER = estrogen receptor; LXR = liver X receptor; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PPAR = peroxisome proliferator activated receptor; WT = wild-type

Sources: Rosen et al. 2017, 2008b, 2013; Wolf et al. 2008b

Gene expression changes typical of CAR and PXR activators (phenobarbital and pregnenolone 16 α-carbonitrile [PCN]) were also observed in rat liver after oral exposure to PFOA and PFOS (Ren et al. 2009). In addition, PFDA was shown to activate CAR-dependent genes in a study comparing wild-type and CAR-null mice exposed by intraperitoneal injection (Cheng and Klaassen 2008b).

These data suggest that perfluoroalkyls may induce gene expression changes through activation of other nuclear receptors including PPARγ, CAR, and ERα. Support for these findings are available from *in vitro* studies demonstrating binding and/or transactivation of PPARγ, CAR, and ERα by perfluoroalkyls. Both PFOA and PFOS activated PPARγ in cultured human, mouse, and rat hepatocytes, albeit with much lower potency than the known agonist rosiglitazone; neither LXRβ nor RXRα was activated in this system (Vanden Heuvel et al. 2006). Zhang et al. (2014) observed binding of PFOA and PFOS to human PPARγ in transfected *Escherichia coli*. However, in experiments conducted by Takacs and Abbott (2007), neither PFOA nor PFOS activated the mouse or human PPARγ.

Oxidative Stress. Perfluoroalkyls increase oxidative stress in the liver, kidney, and brain. Increases in oxidative stress may be mediated in part via PPARα activation, but may also result from activation of the Nrf2 receptor (Xu et al. 2016).

Oxidative stress may contribute to oxidative DNA damage, tumor promotion, perturbation of lipid homeostasis, and stimulation of inflammation, among other changes; thus, increases in oxidative stress can have diverse physiological effects. Evidence that perfluoroalkyls increase oxidative stress is available from *in vivo* and *in vitro* studies. For example, oxidative DNA damage (measured as 8-OH-dG levels) was significantly increased in the liver, but not the kidneys, of male rats exposed to PFOA via feed for 2 weeks (Takagi et al. 1991). In HepG2 cells cultured with PFOA or PFOS, significant increases in reactive oxygen species (ROS) (measured as 2'7'-dichlorofluorescein diacetate fluorescence) were observed, but there was no evidence of DNA damage measured with the comet assay (Eriksen et al. 2010). In this system, PFNA, PFBS, and PFHxA did not induce ROS production, but a significant increase in DNA damage was seen in cells exposed to PFNA (Eriksen et al. 2010).

In male, but not female, KM mouse pups administered a single subcutaneous injection of PFOS at 1, 2, 3, 4, or 5 weeks of age, brain total antioxidant capacity (T-AOC) was lower than controls at most time points, and significantly decreased after treatment on PND 21 (Liu et al. 2009). In the liver, T-AOC was decreased in male pups treated on PNDs 7 and 14, and in females treated on PND 21. Significant decreases in superoxide dismutase (SOD) activity were noted in the brain of males treated on PNDs 7 and 21, and in the liver of females treated on PND 14.

Increases in oxidative stress can lead to NFκB activation (Corton et al. 2014). NFκB activation plays a role in tumorigenesis, and NFκB transcription factors coordinate immune responses. Few studies have examined NFκB activation after exposure to perfluoroalkyls. An increase in NFκB mRNA level was seen in the hippocampus of neonatal rats exposed to PFOS *in utero* (Zeng et al. 2011). In addition, NFκB nuclear translocation was accelerated, and NFκB was activated, in breast cancer cells exposed to PFOA (Zhang et al. 2014). The activation of NFκB was associated with increased invasiveness of the breast cancer cells, as coexposure to an inhibitor of NFκB reduced the invasiveness induced by PFOA.

Gap Junction Intercellular Communication (GJIC) Inhibition. Perfluoroalkyls also have been shown to inhibit GJIC both *in vivo* and *in vitro* in rats (Corton et al. 2014). GJIC plays an important role in maintenance of tissue homeostasis, intercellular transmission of regulatory signals, and metabolic cooperation. Disruption of GJIC is thought to be involved in neurological, reproductive, and endocrine

abnormalities, as well as in carcinogenesis (Corton et al. 2014; EPA 2016h). There are limited data examining the effects of perfluoroalkyls on GJIC. The available studies showed that both PFOA and PFOS inhibited GJIC in the livers of rats exposed via diet for 1 week or 3 or 21 days, respectively (Hu et al. 2002; Upham et al. 1998, 2009). *In vitro* studies in WB-344 rat liver epithelial cells also showed inhibition of GJIC after exposure to PFOS (Hu et al. 2002) and to perfluorinated fatty acids with 7–10 carbons (Upham et al. 1998, 2009). In this system, PFOA activated extracellular receptor kinase, which may play a role in the inhibition of GJIC. In addition, inhibition of phosphatidylcholine-specific phospholipase C partially mitigated the GJIC inhibition, suggesting that PFOA-induced activation of this enzyme may also be involved in GJIC inhibition (Upham et al. 1998, 2009).

PFOS was also shown to inhibit GJIC in dolphin kidney epithelial cells and rat Sertoli cells *in vitro* (Hu et al. 2002; Wan et al. 2014a). In Sertoli cells, GJIC plays an important role in maintenance of the blood:testes barrier and in intercellular communication during spermatogenesis (EPA 2016i).

Impaired Mitochondrial Function. Mitochondrial function, including cellular respiration as well as mitochondrial membrane potential, has been shown to be perturbed by perfluoroalkyls. Available data suggest that PFOA and PFOS are relatively weak mitochondrial toxicants (EPA 2016h, 2016i). Mitochondrial proliferation was observed in rats exposed orally to PFOA for 28 days and in mice exposed to PFOA during gestation and lactation (Quist et al. 2015a, 2015b; Waters et al. 2009). In isolated rat liver mitochondria, higher concentrations of either PFOA or PFOS were noted to slightly increase resting respiration rate and decrease membrane potential, possibly due to these compounds' effects on membrane fluidity (Starkov and Wallace 2002). Testing of other perfluoroalkyls for effects on mitochondrial respiration rate and oxidative phosphorylation showed a wide range of inhibitory activities, with PFOS demonstrating the highest potency (3-fold higher than PFOA and 20–30-fold higher than PFBS and PFHxA) (Wallace et al. 2013).

2.20.2 Hepatic Toxicity Mechanisms

Hepatic effects of perfluoroalkyls in rodents likely result from a combination of PPARα-dependent and independent changes; see Table 2-30. For example, increased liver weight has been observed in both wild-type and PPARα-null mice orally exposed to PFOA or APFO (Nakagawa et al. 2012; Rosen et al. 2008a), PFOS (Qazi et al. 2009b; Rosen et al. 2010), PFNA (Das et al. 2017; Rosen et al. 2017), or PFHxS (Das et al. 2017; Rosen et al. 2017), but not in null mice exposed to PFBA by intraperitoneal injection (Foreman et al. 2009). Similarly, both wild-type and PPARα-null mice exposed to APFO

exhibited increased hepatocyte vacuolation and proliferation, while exposure to WY-14,643 did not induce such changes in the null mice (Wolf et al. 2008b). Das et al. (2017) showed that PFOA, PFNA, and PFHxS also increased hepatocyte cell size, percent lipid, and hepatic triglyceride levels, and decreased hepatic DNA content, in both wild-type and PPARα-null mice, while WY-14,643 did not, indicating that these effects were not dependent on PPARα activation. Similarly, Nakagawa et al. (2012) showed that at a lower APFO dose (1.0 mg/kg/day for 6 weeks), increases in hepatic triglyceride levels were observed in wild-type, PPARα-null, and humanized PPAR (hPPAR) mouse strains; however, at a higher dose (5 mg/kg/day), hepatic triglyceride levels were still increased in PPARα-null and hPPAR mice, but decreased in wild-type mice.

Table 2-30. Hepatic Effects of Perfluoroalkyls in Wild-Type and PPARα-Null Mice Exposed Orally

		↑ Relative liver weight		↑ % Lipid by cell area		↑ Hepatic triglycerides		↑ Hepatocyte cell size		↓ Hepatic DNA content	
	Dose (mg/kg/day)	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null
PFOA	3	+++	+++	+++	-	+++	-	+++	+++	+	+
PFOS	10	++	++	ND							
PFNA	1	++	+	ND							
	3	+++	++								
	10	+++	+++	+++	+++	+++	+++	+++	++	+	+
PFHxS	3	+	+	ND							
	10	+++	+++	+++	+++	+++	–	+++	+++	+	+
WY-14,643[a]	50	+++	–	+++	–	–	–	+++	–	+	–

[a]WY-14,643 is a PPARα agonist.

+ = statistically significant change from control (the number of plus signs indicates degree of change from controls); – = not statistically significantly different from control; DNA = deoxyribonucleic acid; ND = no data; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PPAR = peroxisome proliferator activated receptor; WT = wild type

Sources: Das et al. 2017; Rosen et al. 2008a, 2010, 2017

Lipid homeostasis is maintained through a balance between fatty acid synthesis or accumulation and fatty acid oxidation. Available data indicate that perfluoroalkyls affect both sides of this balance, but a growing body of evidence indicates that fatty acid accumulation induced by perfluoroalkyls tips the balance in favor of hepatic steatosis (Das et al. 2017). As discussed above, perfluoroalkyls alter lipid homeostasis via PPARα activation, which upregulates genes involved in fatty acid oxidation and reduces lipid levels. However, as noted above, Das et al. (2017) indicate that perfluoroalkyls also perturb lipid homeostasis via PPARα-independent mechanisms. In addition to the effects noted in Table 2-30, increased incidences of hepatic steatosis were seen in PPARα-null mice exposed to perfluoroalkyls (Das

et al. 2017; Minata et al. 2010; Nakagawa et al. 2012), but not in those exposed to the PPARα agonist WY-14,643 (Das et al. 2017). Additionally, microvesicular steatosis was observed in hPPAR mice (Nakagawa et al. 2012). The findings are consistent with earlier studies showing triglyceride accumulation in rodent livers after exposure to perfluoroalkyls (Kudo and Kawashima 1997, 2003; Kudo et al. 1999); hepatic steatosis and glucose intolerance in adult rats exposed to PFOS during the prenatal and postnatal periods (Lv et al. 2013); and inhibited hepatic secretion of VLDL, resulting in steatosis, in APOE3-Leiden mice (a rodent model with lipoprotein metabolism similar to humans) exposed to PFOS or PFHxS (Bijland et al. 2011).

Das et al. (2017) investigated whether the steatosis induced by PFOA, PFNA, and PFHxS was mediated by increased fatty acid or triglyceride synthesis or by inhibition of mitochondrial fatty acid transport or β-oxidation. Microarray analysis of mouse liver after exposure to these compounds showed upregulation of genes involved in fatty acid and triglyceride synthesis in both wild-type and PPARα-null mice. In contrast, *in vitro* experiments demonstrated that these perfluoroalkyls did not affect mitochondrial fatty acid oxidation in isolated rat liver mitochondria, and neither PFOA nor PFOS altered fatty acid oxidation in HepG2/C3A human liver cells. The authors suggested that perfluoroalkyls induce hepatic steatosis by perturbing lipid homeostasis in favor of the accumulation of fatty acids and triglycerides in the liver.

Data are also available to suggest that proinflammatory cytokines may also contribute to the hepatotoxicity of perfluoroalkyls. Studies in rodents have shown that *in vivo* exposure to PFOA (Qazi e al. 2013; Yang et al. 2014) or PFNA (Fang et al. 2012b, 2012c) have resulted in increases in IL-6, IL-1β, tumor necrosis factor-α (TNFα), C-reactive protein, and COX-2 at higher perfluoroalkyl doses (Fang et al. 2012b, 2012c; Yang et al. 2014) and decreases in TNFα, interferon-γ (IFN-γ), IL-4, and IL-6 levels at lower doses (Fang et al. 2012b; Qazi et al. 2013). Exposure to PFNA also resulted in increased expression of TNFα, IL-1β, and IL-6 mRNA (Fang et al. 2012b). Nakagawa et al. (2012) found increases in TNFα-mRNA in wild-type (2.9-fold), PPARα-null (1.9-fold), and humanized PPARα (1.9-fold) mouse strains exposed to 5 mg/kg/day doses of PFOA. Fang et al. (2012c) suggested that PFNA exposure stimulated liver Kupffer cells to release large amounts of TNFα and IL-1β and that the release of these cytokines activated the NFκB p65 pathway causing suppression of PPARα promoter activity and resulting in increases in liver triglyceride levels and steatosis.

2.20.3 Developmental Toxicity Mechanisms

Developmental effects observed in laboratory rodents exposed to perfluoroalkyls include prenatal loss, reduced neonate weight and viability, neurodevelopment toxicity, and delays in mammary gland differentiation, eye opening, vaginal opening, and first estrus (see Section 2.17 Developmental). During development, PPARα, PPARβ, and PPARγ mRNA and protein are expressed in the embryo of rodents and humans (Abbott 2009; Abbott et al. 2010). In humans, the fetal expression levels are equivalent to levels in adult tissues (Abbott et al. 2010). PPARα activation also appears to be involved in some, but not all, of the developmental effects of perfluoroalkyls in mice, and the role of PPARα in mediating developmental toxicity differs among the various compounds. For example, a gestational exposure study of PFOA resulted in decreases in postnatal survival in wild-type mice, but not in PPARα-null mice, while the occurrence of full-litter resorptions was similar between the two genotypes (Abbott 2009; Abbott et al. 2007). In contrast, gestational exposure to PFOS resulted in decreased pup survival in both wild-type and PPARα-null mice (Abbot et al. 2009). The developmental effects of PFNA, including reduced pup survival and body weight and delayed eye opening, were seen only in wild-type, and not in PPARα-null mice; however, maternal pregnancy rate was affected only in the null mice (Wolf et al. 2010). No alterations in postnatal survival or growth were observed in wild-type mice exposed to PFBA *in utero* (Das et al. (2008). The investigators suggested that the contrast of these findings to that of PFOA may be due to the shorter half-life of PFBA (daily administration did not result in reaching steady-state) and that PFBA is a less potent agonist of PPARα than PFOA.

Abbott et al. (2012) showed that PFOA altered expression of genes that are involved in homeostatic control of lipids and glucose, and postulated that decreased neonatal survival and body weights may be, in part, due to metabolic disruption. It has been suggested that PFOS interacts with pulmonary surfactants, and that this effect is responsible for neonatal mortality seen in rats. Grasty et al. (2003, 2005) showed that neonatal mortality in PFOS-exposed rats was highest when exposure occurred during the gestational period of lung maturation (GDs 17–20), and that the morphometry of the lungs in exposed neonates was consistent with immaturity. However, treatment of neonates with rescue agents that hasten lung maturation did not prevent neonatal mortality induced by PFOS, and examination of the pulmonary surfactant profile in exposed animals showed no difference from controls, leading Grasty et al. (2005) to conclude that neonatal mortality in neonatal rats exposed to PFOS was not due to immaturity. Other hypotheses pertaining to the mechanisms of developmental toxicity of perfluoroalkyls were not located. However, other molecular- and cellular-level effects of perfluoroalkyls, including increased oxidative

stress, dysregulation of mitochondrial function, and receptor-mediated events, may be involved in the observed developmental effects of these compounds.

2.20.4 Immunotoxicity Mechanisms

NTP (2016b) conducted a systematic review of the human, animal, and *in vitro* data examining immunotoxic effects of PFOA and PFOS. The conclusion of the systematic review was that both PFOA and PFOS are "presumed to be immune hazards to humans." Evidence was considered strong that both compounds were associated with suppression of the antibody response, while there was weaker evidence for PFOA-induced impairment of infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence, and for PFOS-induced suppression of natural killer cell activity. A recent study comparing the T-cell dependent antibody response (TDAR) in female wild-type and PPARα knock-out mice after exposure to PFOA with or without antigen exposure showed that PFOA suppressed TDAR in both wild-type and knock-out mice, indicating that the mechanism for antibody response suppression is independent of PPARα activation (DeWitt et al. 2016). These investigators observed no treatment-related changes in splenic lymphocyte subpopulations in exposed mice of either genotype, suggesting that PFOA suppressed TDAR via impairment of B-cell/plasma cell function rather than by altering lymphocyte numbers. DeWitt et al. (2012) and Corsini et al. (2014) reviewed mechanistic data for perfluoroalkyl-induced suppression of antibody response, and postulated that perfluoroalkyls may modulate cell-signaling responses critical to antibody production, including c-Jun, NF-κB, and IL-6.

2.20.5 Endocrine Mechanisms

Perfluoroalkyls have been shown to induce alterations in thyroid hormone levels in rats, and associations between serum perfluoroalkyl concentrations and thyroid hormone levels have been reported in human epidemiological studies (see Section 2.13). Few data examining mechanisms of thyroid hormone disruption are available, but suggest that effects of perfluoroalkyls on thyroid function may be mediated by binding to the thyroid hormone receptor, and/or by altering expression of genes involved in thyroid function or thyroid hormone regulation. Several perfluoroalkyls were shown to bind to the human thyroid hormone receptor in cultured GH2 cancer cells and in molecular docking experiments (Ren et al. 2015). In the *in vitro* tests, all 16 of the tested compounds exhibited lower affinity for the receptor than T3 (Ren et al. 2015). Among the tested compounds, PFOS exhibited the strongest agonist activity (Ren et al. 2015). Alterations in the mRNA or protein levels of thyroid-regulating genes have been observed after oral exposure of male Sprague-Dawley rats to PFOS. PFOS exposure for 5 or 90 days resulted in

decreased hepatic levels of mRNA type 1 deiodinase (DIO1, which bioactivates T3 by deiodination of T4) (Martin et al. 2007; Yu et al. 2009a); after 5 days of exposure, hepatic mRNA for type 3 deiodinase (DIO3, which inactivates T3) was increased relative to controls (Martin et al. 2007). After 90 days, hepatic levels of uridine diphosphoglucuronosyl transferase 1A1 (UGT1A1, which plays a role in T4 turnover) mRNA and thyroid levels of DOI1 protein were increased, while there were no changes in thyroid levels of the sodium iodide symporter, thyrotropin (THS) receptor, or activity of thyroid peroxidase (Yu et al. 2009a).

Limited data from *in vitro* studies suggest the possibility that perfluoroalkyls may interact with the estrogen and androgen receptors. PFOA, PFOS, PFHxS, PFNA, and PFDA were all shown to be antagonists of the androgen receptor, while PFOA, PFOS, and PFHxS induced transactivation of the estrogen receptor (Kjeldsen and Bonefeld-Jorgensen 2013). Recently, analysis of gene expression data from the livers of wild-type and PPARα-null mice exposed to PFOA, PFOS, PFHxS, and PFNA by gavage for 7 days indicated similarities to gene expression changes induced by known ERα agonists (Rosen et al. 2017), providing indirect evidence for perfluoroalkyl changes in the liver mediated via ER activation. However, at oral doses up to 1 mg/kg, PFOA failed to induce treatment-related alterations in uterine weight, ER-dependent gene expression, or morphology of reproductive organs in uterotrophic assays using immature CD-1 mice (Dixon et al. 2012; Yao et al. 2014), suggesting that PFOA is either inactive *in vivo* or of very low estrogenic potency.

2.20.6 Cancer Mechanisms

PFOA induced hepatocellular adenomas, Leydig cell adenomas, and pancreatic acinar cell adenomas in rats (Biegel et al. 2001). Liver tumors induced by PFOA are believed to be mediated largely through PPARα activation, and considered to be of limited or no relevance to humans (EPA 2016h), based on species differences in response to PPARα (see details above under PPARα activation). An expert panel convened by EPA's Science Advisory Board in 2006 to review issues related to the toxicity of PFOA agreed that the weight of evidence supports the hypothesis that induction of liver tumors in rats by PFOA is mediated by a PPARα agonism mode of action (EPA 2006); this conclusion is also reflected in the EPA Health Effects Support Document for PFOA (EPA 2016h). A recent review by a panel of experts from academia, government, industry, and consulting groups updated the Klaunig et al. (2003) assessment of PPARα agonism as a liver cancer mode of action, and drew the same conclusion: while the PPARα mode of action for liver tumors is biologically plausible, species differences in response to PPARα activation indicate that liver tumors are unlikely to be induced by PPARα induction in humans (Corton et al. 2014).

Studies conducted in rainbow trout, an animal model that is similar to humans in terms of insensitivity to peroxisome proliferators, suggest that some perfluoroalkyls may induce liver cancer by alternate mechanisms (Benninghoff et al. 2011, 2012). The investigators (Benninghoff et al. 2011) found that PFOA, PFNA, PFDA, and PFUnA were potent inducers of vitellogenin, an estrogen-responsive biomarker protein at fairly high doses. Neither PFOA nor PFDA exposure resulted in vitellogenin expression at serum levels corresponding to general population serum levels of 2–7 ng/L. *In vitro*, PFOA, PFOS, PFHpA, PFNA, PFUnA, and PFDA also had weak to very weak affinities for estrogen receptors (ERα) for several species including humans, mice, and rats (Benninghoff et al. 2011). *In vivo* studies demonstrated that PFOA, PFOS, PFNA, and PFDA enhanced liver carcinogenesis in AFB_1 initiated fish via a mechanism that likely involves interactions with hepatic estrogen receptors (Benninghoff et al. 2012).

Although Leydig cell tumors are commonly induced by peroxisome proliferating agents such as perfluoroalkyls, the mode of action by which these tumors are induced, and thus their relevance to humans, is much less clear (Corton et al. 2014; EPA 2016h; Klaunig et al. 2003). One mode of action proposed for the induction of Leydig cell tumors involves PFOA-induced inhibition of testosterone biosynthesis, leading to increased production of gonadotropin releasing hormone and circulating LH, which promotes Leydig cell proliferation. Activation of PPARα may be involved in the decreased serum testosterone levels; PPARα-null mice did not exhibit the reduction in testosterone concentration seen in wild-type mice exposed to PFOA (Li et al. 2011). Evidence of decreased serum testosterone and increased serum estradiol was seen in studies of male rats exposed orally to PFOA for 14 days (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996). Reduced testosterone levels may occur through the conversion of testosterone to estradiol via the enzyme aromatase. Hepatic aromatase activity was shown to be markedly increased in male rats exposed to APFO by gavage for 14 days, and aromatase activity was positively correlated with serum estradiol levels in these animals (Liu et al. 1996). The relevance of Leydig cell tumors induced by PFOA to human risk assessment is uncertain. For example, an intermediate-duration study in Cynomolgus monkeys exposed to PFOA did not find treatment-related alterations in serum estradiol, estrone, estriol, or testosterone (Butenhoff et al. 2002). Studies of humans occupationally exposed to PFOA have not consistently reported alterations in estradiol or testosterone levels (Klaunig et al. 2012). In addition, humans are less sensitive than rats to LH stimulation, and the average number of LH receptors per Leydig cell is 13-fold higher in rats than humans (Klaunig et al. 2012). In summary, the induction of Leydig cell tumors by PFOA may be mediated by effects on aromatase activity or testosterone biosynthesis, both of which may be related to PPARα activation (EPA

2016h). While the relevance of the PPARα mode of action to humans is uncertain, the data supporting this mode of action for Leydig cell tumors is not sufficient to rule out human relevance (EPA 2016h).

The mechanism of PFOA-induced pancreatic acinar cell tumors in rodents has not been elucidated, and relevant data are limited. A proposed mode of action involves stimulation of PPARα leading to reduced bile flow and/or changes in bile acid composition with subsequent increase in cholecystokinin (CCK), which stimulates pancreatic cell proliferation and tumor formation (EPA 2016h). Effects on bile acid composition induced by PFOA may be mediated by effects on bile acid transporters. PFOA exposure has been shown to decrease expression of OATPs and increase expression of MRP3 and MRP4 (Cheng and Klassen 2008a; Maher et al. 2008). In a study using wild-type and PPARα-null mice, increased biliary excretion of PFOA was seen in wild-type mice compared with null mice, and biliary excretion of bile acids was highest in the null mice (Minata et al. 2010). These observations suggest the possibility that increased excretion of PFOA could diminish the excretion of bile acids that require the same transporters. However, given the limitations in available data, information is insufficient to fully characterize the mode of action for PFOA-induced pancreatic tumors (EPA 2016e).

Mechanisms of carcinogenicity of PFOA are unknown. Liver and Leydig cell tumors produced by PFOS may be associated with PPARα activation or may involve other mechanisms. PFOS activates PPARβ/δ, γ, and CAR and PXR (Ren et al. 2009).

2.21 GENOTOXICITY

The genotoxicity of perfluoroalkyls has not been extensively studied, with the most information available for PFOA and PFOS. To supplement the information reported in the published literature, results of unpublished studies taken from publicly available reviews have been included in the following discussions. No studies of genotoxicity in humans exposed to perfluoroalkyls were located.

PFOA

The genotoxicity of PFOA has been examined in bacterial and mammalian *in vitro* systems and in mammalian *in vivo* assays. In general, results show that PFOA can produce DNA damage, but is not mutagenic at noncytotoxic concentrations.

Results of *in vitro* studies in bacteria show that PFOA induces DNA damage but is not mutagenic. DNA damage was observed in *Paramecium caudatum* following exposure to 100 µM for 12 and 24 hours (Kawamoto et al. 2010). Intracellular ROS was significantly increased but DNA damage was not reversed by the application of glutathione, a ROS inhibitor, indicating that intracellular ROS may not be the cause of PFOA-induced DNA damage. PFOA was not mutagenic in *Salmonella typhimurium* TA1535/pSK1002 (*hisG46, rfa, uvrB*) with or without metabolic activation using the *umu* test (Oda et al. 2007) or in *S. typhimurium* TA98, TA100, TA102, and TA104 strains with or without metabolic activation using an Ames assay (Fernández Freire et al. 2008). Butenhoff et al. (2014) and Kennedy et al. (2004) summarized the results of various unpublished mutagenicity studies with PFOA showing negative results in reverse mutation assays using *S. typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538), *Saccharomyces cerevisiae*, and *Escherichia coli* (WP2uvrA strain) with or without metabolic activation.

In vitro genotoxicity assays in mammalian cells show that PFOA induced DNA damage, although conflicting results have been reported for mutagenicity and increased micronuclei formation. Incubation of human hepatoma HepG2 cells with 50–400 µM PFOA caused DNA strand breaks and 100–400 µM increased the incidence of micronuclei, in a dose-related manner in both cases (Yao and Zhong 2005). These effects were accompanied by a significant increase in ROS, which the investigators suggested caused the DNA damage. Bjork and Wallace (2009) measured mRNA expression for DNA damage inducible *Ddit3* to assess DNA damage in primary rat and human hepatocyte cultures and in HepG2/C3a hepatoma cells. Significant increases in mRNA transcription for *Ddit3* were found in primary rat hepatocytes at 100 µM PFOA and in primary human hepatocytes and HepG2/C3a hepatoma cells at 200 µM PFOA. Although both studies provide evidence of DNA damage, the tested concentrations were very high as compared to what could be expected to occur in the environment. A significant increase in mutation frequencies was observed in hamster-human hybrid cells exposed to 200 µM PFOA for 1–16 days; a 79% decrease in cell viability was also observed at this concentration (Zhao et al. 2011). Concurrent treatment with a ROS inhibitor significantly decreased mutations, indicating that ROS may play an important role in mediating the genotoxic effects of PFOA. In contrast, Butenhoff et al. (2014) and Kennedy et al. (2004) summarized the results of various unpublished mutagenicity studies with PFOA. In mammalian cells, PFOA was negative for forward mutations using Chinese hamster ovary cells, for chromosomal aberrations in Chinese hamster ovary cells and human lymphocytes, and for cell transformation in C3H 10T1/2 cells.

Results of *in vivo* exposure of laboratory animals show that PFOA induced DNA damage, but not micronuclei formation. Administration of a single intraperitoneal injection of 100 mg/kg PFOA to male Fischer-344 rats resulted in a significant increase in the levels of 8-hydroxydeoxyguanosine (a marker of oxidative DNA damage) in liver DNA, but not in kidney DNA (Takagi et al. 1991). Oral administration of approximately 20 mg/kg/day PFOA in the diet for 2 weeks to male Fischer-344 rats induced hepatomegaly and increased the levels of 8-hydroxydeoxyguanosine in liver DNA but not in kidney DNA (Takagi et al. 1991). Unpublished studies summarized by Butenhoff et al. (2014) and Kennedy et al. (2004) did not find increased micronuclei formation in mice orally exposed to PFOA.

PFOS

The genotoxicity of PFOS has been examined in bacterial and mammalian *in vitro* systems and in mammalian *in vivo* assays. However, compared to PFOA, less information is available. Results do not provide evidence for genotoxicity of PFOS, except for one *in vitro* study showing cell transformation and one report of increased micronuclei formation following *in vivo* exposure.

Results of *in vitro* studies in bacteria and mammalian cells show that PFOS did not induce DNA damage, mutagenicity or chromosome damage. In bacterial cell assays, as reviewed by OECD (2002), PFOS did not induce reverse mutations in *S. typhimurium* or *E. coli* with or without metabolic activation. A study published after this review also found that PFOS was not mutagenic in *S. typhimurium* TA1535/pSK1002 (*hisG46, rfa, uvrB*) with or without metabolic activation using the *umu* test (Oda et al. 2007).

In vitro genotoxicity assays of PFOS in mammalian cells were negative for DNA damage, mutagenicity, micronuclei formation, and chromosome damage, although one *in vitro* study reported cell transformation. PFOS did not result in DNA damage in Syrian hamster embryo cells at concentrations up to 50 µg/mL but did induce cell transformation at noncytotoxic concentrations (0.2 and 2 µg/mL) following 5 and 24 hours of exposure (Jacquet et al. 2012). Similarly, PFOS did not induce DNA damage or increased micronuclei formation in human hepatoma HepG2 cells following a 24-hour exposure to PFOS concentrations as high as 600 µM; cytotoxicity was observed at ≥300 µM (Florentin et al. 2011). Another study of with HepG2 cells did not find evidence of DNA damage at concentrations of 100 and 400 µM PFOS (Eriksen et al. 2010). As summarized by OECD (2002), PFOS did not induce chromosomal aberrations in human lymphocytes with or without metabolic activation and did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes.

Conflicting results have been reported on micronuclei formation following *in vivo* exposure to PFOS. Micronuclei frequency was increased and the ratio of polychromatic erythrocytes to normochromatic erythrocytes was decreased in bone marrow of rats following oral exposure to 0.6–2.5 mg/kg PFOS for 30 days (Celik et al. 2013; Eke and Celik 2016). As summarized by OECD (2002), PFOS did not induce micronuclei in the bone marrow of CD-1 mice in an *in vivo* assay.

Other Perfluoroalkyls

Little information is available on the genotoxicity of other perfluoroalkyl compounds, with available studies focused on DNA damage. No DNA damage was found in HepG2 cells incubated with 100 or 400 µM PFHxS or PFBS for 24 hours, although a "modest" increase in DNA damage was observed at 400 µM PFNA, a cytotoxic concentration (Eriksen et al. 2010). Oral administration of approximately 10 mg/kg/day PFDA in the diet for 2 weeks to male Fischer-344 rats induced hepatomegaly and also increased the levels of 8-hydroxydeoxyguanosine in liver DNA but not in kidney DNA (Takagi et al. 1991). In contrast, no DNA damage in liver or kidney was observed following administration of a single intraperitoneal injection of 100 mg/kg PFBA to male Fischer-344 rats (Takagi et al. 1991).

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Toxicokinetic data on perfluoroalkyls examined in this profile are available from studies in humans and animals. Most studies in animals administered perfluoroalkyls by the oral route. These data are briefly summarized below.

- Absorption
  - Perfluoroalkyls are absorbed following oral, inhalation, and dermal exposure.
  - Quantitative estimates of the fractional absorption of orally administered perfluoroalkyls in animals range from >50% for PFHxS to >95% for PFOA, PFBA, PFNA, PFDA, PFUnA, and PFDoDA.
  - No quantitative estimates of the fractional absorption of perfluoroalkyls following inhalation or dermal exposure were identified.
- Distribution
  - Perfluoroalkyls are widely distributed in the body, with the highest concentrations in the liver, kidneys, and blood.
  - In the blood, perfluoroalkyls bind to albumin and other proteins.
  - Perfluoroalkyls can be transferred to the fetus during pregnancy and to nursing infants.
- Metabolism
  - Results of available oral and *in vitro* studies suggest that perfluoroalkyls are not metabolized and do not undergo chemical reactions in the body.
  - Although no studies examining metabolism of perfluoroalkyls following inhalation or dermal exposure were identified, metabolism by these exposure routes is not expected.
- Excretion
  - Studies of elimination rates (i.e., half-lives) of perfluoroalkyls show that elimination $t_{1/2}$ values are similar following intravenous, intraperitoneal, and oral exposures. Findings suggest that the route of absorption has no substantial effect on rates of elimination of absorbed perfluoroalkyls.
  - Perfluoroalkyls are primarily eliminated in the urine, with smaller amounts eliminated in feces and breast milk.

- Perfluoroalkyls undergo biliary excretion, but substantial reabsorption occurs; therefore, biliary excretion is not a major elimination pathway.
- Rates of elimination of perfluoroalkyls vary substantially across chemical species and animal species, and show sex differences and age-dependencies within certain species.
- In general, perfluoroalkyl sulfonates are eliminated more slowly than perfluoroalkyl carboxylates; elimination rate decreases with increasing chain length, and increases with increased branching.
- In humans, estimates for elimination $t_{1/2}$ range from hours (PFBA: 72–81 hours) to several years (PFOA: 2.1–8.5 years; PFOS: 3.1–7.4 years; PFHxS: 4.7–15.5 years).
- Evidence for sex differences in elimination of perfluoroalkyls in humans is not as strong as in rats. Menstruation may contribute to faster elimination of PFOS in younger women (≤50 years) when compared to men and older women.

3.1.1 Absorption

Inhalation Exposure. Studies of the absorption of perfluoroalkyls in humans following inhalation exposure were not located; elevated serum concentrations of perfluoroalkyls in workers in fluorochemical production industry have been reported (see Table 5-22), indicating that perfluoroalkyls are absorbed following inhalation exposure. Occupational exposures in these workers are likely to have included inhalation of aerosols of perfluoroalkyls complexed with airborne dusts. Higher serum levels in workers compared to the general population (see Table 5-20) probably reflect a predominant contribution from inhaled perfluoroalkyls.

Studies conducted in rodents provide direct evidence for absorption of inhaled perfluoroalkyls. PFOA was detected in plasma of rats within 30 minutes of initiating nose-only exposures to aerosols (mass median aerodynamic diameter [MMAD]=1.9–2.1 µm) of 1–25 mg ammonium PFOA/m^3. Plasma concentrations increased during the 6-hour exposure, with the highest concentrations observed at 9 hours (3 hours after cessation of exposure) in male rats and at 7 hours (1 hour after cessation of exposure) in females (Hinderliter et al. 2006a). Assuming an elimination $t_{1/2}$ of absorbed PFOA of approximately 160 hours in male rats, a peak plasma concentration at 9 hours would correspond to an absorption $t_{1/2}$ of approximately 1.3 hours (see discussion below, Equations 3-1 and 3-2). The earlier time of highest plasma concentration observed in female rats appears to be associated with faster elimination of absorbed PFOA in female rats, compared to male rats (see Section 3.1.4).

Nose-only exposure of male rats to dusts of ammonium perfluorononanoate induced significant increases in absolute and relative liver weight, assessed 5 and 12 days after exposure, providing indirect evidence of absorption of this compound through the respiratory airways (Kinney et al. 1989).

Oral Exposure. Studies of absorption of perfluoroalkyls through the gastrointestinal tract in humans are not available. A study of the general population of Europe and North America estimated that the greatest portion of the chronic exposure to PFOS and PFOA results from the intake of contaminated food, including drinking water (Trudel et al. 2008). Direct evidence of oral absorption of perfluoroalkyls was provided in studies that found associations between environmental levels (e.g., drinking water) and perfluoroalkyl concentrations in human serum (Emmett et al. 2006a; Hoffman et al. 2011; Hölzer et al. 2008; Seals et al. 2011; Wilhelm et al. 2008) and by reductions in serum levels after exposures from water were eliminated or reduced (Bartell et al. 2010; Emmett et al. 2009).

Animal data provide quantitative estimates of the fractional absorption of orally administered PFOA, PFOS, PFBA, PFHxA, PFHxS, PFHpA, PFNA, PFDA, PFUnA, and PFDoDA, with estimates ranging from >50% for PFHxS to >95% for PFOA, PFBA, PFNA, PFDA, PFUnA, and PFDoDA. Greater than 95% of an oral dose of ammonium [^{14}C]PFOA was absorbed in rats that received single gavage doses ranging from 0.1 to 25 mg/kg (Kemper 2003). In male and female mice, comparison of the 24-hour area under the curve (AUC) for oral and intravenous administration showed that 90–100% of the oral dose was absorbed for PFOA (females), PFNA (males and female), PFDA (males and females), PFUnA (males and females), and PFDoDA (males and female); however, absorption of PFOA in males was 80%, compared to 100% in females (Fujii et al. 2015a, 2015b). Gannon et al. (2011) estimated an absorption fraction of 99% based on 168-hour urinary excretion of ^{14}C in male and female rats and mice following single oral doses of 2 or 100 mg/kg ^{14}C-PFHxA. Based on comparison of the AUC for oral and intravenous administration, the estimated oral absorption fractions were 50% in female rats administered a single 10 mg/kg dose of potassium [$^{18}O_{3-}$]PFHxS (Sundström et al. 2012) and 79 and 55% in male and female rats administered a single dose of 4 mg/kg sodium [$^{18}O_{3-}$]PFHxS (Kim et al. 2016b). Sundström et al. (2012) stated that this estimate may not be reliable due to the short (24 hours) observation period. Based on 72-hour urinary excretion of ^{14}C, the estimated fractional absorption of a single dose (50 mg/kg) of ^{14}C-PFHxA was approximately 74% in male rats, 90% in female rats, and 80% in male and female mice (Iwai et al. 2011). A comparison of ^{14}C disposition in rats, mice, hamsters, and rabbits following an oral dose of 10 mg ammonium [^{14}C]PFOA/kg showed that similar fractions of the dose were absorbed (Hundley et al. 2006). The estimated absorbed fractions (i.e., ^{14}C in tissues, urine, and exhaled air measured 120–168 hours after the dose) in males were 89% in rats, 82% in mice, 92% in hamsters, and

88% in rabbits. Corresponding values for females were 76% in rats, 61%, in mice, 75% in hamsters, and 88% in rabbits. These estimates exclude ^{14}C excreted in feces, which may have been absorbed and secreted in bile before excretion (see Section 3.1.4). Fasting appears to increase absorption of PFOA. Plasma PFOA concentrations in rats, 24 hours following a gavage dose of 10 mg ammonium PFOA/kg, were 2–3 times higher when administered to fasted rats, compared to fed rats (Hinderliter et al. 2006b). The estimated absorption fractions of ingested ammonium [^{14}C]PFOA or potassium [^{14}C]PFOS (administered as a 4.2 mg/kg oral dose) were >93 and >95% in rats, respectively (Johnson and Ober 1979, 1999a). Based on combined urinary excretion and retention in the carcass (excluding the gastrointestinal tract and its contents), the estimated oral absorption fraction of [^{14}C]PFOS (administered as a single 4.2 mg/kg dose of potassium [^{14}C]PFOS) in male rats was >95% (Chang et al. 2012). The estimated absorption fraction of PFBA (administered as 30 mg/kg oral dose of PFBA) was >95% in rats (Chang et al. 2008a). Cumulative excretion of PFBA 24 hours after an oral dose (administered as 10, 30, or 100 mg/kg ammonium PFBA) was approximately 35% in urine and 4–11% in feces in male mice; and 65–69% in urine, and 5–7% in feces in female mice (Chang et al. 2008a).

Studies examining the rate of absorption of PFOA, PFHxA, PFBA, and PFBS show rapid absorption from the gastrointestinal tract, with values for absorption $t_{1/2}$ of <2 hours. For PFOA, the highest observed concentrations of ^{14}C in plasma occurred in male rats at approximately 10 hours (range 7.5–15 hours) following single oral doses ranging from 0.1 to 25 mg ammonium PFOA/kg (Kemper 2003). The elimination $t_{1/2}$ of ^{14}C in plasma estimated in these same animals was approximately 170 hours (range 138–202 hours), corresponding to an elimination rate constant (k_e) of 0.0044 hour^{-1} (range 0.004–0.005 hour^{-1}). The corresponding absorption $t_{1/2}$ of approximately 1.5 hours (k_a=0.45 hour^{-1}) can be calculated from these observations (Equations 3-1 and 3-2):

$$t_{\max} = \ln\frac{k_a}{k_e} \cdot \frac{1}{(k_a - k_e)} \qquad \text{Eq. (3-1)}$$

$$t_{1/2} = \frac{\ln(2)}{k_e} \qquad \text{Eq. (3-2)}$$

Where t_{max} = time of maximum concentration of ^{14}C; k_e = elimination rate constant; and k_a = absorption constant. The absorption rate of PFOA appears to be greater in female rats compared to male rats. The time to peak concentrations of ^{14}C in plasma occurred at approximately 1.1 hour (range 0.6–1.5 hours) in female rats and 10 hours (range 7–15 hours) in male rats following single oral doses ranging from 0.1 to 25 mg ammonium PFOA/kg (Kemper 2003). The elimination $t_{1/2}$ of ^{14}C in plasma estimated in these same animals varied with dose and ranged from 3.2 hours at the lowest dose (k_e=0.23 hour^{-1}) to 16.2 hours at the highest dose (k_e=0.059 hour^{-1}). The estimated absorption $t_{1/2}$ from the observations

made at all doses (0.1, 1, 5, and 25 mg/kg), based on Equations 3-1 and 3-2, was approximately 0.25 hours (range 0.12–0.38 hours). The absorption $t_{1/2}$ of PFBA in male and female rats following administration of a single oral dose (30 mg/kg ammonium PFBA) was 0.23 hours (3.04 $hour^{-1}$) in males and 0.17 hours (4.15 $hour^{-1}$) in females (Chang et al. 2008a). In male and female mice administered 10–30 mg/kg ammonium PFBA, the absorption $t_{1/2}$ was <1 hour, although the absorption rate may be dose-dependent in males, with higher absorption $t_{1/2}$ at doses >30 mg/kg (Chang et al. 2008a). Estimated t_{max} values following administration of single doses (2 or 100 mg/kg) of ^{14}C-PFHxA to rats and mice ranged from 0.3 to 0.8 hours (Gannon et al. 2011). Similar results for were reported by Olsen et al. (2009) based on estimated compartmental pharmacokinetic parameters for PFBS in serum of male and female rats following a single intravenous or gavage dose of 30 mg potassium PFBS. Plasma concentration-time profiles were fit to a two-compartment elimination model. The absorption $t_{1/2}$ can be approximated from these data using Equation 3-1, with the elimination rate constant represented by the fast-phase elimination rate constant estimated for either the oral or intravenous dose. Using the oral or intravenous parameters yielded similar values for the absorption $t_{1/2}$ (0.12–0.16 hours). The estimated t_{max} values following the gavage dose were 0.42 hours in males and 0.33 hours in females. The fast-phase elimination rate constants following the gavage dose were 0.892 $hours^{-1}$ ($t_{1/2}$=0.79 hours) in males and 1.308 $hours^{-1}$ ($t_{1/2}$=0.53 hours) in females. The corresponding values for absorption $t_{1/2}$ were 0.14 hours (k_a=5.0 $hours^{-1}$) in males and 0.12 hours (k_a=5.8 $hours^{-1}$) in females. Use of the fast-phase elimination rate constants estimated following intravenous administration (male: 1.143 $hours^{-1}$; female: 1.956 $hours^{-1}$) yielded values for the absorption $t_{1/2}$ of 0.16 hours in males (k_a=4.30 $hours^{-1}$) and females (k_a=4.45 $hours^{-1}$).

Mechanisms of oral absorption of perfluoroalkyls have not been elucidated.

Dermal Exposure. Dermal exposures of rats to ammonium PFOA have been shown to produce systemic (e.g., liver, immunotoxicity) toxicity in animals (see Chapter 2). Estimates of the amount or rates of dermal absorption in humans or animals have not been reported. PFOA was detected in serum of mice following dermal application of PFOA dissolved in acetone (Franko et al. 2012). The investigators noted PFOA ingestion may have occurred during grooming and may have contributed to the body burden. Dermal absorption of PFOS was assessed following application of single doses of potassium PFOS (doses up to 0.30 mg/kg) and the diethanolamine salt of PFOS (doses up to 20 µg/kg) to clipped, intact skin of rabbits (Johnson 1995a, 1995b). Analysis of the liver 28 days after application showed no increase in content of total organic fluoride compared to controls, indicating that dermal absorption was not detectable at low dose levels using this methodology. Dermal penetration of PFOA has been studied in preparations of isolated rat, mouse, and human epidermis (Fasano et al. 2005; Franko et al. 2012). These

studies indicate that the rat and mouse skin may be more permeable to PFOA than human skin. Approximately 24% of a dermal dose of PFOA (0.5 mg in 1% acetone) was absorbed across isolated full thickness human skin in 24 hours and 45% of the dose was retained in skin (Franko et al. 2012); it is noted that the acetone, as well as the glycerol used to pretreat the skin may have enhanced PFOA absorption. Permeability was sensitive to pH and was higher when the skin was buffered at pH 2.5 ($5.5x10^{-2}$ cm/hour) compared to pH 5.5 ($4.4x10^{-5}$ cm/hour), well above the pKa for the terminal carboxylic acid of PFOA (Franko et al. 2012). This suggests that permeability of the unionized acid is greater than that of the dissociated anion. Lower permeability of ionized PFOA is also suggested by relatively low permeability of the ammonium salt of PFOA in isolated preparations of rat and human skin. Following application of the ammonium salt of PFOA to isolated human or rat epidermis (150 µL/cm^2 of a 20% aqueous solution of ammonium PFOA; approximately 30 mg ammonium PFOA/cm^2), approximately 0.048% of the dose was absorbed across human epidermis and 1.44% was absorbed across rat epidermis in 40 hours. The estimated dermal penetration coefficients were $9.49x10^{-7}$ cm/hour in the isolated human epidermis and $3.25x10^{-5}$ cm/hour in the isolated rat epidermis.

The available data suggest that absorption of PFOA and PFOS through the skin is limited and is of minimal concern as an exposure route. No dermal absorption data were located for other perfluoroalkyls.

3.1.2 Distribution

Available information on the distribution of perfluoroalkyls is obtained from oral exposure studies in laboratory animals and occupational exposure studies in which exposure is predominantly by inhalation. Studies specifically examining the distribution of perfluoroalkyls by inhalation or dermal exposure were not identified. As discussed in Section 3.1.3 (Metabolism), perfluoroalkyls do not undergo metabolism. Therefore, distribution is expected to be the same regardless of the route of administration.

Distribution in Blood. In a study of 60 healthy Chinese participants from the general population, whole blood:plasma ratios for PFOS, PFOA, PFHxA, and PFHxS were 0.65, 0.83, 3.0, and 0.57 (Jin et al. 2016). These results indicate that PFHxA, but not PFOA, PFOS, or PFHxS, enters cellular components of blood. In a study of perfluoroalkyl workers, serum:plasma ratios for PFHxS, PFOS, and PFOA were 1:1, and this ratio was independent of the concentrations measured (Ehresman et al. 2007). The ratio of whole blood:plasma (or serum) was approximately one-half, which corresponded to volume displacement by red blood cells, suggesting that these perfluoroalkyls do not enter cellular components of blood. In studies conducted in animals, most of the PFOA in blood is in the plasma fraction. In rats, 24 or 48 hours

following an oral dose of 11.4 mg ammonium [^{14}C]PFOA/kg, the red blood cell:plasma PFOA concentration ratio ranged from 0.2 to 0.3, suggesting that there was no selective retention of PFOA by red blood cells (Johnson and Ober 1999a). Blood:plasma (or serum) ratios of approximately 0.5 have also been observed in rats following intravenous injection of PFOA (Kudo et al. 2007).

Perfluoroalkyls in plasma bind to serum albumin. The dissociation constant for binding of PFOA to serum albumin is approximately 0.4 mM (0.38 mM, ±0.04 standard deviation [SD] for human serum albumin; 0.36 mM, ±0.08 SD for rat serum albumin) and involves 6–9 binding sites (Han et al. 2003). Given a dissociation constant (K_D) of 0.4 mM and an albumin concentration of approximately 0.6 mM, >90% of PFOA in serum would be expected to be bound to albumin when the serum concentration of PFOA is <1 mM (<440 mg/L). This is consistent with observations of the bound fraction of perfluoroalkyls in plasma of rats that received a gavage dose of 25 mg PFOA/kg (Han et al. 2003, 2005; Ylinen and Auriola 1990), and in human, rat, and monkey plasma incubated *in vitro* with perfluoroalkyls (e.g., PFHxA, PFOA, PFOS, PFNA, PFDA) (Kerstner-Wood et al. 2003; Ohmori et al. 2003). Comparison of dissociation constants for binding of PFOA and PFOS to human serum albumin indicates that PFOS (K_D: $8x10^{-8}$) has a higher binding affinity than PFOA (K_D: $1x10^{-4}$) for albumin, consistent with the longer $t_{1/2}$ of PFOS versus PFOA in humans (Beesoon and Martin 2015; see Section 3.1.4 for additional information). PFOS has also been shown to bind to human hemoglobin *in vitro* (Wang et al. 2016). PFBS was found to bind only to albumin, whereas PFOS, PFOA, and PFHxS were found to have the potential to bind to other human serum binding proteins, including plasma gamma-globulin, alpha-globulin, alpha-2-macroglobulin, transferrin, and beta-lipoproteins (Kerstner-Wood et al. 2003).

Distribution to Extravascular Tissues. Absorbed perfluoroalkyls distribute from plasma to soft tissues, with the highest extravascular concentrations achieved in liver. An analysis of samples from human cadavers attempted to quantify PFOA, PFOS, FOSA, and PFHxA concentrations in serum and liver (Olsen et al. 2003c). The route of exposure was unknown. Mean serum PFOS concentration was 17.7 ng/mL (95% CI 13.0–22.5, range of <6.9 [limit of quantification] to 57 ng/mL, n=24) and was not different in males (18.2 ng/mL, n=13) and females (17.2 ng/mL, n=11). The mean liver concentration was 18.8 ng/g (95% CI 14.1–23.5; range <7.3–53.8 ng/g, n=30). The mean liver:serum concentration ratio was 1.3 (95% CI 0.9–1.7, n=23) and was not different in males (1.3, n=13) and females (1.3, n=10). Most liver and serum concentrations for PFOA, FOSA, and PFHxA were below the limit of quantification; these limits were <17.9–<35.9 ng/mL for PFOA, <7.5–<19.6 ng/g for FOSA, and <3.4–<18.5 ng/mL for PFHxA.

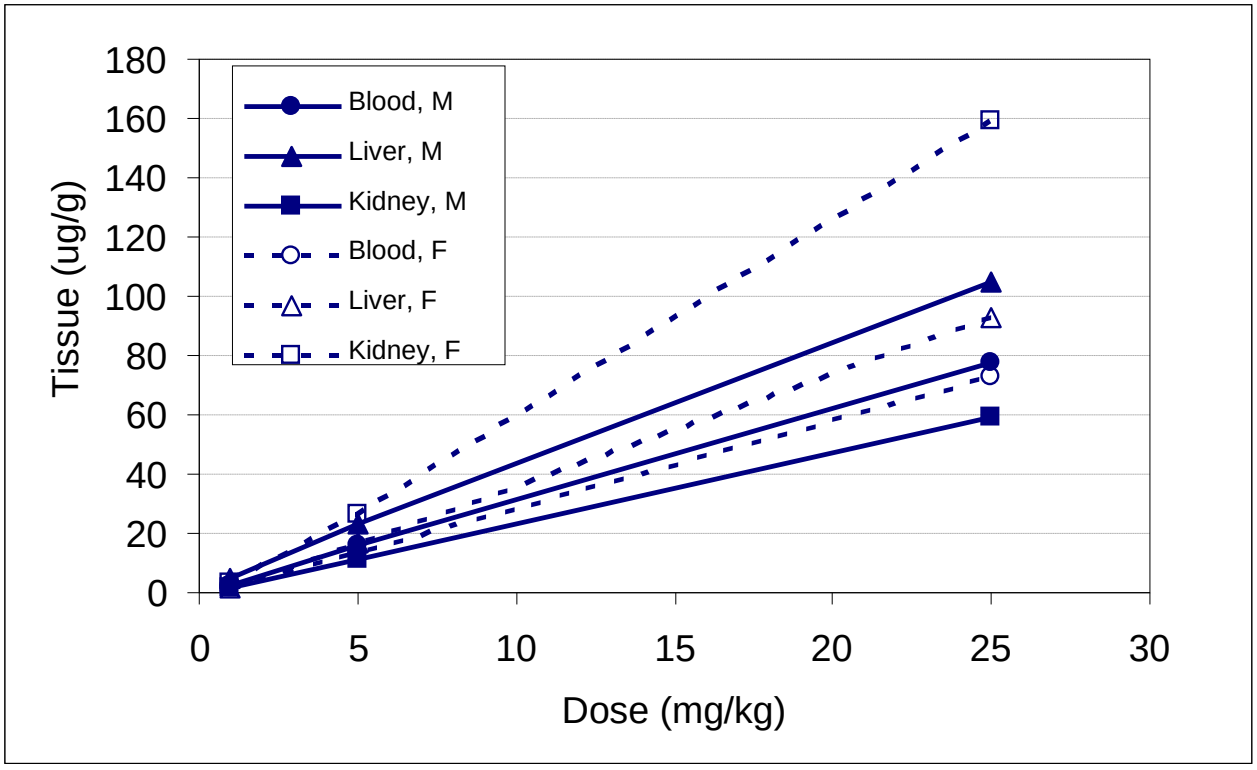
Studies conducted in nonhuman primates and rodents have provided additional information on the distribution of absorbed perfluoroalkyls to extravascular tissues. Distribution, as assessed from tissue perfluoroalkyl concentrations and tissue:serum ratios, exhibits profound species and sex differences as well as dose-dependencies (e.g., tissue levels that change disproportionately with dose). These differences have been attributed, in part, to species and sex differences in elimination kinetics of absorbed perfluoroalkyls and dose-dependence of elimination kinetics (see Section 3.1.4). In general, a consistent finding across species is that the liver receives a relatively high fraction of the absorbed dose and may also experience relatively high tissue concentrations compared with other tissues, with blood (i.e., plasma) and kidney also showing relatively high concentrations. The most extensive investigations of tissue distribution have been conducted in rodents.

Bogdanska et al. (2011) examined distribution of ^{35}S following dietary exposure to adult male C57/BL6 mice to low (environmentally relevant; 0.031 mg/kg/day) and high (experimentally relevant; 23 mg/kg/day) doses of [^{35}S]PFOS for 1–5 days. For both low and high doses after 1, 3, and 5 days of exposure, ^{35}S was distributed to the following tissues: blood, liver, lung, kidney, skin, whole bone, pancreas, spleen, thymus, heart, testes, epididymal fat, fat pads, brain, and muscle; ^{35}S was also detected in tissues throughout the gastrointestinal tract. Similar tissue:blood ratios were observed in both dose groups. In low-dose animals after 5 days of treatment, the highest tissue concentrations (excluding the gastrointestinal tract) were liver (tissue:blood=5.8), followed by lung (tissue:blood=1.4), whole bone, including marrow (tissue:blood=1.1), blood, and kidney (tissue:blood=0.94). In high-dose animals, the highest tissue concentrations were liver (tissue:blood=3.6), followed by lung (tissue:blood=1.6), blood, kidney (tissue:blood=0.81), and whole bone, including marrow (tissue:blood=0.72). A similar pattern of distribution was observed following intravenous administration of [^{14}C]potassium PFOS (4.2 mg/kg) to male rats (Johnson and Ober 1980). For both dose groups, the tissue:blood ratios for all other tissues were <1. In male and female CD-1 mice administered a single oral dose (4.2 mg/kg) of [^{14}C]PFOS, the highest concentrations of ^{14}C was observed in the liver, followed by serum, and then kidney, with similar tissue levels observed in males and females (Chang et al. 2012). In male and female rats fed diets containing 0, 2, 20, 50, or 100 mg/kg [^{13}C]sodium PFOS (equivalent to 0, 0.14, 1.33, 3.21, and 6.34 and 0, 0.15, 1.43, 3.73, and 7.58 mg/kg/day in males and females, respectively) for 28 days, PFOS levels were highest in liver, followed by spleen, heart, and serum. Liver:serum ratios for the 2, 20, 50, and 100 mg/kg/day diets were approximately 52, 42, 41, and 35, respectively, in males and 30, 47, 20, and 23, respectively, in females (Curran et al. 2008). Except for rats fed diets containing 20 mg/kg, the liver:serum ratio in males was higher than in females. No additional data were reported to determine if PFOS distribution differed between male and female rats.

Kemper (2003) determined the distribution of ^{14}C in male and female rats at the approximate time of maximum plasma concentration in both sexes, following single gavage doses of [^{14}C]PFOA (as ammonium PFOA, 0.1–25 mg/kg). This design allows a more direct comparison of patterns of tissue distribution in male and female rats at similar plasma concentrations, even though the elimination kinetics in the female rat are substantially faster than in male rats (see Section 3.1.4). The highest concentrations of ^{14}C were observed in blood, liver, and kidney (Figure 3-1). Liver, blood, and kidney accounted for approximately 22, 22, and 2% of the administered dose of 1 mg/kg in male rats; and 6, 7, and, 3% in female rats (the sex difference reflected more rapid excretory elimination in females). Although blood, liver, and kidney concentrations appeared to increase proportionately with increasing dose in male rats, in female rats, a disproportionately higher concentration in kidney was observed following the 25 mg/kg dose (Figure 3-1). Concentrations in other tissues ranged from 0.1 to 0.25 of that in liver or kidney; concentrations in bone and fat were <0.1 of that in liver or kidney. Profound sex differences and dose-dependencies in tissue concentrations of PFOA were also observed in rats that received oral doses of PFOA for 28 days at doses of 3, 10, or 30 mg PFOA/kg/day (Ylinen et al. 1990; Figure 3-2). Mean serum, kidney, or liver concentrations did not increase proportionally with dose in either sex. Kidney concentrations exhibited a disproportionate increase as the dose increased from 3 to 10 mg/kg/day, with little further increase at the 30 mg/kg/day dose. Sex differences in tissue distribution of PFOA in rats are not explained by sex differences in bioavailability since the differences persist in animals that received parenteral doses of PFOA (Johnson and Ober 1999b; Vanden Heuvel et al. 1991b, 1991c). The differences have been attributed to more rapid elimination of PFOA in female rats, compared to male rats (see Section 3.1.4).

A comparison of PFOA disposition in rats, mice, hamsters, and rabbits showed pronounced species and sex differences (Hundley et al. 2006; Table 3-1). In this study, rats, mice, hamsters, or rabbits received an oral dose of 10 mg ammonium [^{14}C]PFOA/kg and ^{14}C in tissues was measured at 120 or 168 hours (rabbits) hours post-dosing. In male rats, the highest concentrations of ^{14}C occurred in blood, liver and kidney, and all tissues combined accounted for approximately 60% of the dose. However, in female rats, concentrations of ^{14}C in all tissues were below limits of quantification. In mice, liver concentrations were similar in males and females, and liver showed the highest concentrations; ^{14}C levels in all tissues combined were lower in females compared to males. The opposite pattern was evident in hamsters and rabbits, with males having lower tissue levels than females, although, in common with rats and mice,

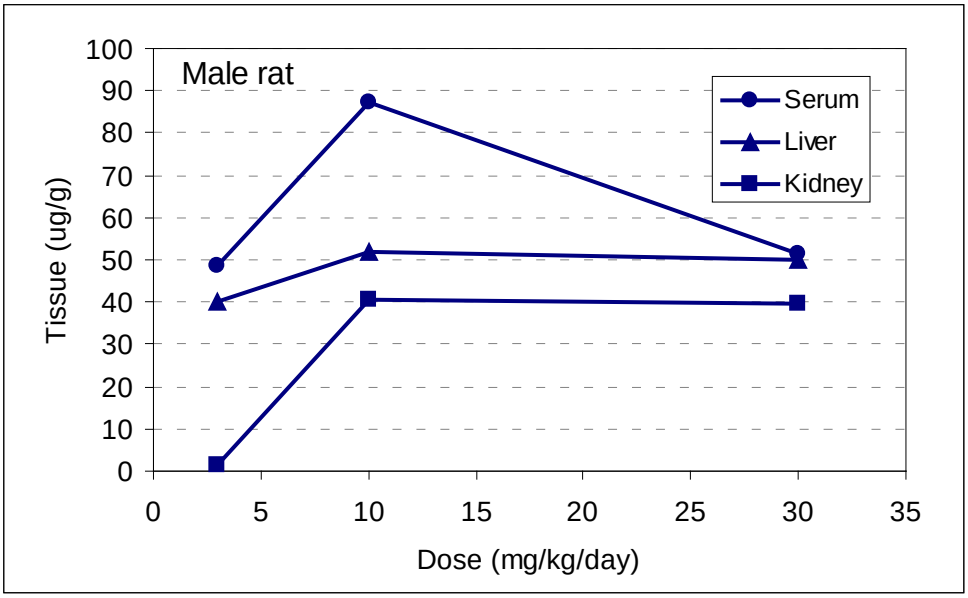
Figure 3-1. Tissue Concentrations of ^{14}C in Male and Female Rats Following a Single Gavage Dose of [^{14}C]PFOA at 1, 5, or 25 mg/kg*

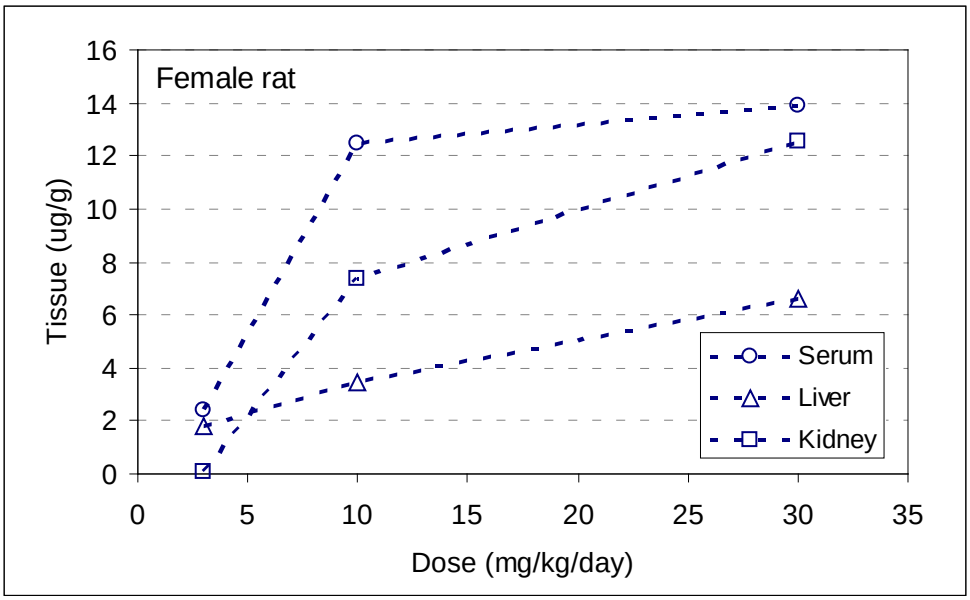


*Tissue levels are measured at time of maximum concentration in each tissue.

Source: Kemper 2003

Figure 3-2. Tissue Concentrations of ^{14}C in Male (Upper Panel) and Female (Lower Panel) Rats Following Oral Doses of PFOA for 28 Days at Doses of 3, 10, or 30 mg/kg/day





Source: Ylinen et al. 1990

Table 3-1. Tissue Distribution and Excretion of ^{14}C-Radioactivity from Both Sexes of Rats, Mice, Hamsters, and Rabbits Dosed with ^{14}C-Labeled APFO[a]

	µg Equivalent per g (mL) wet weight[b]							
	Rat		Mouse		Hamster		Rabbit	
Sample	Male	Female	Male	Female	Male	Female	Male	Female
Blood	23.5	<0.1	13.8	10.1	0.1	8.8	<0.1	0.1
Liver	40.0	<0.1	43.2	45.3	0.3	7.3	0.1	1.5
Kidneys	24.0	<0.1	2.9[c]	2.2[c]	0.2	7.1	0.1	0.4
Lungs	8.7	<0.1	1.4[c]	1.3[c]	<0.1	3.8	<0.1	0.1
Heart	6.4	<0.1	1.2[c]	0.6[c]	<0.1	2.9	<0.1	<0.1
Skin	4.8	<0.01	3.5	0.2	<0.1	3.4	<0.1	<0.1
Testes	3.2	–	0.9[c]	–	<0.1	–	<0.1	–
Muscle	1.9	<0.1	1.1	0.5	<0.1	0.9	<0.1	<0.1
Fat	1.7	<0.1	1.6	1.3	<0.1	1.5	<0.1	<0.1
Brain	0.6	<0.1	0.2[c]	0.8[c]	<0.1	0.3	<0.1	<0.1
	Percent of dose							
Tissues	59.6	0.6	73.6	50.0	0.7	26.5	<0.1	0.3
Urine	25.6	73.9	3.4	6.7	90.3	45.3	76.8	87.9
Feces	9.2	27.8	8.3	5.4	8.2	9.3	4.2	4.6
Expiration	3.6	1.5	5.2	4.4	1.3	2.9	No data	No data
Cage wash	0.6	0.8	4.9	4.9	0.6	2.1	0.5	4.8
Percent recovered	98.5	104.6	95.4	71.4	101.1	86.1	81.6	97.6

[a]The rabbits were sacrificed 168 hours after dosing; all other animals were sacrificed 120 hours after dosing.
[b]The µg equivalent calculations were based on the specific activity of ^{14}C-labeled APFO, which was 1.1×10^6 DPM/mg. The µg equivalent per g wet weight could not accurately be determined below 0.1 µg/g.
[c]Represents the µg equivalents for the entire organ.

APFO = ammonium perfluorooctanoate

Source: Hundley et al. 2006

blood, liver and kidney had the highest concentrations. Male rats that received a single oral dose of 5 mg FOSA/kg had liver FOSA concentrations that were 3–5 times higher than serum concentrations 1 day post-dosing (Seacat and Luebker 2000).

Sex differences in elimination that give rise to sex differences in tissue levels following oral exposure to perfluoroalkyls in rats are not evident in studies conducted with nonhuman primates. Rhesus monkeys that received 3 or 10 mg ammonium PFOA/kg/day for 90 days had liver concentrations of 48 µg/g (one male) or 50 µg/g (one female) at the low dose and 45 µg/g (one male) and 72 µg/g (one female) at the higher dose, with corresponding serum concentrations of 3 and 7 µg/mL, and 9 and 10 µg/mL, respectively (Griffith and Long 1980). Although limited to only one animal per sex, these results suggest

that liver levels did not increase proportionately with increasing dose. A similar observation was made in a study of male Cynomolgus monkeys (Butenhoff et al. 2004c). In male monkeys that received daily oral doses of 3 or 10 mg ammonium PFOA kg/day for 27 weeks, liver PFOA concentrations ranged from 11 to 18 µg/g at the low dose and from 6 to 22 µg/g at the higher dose. Mean serum concentrations measured after 6 weeks of exposure (which may have represented steady-state concentrations) were 77,000 ng/mL in the low-dose group and 86,000 ng/mL in the higher dose group. In this same study, an analysis of serum PFOA kinetics following an intravenous dose of PFOA revealed similar elimination kinetics in males and females (Butenhoff et al. 2004c; see Section 3.1.4). In Cynomolgus monkeys that received daily oral doses of PFOS (0, 0.03, 0.15, or 0.75 mg PFOS/kg/day) for 26 weeks, liver concentrations of PFOS and serum concentration were similar in males and females (liver:serum ratios ranged from 1 to 2) and increased in approximate proportion to the administered dose (Seacat et al. 2002).

Bogdanska et al. (2014) examined distribution of ^{35}S in 20 tissues following dietary exposure of adult male C57/BL6 mice to PFBS (16 mg/kg/day) for 1–5 days. ^{35}S was detected in all tissues and concentrations reached plateau levels after 3 days of exposure. After 5 days, tissue:blood ratios (excluding stomach and small intestine) were >1 for liver (tissue:blood=1.6), kidney (tissue:blood=1.3), whole bone (tissue:blood=1.1), and cartilage (tissue:blood=1.1). At all-time points, approximately 90% of the ingested ^{35}S was recovered in combined blood, liver, bone, skin, and muscle.

Iwabuchi et al. (2017) compared tissue distribution following single doses or 3-month dosing of PFOS (100 µg/kg), PFOA (100 µg/kg), PFHxA (100 µg/kg), and PFNA (50 µg/kg). Following administration of single doses, the tissue:serum (and/or whole blood) ratio was >1 for the liver for PFOS, PFOA, and PFNA, with tissue:serum ratios <1 for kidney, spleen, heart, and brain. For PFNA, the only tissue with a tissue:serum ratio >1 was kidney. After 3 months of exposure, tissue:serum ratios >1 were observed for the liver for PFOA and PFNA, and the liver and kidney for PFOS. For PFHxA, all tissue:serum ratios were <1. Similar to the single dose study, the lowest serum:tissue ratio for all compounds was observed for brain.

Subcellular Distribution. The subcellular distribution of perfluoroalkyls has been examined in rats (Han et al. 2004, 2005; Kudo et al. 2007; Vanden Heuvel et al. 1992b). Two hours following an oral dose of 25 mg ammonium [^{14}C]PFOA/kg, sex differences were noted in the subcellular distribution of ^{14}C in liver; females had approximately 50% of total ^{14}C in the cytosolic fraction compared to 26% in males (Han et al. 2005). The distributions to other cell fractions were: nuclear/cell debris fraction, 30% females, 40% males; lysosomes, 12% females, 14% males; mitochondria, 8% females, 16% males; and

ribosomes, <3% males and females. In kidney, 80 and 70% of the ^{14}C was associated with the cytosolic fraction in males and females, respectively, 16–22% in the nuclear/cell debris fraction, and the remainder in lysosome/mitochondria/ribosome fractions. In liver, approximately 55% of cytosolic ^{14}C was bound to proteins (>6,000 Da) in both males and females, whereas in kidney, 42% of the cytosolic fraction was bound to protein in males and 17% in females. The subcellular distribution of PFOA is dose-dependent. In rats, 2 hours following an intravenous dose of 0.041 mg [^{14}C]PFOA/kg, approximately 5% ^{14}C in the liver was associated with the cytosolic fraction, whereas approximately 45% was in the cytosolic fraction following a dose of 16.6 mg/kg (Kudo et al. 2007). A small component of tissue-associated PFOA and PFDA appeared to be bound covalently to protein. Following an intraperitoneal dose of 9.4 µmol/kg [^{14}C]PFDA or [^{14}C]PFOA (4.2 mg/kg), approximately 0.1–0.5% of liver ^{14}C was bound covalently (i.e., was not removed by repeated extraction with a methanol/ether and ethyl acetate; Vanden Heuvel et al. 1992b). Covalent binding was detected when cytosolic or microsomal fractions of rat liver were incubated *in vitro* with [^{14}C]PFDA (Vanden Heuvel et al. 1992b).

PFOA binds to rat kidney and urine α2u-globulin; dissociation constants were estimated to be approximately 1.5 and >2 mM (for a single binding site) for the proteins isolated from rat kidney and urine, respectively. These values suggest relatively low affinity for the protein, compared to other ligands that are known to induce hyaline droplet nephropathy (10^{-4}–10^{-7} M; Han et al. 2004).

Maternal-fetal Transfer. Perfluoroalkyls can be transferred to the fetus during pregnancy (Cariou et al. 2015; Chen et al. 2017a; Fei et al. 2007; Fisher et al. 2016; Fromme et al. 2010; Glynn et al. 2012; Gützkow et al. 2012; Hanssen et al. 2010, 2013; Inoue et al. 2004; Kato et al. 2014; Kim et al. 2011, Lee et al. 2013; Lien et al. 2013; Liu et al. 2011; Manzano-Salgado et al. 2015; Midasch et al. 2007; Monroy et al. 2008; Needham et al. 2011; Ode et al. 2013; Porpora et al. 2013; Yang et al. 2016a, 2016b). Studies that measured perfluoroalkyls in maternal and fetal cord blood of matched mother-infant pairs found relatively strong correlations (r>0.8) between maternal and fetal serum (or plasma); however, fetal/maternal serum ratios vary depending on the structure of the perfluoroalkyl (Table 3-2). With some exceptions, longer fluoroalkyl chain length and a terminal sulfonate group are associated with lower fetal/maternal ratios (Glynn et al. 2012; Gützkow et al. 2012; Hanssen et al. 2013; Kim et al. 2011; Liu et al. 2011; Needham et al. 2011). PFOS was detected in amniotic fluid obtained from amniocentesis (Jensen et al. 2012). The median concentration in amniotic fluid samples from 300 pregnancies (from the Danish amniotic fluid pregnancy-screening biobank) was 1.1 ng/mL.

Table 3-2. Serum (or Plasma) Concentrations in Matched Human Maternal-Infant Pairs

Study	Perfluoro-alkyl	Perfluoroalkyl chain length	N	Maternal (ng/mL)	Cord (ng/mL)	Ratio[a]	r
Glynn et al. 2012	PFOA	7	413	4	1	NR	0.89
	PFOS	8	413	29	5	NR	0.86
	PFNA	8	413	0.6	0.1	NR	0.53
Cariou et al. 2015	PFHxS	6	59	0.62	0.34	0.56	0.99
	PFOA	7	89	1.05	0.86	0.78	0.83
	PFOS	8	94	3.07	1.11	0.38	0.88
	PFNA	8	22	0.43	0.27	0.51	0.92
Chen et al. 2017a	PFHxS	6	32	0.53	0.33	0.62	ND
	PFOA	7	32	8.67	3.67	0.42	ND
	PFOS	8	32	1.56	1.24	0.79	ND
Fisher et al. 2016	PFHxS	6	315	NR	NR	0.23	NR
	PFOA	7	865	NR	NR	0.28	NR
	PFOS	8	648	NR	NR	0.14	NR
Fromme et al. 2010	PFHxS	6	53	0.60	0.30	0.50	0.89
	PFOA	7	53	2.60	1.70	0.65	0.94
	PFOS	8	53	3.50	1.10	0.31	0.89
	PFNA	8	53	0.60	<0.4	ND	ND
Gützkow et al. 2012	PFHxS	6	123	0.34	0.23	0.68	0.70
	PFOA	7	123	1.25	1.03	0.82	0.82
	PFOS	8	123	5.37	1.78	0.33	0.74
	PFNA	8	123	0.40	0.16	0.40	0.64
	PFDA	9	123	0.10	0.04	ND	ND
	PFUnA	10	123	0.19	0.06	0.32	0.67
Hanssen et al. 2013	PFHxS	6	7	0.26	0.17	0.65	ND
	PFOA	7	7	1.50	1.26	0.84	ND
	PFOS	8	7	10.70	3.93	0.37	ND
	PFNA	8	7	0.89	0.50	0.56	ND
	FOSA	8	7	0.41	0.45	1.10	ND
	PFUnA	10	7	0.33	0.16	0.48	ND
Han et al. 2018	PFBS	4	369	0.19	0.19	1.00	ND
	PFHxS	6	369	0.32	0.31	1.03	ND
	PFHpA	6	369	0.06	0.09	1.50	ND
	PFOA	7	369	42.83	34.67	0.81	ND
	PFOS	8	369	4.55	1.39	0.31	ND
	FOSA	8	369	0.13	0.13	1.00	ND
	PFNA	8	369	0.81	0.44	0.54	ND
	PFDA	9	369	0.55	0.21	0.38	ND
	PFUnA	10	369	0.47	0.17	0.36	ND
	PFDoDA	11	369	0.17	0.14	0.82	ND

Table 3-2. Serum (or Plasma) Concentrations in Matched Human Maternal-Infant Pairs

Study	Perfluoro-alkyl	Perfluoroalkyl chain length	N	Maternal (ng/mL)	Cord (ng/mL)	Ratio[a]	r
Inoue et al. 2004	PFOA	7	15	8.90	2.90	0.32	0.94
Kim et al. 2011	PFHxS	6	20	0.89	0.58	0.65	ND
	PFOA	7	20	1.60	1.10	0.69	ND
	PFOS	8	20	5.60	2.00	0.36	ND
	PFNA	8	20	0.79	0.37	0.47	ND
	PFDA	9	20	0.36	0.01	0.03	ND
	PFUnA	10	20	1.60	0.46	0.29	ND
Kato et al. 2014	PFHxS	6	78	1.20	0.60	0.50	0.89
	PFOA	7	78	3.30	3.10	0.89	0.88
	PFOS	8	78	8.50	3.50	0.31	0.82
	PFNA	8	78	0.66	0.41	0.62	0.79
	PFDA	9	78	0.20	ND	ND	ND
Lee et al. 2013	PFHS	6	70	1.35	0.67	0.57	ND
	PFOA	7	70	2.73	2.09	0.84	ND
	PFOS	8	70	10.77	3.44	0.35	ND
Liu et al. 2011	PFHxS	6	50	0.08	0.06	0.79	0.59
	PFOA	7	50	1.66	1.50	0.91	0.91
	PFOS	8	50	3.18	1.69	0.53	0.75
	PFNA	8	50	0.55	0.33	0.61	0.82
	PFDA	9	50	0.58	0.24	0.41	0.82
	PFUnA	10	50	0.56	0.30	0.53	0.70
	PFDoDA	11	50	0.08	ND	ND	ND
Manzano-Salgado et al. 2015	PFHxS	6	66	0.84	0.40	0.446	NR
	PFOA	7	66	2.97	1.90	0.746	NR
	PFOS	8	66	6.99	1.86	0.299	NR
	PFNA	8	66	0.85	0.32	0.4	NR
Midasch et al. 2007	PFOA	7	11	2.70	3.40	1.30	0.42
	PFOS	8	11	12.10	7.20	0.60	0.72
Monroy et al. 2008	PFHxS	6	101	4.05	5.05	1.25	ND
	PFOA	7	101	2.24	1.94	0.87	0.94
	PFOS	8	101	16.19	7.19	0.44	0.91
	PFNA	8	101	0.80	0.94	1.18	ND
Needham et al. 2011	PFHxS	6	12	12.30	9.10	0.74	0.05
	PFOA	7	12	4.20	3.10	0.72	0.91
	PFOS	8	12	19.70	6.60	0.34	0.82
	PFNA	8	12	0.76	0.37	0.50	0.84
	PFDA	9	12	0.34	0.10	0.29	0.91

Table 3-2. Serum (or Plasma) Concentrations in Matched Human Maternal-Infant Pairs

Study	Perfluoro-alkyl	Perfluoroalkyl chain length	N	Maternal (ng/mL)	Cord (ng/mL)	Ratio[a]	r
Ode et al. 2013	PFOA	7	263	2.30	2.80	1.30	0.74
	PFOS	8	263	17.00	7.40	0.45	0.76
	PFNA	8	263	0.31	0.26	0.93	0.51
Porpora et al. 2013	PFOA	7	38	2.90	1.60	0.55	0.70
	PFOS	8	38	3.20	1.40	0.44	0.72
Yang et al. 2016a	PFHxS	6	50	0.064	0.033	0.52	0.80
	PFOA	7	50	1.24	1.03	0.83	0.93
	PFOS	8	50	2.98	1.23	0.41	0.88
	PFNA	8	50	0.55	0.35	0.64	0.89
	PFDA	9	50	0.56	0.22	0.39	0.92
	PFUnA	10	50	0.55	0.23	0.42	0.88
	PFDoDA	11	50	0.085	0.058	0.68	0.76
Yang et al. 2016b	PFHxS	6	157	0.53	0.26	0.43	0.68
	PFOA	7	157	1.74	1.32	0.71	0.81
	PFOS	8	157	4.23	1.52	0.36	0.63
	PFNA	8	157	0.46	0.23	0.49	0.70
	PFDA	9	157	0.37	0.13	0.35	0.65
	PFUnA	10	157	0.38	0.14	0.36	0.63
	PFDoDA	11	157	0.040	0.026	0.61	0.52

[a]Ratio of cord:maternal perfluoroalkyl level.

FOSA = perfluorooctane sulfonamide; ND = no data (detected but below limit of quantification); NR = not reported; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFHpA = Perfluoroheptanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFTrDA = perfluorotridecanoic acid; PFUnA = perfluoroundecanoic acid

Studies in rats and mice provide further support for maternal-fetal transfer of perfluoroalkyls. Following gavage administration of 0.1–10 mg/kg/day PFOS to rats during gestation, PFOS was distributed to fetal serum, liver, and brain, with fetal concentrations increasing with maternal dose (Chang et al. 2009; Lau et al. 2003; Luebker et al. 2005a, 2005b; Thibodeaux et al. 2003). Levels in fetal serum and liver generally were similar and higher than in brain. Studies did not report on concentrations of PFOS in other fetal tissues. Paired fetal-maternal levels of PFOS were examined in rats following exposure (gavage) to potassium PFOS at doses of 0.1, 0.4, 1.6, or 3.2 mg/kg/day on GDs 0–20 (Luebker et al. 2005b). On GD 21, fetal:maternal serum ratios were 2.1, 1.7, 1.6, and 1.1 at doses of 0.1, 0.4, 1.6, and 3.2 mg/kg/day, respectively; these results suggest that fetal:maternal serum ratios varied inversely with dose. Fetal:maternal liver ratios (0.37–0.44) were similar across the dose range. In mice administered a single

gavage dose of 12.5 mg/kg [^{35}S]PFOS on GD 16, fetal organ:maternal blood ratios of ^{35}S on GD 18 were 2.8 for kidneys, 2.6 for liver, 2.3 for blood, 2.1 for lungs, and 1.2 for brain (Borg et al. 2010).

Maternal-fetal transfer of PFOA has also been studied in rats and mice (Das et al. 2008; Hinderliter et al. 2005). In rats, PFOA concentrations in amniotic fluid, placenta, and fetus (measured on days 10, 15, or 21 of gestation) increased with increasing maternal oral dose (3, 10, or 30 mg/kg/day, administered daily beginning on GD 4) (Hinderliter et al. 2005). Fetal plasma concentrations of PFOA measured on GD 21 were approximately 40% of maternal plasma concentration. Following gavage administration of 0.01, 1, or 5 mg/kg ammonium PFOA on GD 17 in mice, PFOA was detected in amniotic fluid and pup serum, with dose-dependent increases (Fenton et al. 2009). On PND 1, pup serum PFOA concentrations were approximately 1.7–2.0-fold greater than levels in maternal serum.

Following administration of ammonium PFBA (35, 175, or 350 mg/kg) to pregnant mice on GDs 0–17, fetal serum and liver levels of PFBA were determined on PND 1 (Das et al. 2008). The fetal:maternal serum ratio of PFBA was approximately 0.15 and did not vary with maternal dose. Fetal liver:serum ratios were 0.44, 0.75, and 0.78 at maternal doses of 35, 175, and 350 mg/kg, respectively. PFHxS was detected in fetal blood and in the liver of neonates following exposure of dams to potassium PFHxS (0.3, 1, 3, and 10 mg/kg) throughout gestation (Butenhoff et al. 2009a); concentrations in serum and liver increased with dose.

Maternal-infant Transfer. Perfluoroalkyls can be transferred to nursing infants (Barbarossa et al. 2013; Cariou et al. 2015; Fromme et al. 2010; Kärrman et al. 2007; Kim et al. 2011; Kuklenyik et al. 2004; Liu et al. 2011; Tao et al. 2008a, 2008b). Studies that measured perfluoroalkyls in maternal serum (or plasma) and breast milk in matched mother-infant pairs found highly variable correlations (Table 3-3). Relatively high correlations have been reported for PFOA (Kärrman et al. 2007; Liu et al. 2011). Transfer to breast milk appears to be a significant route of elimination of perfluoroalkyls during breastfeeding. Comparisons of serum concentrations of women who did or did not breastfeed their infants showed that breastfeeding significantly decreases maternal serum concentrations of PFOA, PFOS, PFHxS, and PFNA (Bjermo et al. 2013; Brantsaeter et al. 2013; Mondal et al. 2012, 2014; von Ehrenstein et al. 2009). The decrease was estimated to be 2–3% decrease per month of breastfeeding (Brantsaeter et al. 2013; Mondal et al. 2012, 2014). Concentrations of perfluoroalkyls in breast milk also decrease with breastfeeding duration (Tao et al. 2008b; Thomsen et al. 2010). Numerous perfluoroalkyls (including PFOS, PFOA, PFBS, PFHxS, PFNA, PFDA, PFDoDA, PFUnA, and FOSA) have been detected in breast milk samples in women in China, Korea, Japan, Malaysia, Cambodia, India, Korea, Vietnam, Indonesia,

Norway, Philippines, Sweden, and the United States (Forns et al. 2015; Fujii et al. 2012; Kang et al. 2016; Kärrman et al. 2007; Kim et al. 2011; Liu et al. 2010, 2011; Mondal et al. 2014; So et al. 2006b; Tao et al. 2008a). The mean concentrations for perfluoroalkyls in breast milk collected from 45 women in Massachusetts were 0.131 ng/mL (range of <0.032.0–617 ng/mL) for PFOS, 0.043.8 ng/mL (<0.0301–0.161 ng/mL) for PFOA, and 0.0145 ng/mL (<0.0120–0.0638 ng/mL) for PFHxS (Tao et al. 2008b). PFHpA, PFDA, PFUnA, PFDoDA, and PFBS were also detected in the breast milk; however, ≤4 samples had levels that exceeded the limit of quantitation. Serum concentrations in breastfed infants can be higher than maternal levels. Although cord:maternal serum ratios of PFOA, PFOS, and PFNA at birth are typically lower than 1 (see Table 3-2), infant serum levels increase several-fold during the first 6 months after birth (Fromme et al. 2010; Mondal et al. 2014; Post et al. 2012; Verner 2016a, 2016b). This increase is likely because breast milk concentrations of perfluoroalkyls and fluid intake per infant body weight are highest during this time period. Fromme et al. (2010) also showed increases in serum levels of PFNA in infants fed formula made with contaminated drinking water. Mogensen et al. (2015b) reported that following weaning, significant (<0.0001) decreases were observed in infant serum concentrations of PFOS, PFOA, and PFHxS.

Table 3-3. Matched Serum (or Plasma) and Breast Milk Concentrations in Humans

Study	Perfluoroalkyl	Perfluoroalkyl chain length	N	Serum (ng/mL)	Milk (ng/mL)	Ratio[a]	r
Cariou et al. 2015	PFHxS	6	9	2.28	0.026	0.011	0.36
	PFOA	7	10	1.22	0.041	0.034	0.72
	PFOS	8	19	3.62	0.040	0.011	0.85
Kärrman et al. 2007a	PFHxS	6	12	4.7	0.085	0.020	ND
	PFOA	7	12	3.8	0.49	0.120	0.88
	PFOS	8	12	20.7	0.20	0.010	0.83
	FOSA	8	12	0.24	0.013	0.070	ND
	PFNA	8	12	0.80	0.017	0.010	ND
Kim et al. 2011	PFHxS	6	20	0.89	0.007	0.008	NS
	PFOA	7	20	1.60	0.041	0.026	NS
	PFOS	8	20	5.60	0.061	0.011	0.60
	PFNA	8	20	0.79	<0.0088	ND	ND
	PFDA	9	20	0.36	<0.018	ND	ND
	PFUnA	10	20	1.60	<0.024	ND	ND

Table 3-3. Matched Serum (or Plasma) and Breast Milk Concentrations in Humans

Study	Perfluoroalkyl	Perfluoroalkyl chain length	N	Serum (ng/mL)	Milk (ng/mL)	Ratio[a]	r
Liu et al. 2011	PFHxS	6	50	0.08	ND	ND	ND
	PFOA	7	50	1.66	0.181	0.109	0.77
	PFOS	8	50	3.18	0.056	0.018	0.57
	PFNA	8	50	0.55	0.026	0.048	0.62
	PFDA	9	50	0.58	0.02	0.034	0.54
	PFUnA	10	50	0.56	0.026	0.046	0.44
	PFDoDA	11	50	0.08	ND	ND	ND
	PFTrDA	12	50	0.08	ND	ND	ND

[a]Milk to serum ratio.

FOSA = perfluorooctane sulfonamide; ND = no data (detected but below limit of quantification); NS = not significantly correlated; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFTrDA = perfluorotridecanoic acid; PFUnA = perfluoroundecanoic acid

Studies conducted in rats and mice provide further support for maternal-infant transfer of perfluoroalkyls through breast milk (Fenton et al. 2009; Hinderliter et al. 2005; Lau et al. 2003; Luebker et al. 2005a; Yu et al. 2009b). PFOA concentrations in breast milk of nursing rats increased with increasing maternal oral dose (3, 10, or 30 mg/kg/day, administered daily beginning on GD 4) (Hinderliter et al. 2005). Milk concentrations of PFOA measured on postpartum days 3, 7, 14, or 21 in rats were approximately 0.1 of maternal plasma concentration. In dams exposed to 0.1, 1, or 5 mg/kg PFOA by gavage on GD 17, a dose-dependent increase in PFOA concentrations in breast milk was observed on PND 2, with breast milk:serum ratios of approximately 0.15, 0.38, and 0.25 at 0.1, 1, and 5 mg/kg doses, respectively; milk/serum concentration ratios for PFOA ranged from 0.15 to 0.56 (Fenton et al. 2009). Following lactational exposure of control rat pups to PFOS in breast milk of dams treated with dietary PFOS (3.2 mg/kg diet; approximately equivalent to 0.33 mg/kg/day), pup serum and liver concentrations increased throughout the 35-day lactation period (Yu et al. 2009b). At PND 35, the pup liver:serum PFOS ratios were 2.55 and 2.43 in male and female pups, respectively. Results of a cross-foster study show that pups are exposed to PFOS through breast milk (Luebker et al. 2005a). Postnatal toxicity observed in cross-fostered pups that nursed from exposed dams provides additional evidence of maternal-infant transfer of PFOS in rats and mice (see Section 2.17).

Mechanisms of Distribution. Perfluoroalkyls in plasma bind to serum albumin and various other plasma proteins including gamma-globulin, alpha-globulin, alpha-2-macroglobulin, transferrin, and beta-lipoproteins (Bischel et al. 2011; Butenhoff et al. 2012d; Chen and Guo 2009; Han et al. 2003, 2005; Kerstner-Wood et al. 2003; Luo et al. 2012; Ohmori et al. 2003; Salvalaglio et al. 2010; Vanden Heuvel et al. 1992b; Wu et al. 2009; Ylinen and Auriola 1990; Zhang et al. 2009). The dissociation constant for albumin-bound PFOA in serum is approximately 0.4 mM (0.38 mM, ±0.04 SD for human serum albumin; 0.36 nM, ±0.08 SD for rat serum albumin) and involves 6–9 biding sites (Han et al. 2003). Noncovalent binding appears to be at the same sites as fatty acids (Chen and Guo 2009). Interactions between PFOS and human serum albumin include interaction of PFOS polar sulfonyl groups with albumin hydrophilic sites and interaction of perfluorinated groups with albumin hydrophobic sites (Luo et al. 2012).

Absorbed perfluoroalkyls distribute from plasma to soft tissues, with the highest extravascular concentrations achieved in liver. Mechanisms by which perfluoroalkyls enter the liver have not been elucidated and may involve interactions with organic anion transporters that function in the distribution of fatty acids or other organic anions (Andersen et al. 2008). PFOA appears to be a substrate for organic anion transporters in the luminal and basolateral membranes of renal tubular epithelial cells, which facilitates entry of PFOA into renal tubular cells (Kudo et al. 2002; Nakagawa et al. 2008; Vanden Heuvel et al. 1992b; Weaver et al. 2010). The subcellular distribution of PFOA is sex- and dose-dependent in rats (Han et al. 2005; Kudo et al. 2007) and the association with the membrane fraction of liver cells decreases with increasing dose (Kudo et al. 2007), consistent with limited capacity of membrane proteins that bind PFOA (e.g., membrane transport proteins). Intracellular PFOA binds to proteins; protein complexes formed have not been fully characterized. PFOA exhibits a low affinity for binding to rat kidney and urine alpha-2μ-globulin (dissociation constants 1.5 and >2 mM, respectively) (Han et al. 2004).

3.1.3 Metabolism

Results of available intraperitoneal and *in vitro* studies suggest that the perfluoroalkyls discussed in this profile are not metabolized and do not undergo chemical reactions in the body. The absence of significant metabolism is attributed to the high stability and low reactivity of carbon-fluorine bonds in perfluoroalkyls. Studies conducted in male and female rats did not detect fluorine metabolites in the urine, plasma, or liver following a single injection of 4–150 mg/kg PFOA or 5–50 mg/kg PFDA (Goecke et al. 1992; Vanden Heuvel et al. 1991b, 1991c; Ylinen and Auriola 1990). Following a single intraperitoneal dose of approximately 4 mg/kg of ^{14}C-PFOA, only parent compound was excreted in the

urine and bile (Vanden Heuvel et al. 1991c). PFOA was not metabolized when incubated with microsomal fractions of human or rat intestine, kidney, or liver homogenates (Kemper and Nabb 2005). Although no studies examining metabolism of other perfluoroalkyls, including PFOS, following inhalation, oral, or dermal exposure were identified, metabolism by these exposure routes is not anticipated.

3.1.4 Excretion

As noted in Section 3.1.3 (Metabolism), there is presently no evidence that perfluoroalkyls undergo metabolism. The absence of significant metabolism is attributed to the high stability and low reactivity of carbon-fluorine bonds in perfluoroalkyls. Therefore, route-specific differences in excretion patterns are not expected. Selected studies in which elimination half-lives rates (i.e., $t_{1/2}$) of perfluoroalkyls have been determined (see summaries in Table 3-5) show that, in general, elimination $t_{1/2}$ values are similar following intravenous, intraperitoneal, and oral exposures. Findings suggest that the route of absorption has no substantial effect of rates of elimination of absorbed perfluoroalkyls (Butenhoff et al. 2004c; Chang et al. 2008a; Kemper 2003; Kudo et al. 2002; Ohmori et al. 2003; Vanden Heuvel et al. 1991b; Ylinen et al. 1990). As discussed in this section, perfluoroalkyls are primarily eliminated in the urine, with smaller amounts eliminated in the feces, breast milk (see Section 3.1.2; Distribution, Maternal-fetal Transfer), and menstrual fluid. Perfluoroalkyls undergo biliary excretion, but substantial reabsorption occurs; therefore, biliary excretion does not represent a major elimination pathway. Perfluoroalkyls do not appear to be eliminated in sweat, as induction of perspiration by exercise or sauna does not alter clearance of PFOA, PFOA, PFHxA, or PFNA (Genuis et al. 2013). The elimination of perfluoroalkyls in menstrual fluid appears to contribute to sex differences in serum elimination rates (Wong et al. 2014, 2015; Zhang et al. 2013). Only free (unbound) perfluoroalkyls are available for redistribution, excretion, and renal reabsorption; the interaction of perfluoroalkyls with proteins plays a critical role in bioaccumulation, and the tissue environment highly favors protein bonding.

In humans, absorbed perfluoroalkyls are excreted in urine. Estimates of renal clearance of PFOA and PFOS from serum in humans ranged from 0.8 to 3.3 mL/day for PFOA (serum concentration range: 5–16 ng/mL) and 0.1–1.5 mL/day for PFOS (serum concentration range 9–49 ng/mL). These clearance values were <0.001% of glomerular filtration rate (Harada et al. 2005a). Assuming that 99% of the serum PFOA and PFOS was bound to albumin (see Section 3.1.2), <0.1% of filtered perfluoroalkyls were excreted in urine, suggesting extensive reabsorption of filtered PFOA and PFOS in the renal tubule. Renal clearance was not different in males and females. Mean renal clearances for PFOA were

2.12 mL/day (±0.80 SD, n=5) in males and 1.15 (±0.33 SD, n=5) in five females (mean age 22 and 23 years, respectively). Mean renal clearances for PFOS were 0.66 mL/day (±0.48 SD, n=5) in males and 0.91 (±0.56 SD, n=5) for females. Fujii et al. (2015a) reported renal clearances (mL/day/kg; mean±SD) for several perfluoroalkyls in humans (three males and five females), including PFOA (0.044±0.01), PFNA (0.038±0.01), PFDA (0.015±0.01), PFUnA (0.005±0.00), and PFDoDA (0.005±0.00). Zhang et al. (2013) reported renal clearances for several perfluoroalkyls (mL/day/kg) and found that clearance of PFOS was similar in younger females (≤50 years, 0.050 mL/day/kg, 95% CI 0.037–0.064) and a combined group of males and older females (grouped together since there were no significant differences in serum concentrations) (0.037 mL/day/kg, 95% CI 0.026–0.049). However, there appeared to be differences in renal clearance for PFOA; clearance rates were 0.30 mL/day/kg (95% CI 0.11–0.49) in young females and 0.77 mL/day/kg (95% CI 0.47–1.1) in the combined older women and all males group. Urinary excretion of perfluoroalkyls may show sex and age differences (Zhang et al. 2015b). Urinary excretion of PFOA as a fraction of estimated intake in male adults (n=29) was 31% (p=0.002) higher than in nonpregnant female adults (n=25). In addition, urinary excretion of PFOS was inversely correlated with age (r=0.334; p=0.015).

Absorbed PFOA and PFOS are also secreted into bile in humans, but the biliary pathway is not a major excretory pathway because PFOA and PFOS are reabsorbed after biliary secretion. Estimates of total body clearance, serum-to-urine clearance, and serum-to-bile clearance of PFOA and PFOS in humans are presented in Table 3-4 (Harada et al. 2007). Biliary clearances of PFOA and PFOS were 1.06 and 2.98 mL/kg body weight/day, respectively, and greatly exceeded total body clearance (0.150 and 0.106 mL/kg/day) and urinary clearance (0.030 and 0.015 mL/kg/day). Based on these estimates, approximately 89% of the PFOA secreted into bile and 97% of secreted PFOS was estimated to have been reabsorbed from the gastrointestinal tract. Fujii et al. (2015a) also reported that biliary clearances of several perfluoroalkyls (PFOA, PFNA, PFDA, PFUnA, PFDoDA) were much higher than total body clearance in humans, further supporting that perfluoroalkyls excreted in bile undergo extensive reabsorption.

Table 3-4. Excretory Clearance of PFOA and PFOS in Humans

Parameter	Units	PFOA	PFOS
Serum $t_{1/2}$[a]	day	1,387	1,971
Total clearance[b]	mL/kg/day	0.150	0.106
Urinary clearance[c]	mL/kg/day	0.030	0.150

Table 3-4. Excretory Clearance of PFOA and PFOS in Humans

Parameter	Units	PFOA	PFOS
Biliary clearance[d]	mL/kg/day	1.06	2.98
Reabsorbed from bile[e]	%	89	97

[a]Estimates from Olsen et al. (2005).
[b]$\ln(t_{1/2})$xVd, where Vd is the volume of distribution (300 mL/kg).
[c]Estimates from Harada et al. (2005a).
[d]Estimates from Harada et al. (2007).
[e]1-(Total-Urinary)/Biliary.

PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: Harada et al. (2007)

Studies conducted in nonhuman primates and rodents provide further evidence that urine is the major route of excretion of perfluoroalkyls, accounting for >93% of absorbed PFOA and PFOS (Benskin et al. 2009; Butenhoff et al. 2004c; Chang et al. 2008a, 2012; Chengelis et al. 2009a; Hanhijarvi et al. 1982, 1987; Hundley et al. 2006; Johnson and Ober 1979, 1980, 1999a, 1999b; Kemper 2003; Kudo et al. 2001; Olsen et al. 2009; Sundström et al. 2012; Vanden Heuvel et al. 1991b, 1991c). Studies conducted in rats have shown that PFDA, PFNA, PFOA, and PFHxA are secreted in bile and undergo extensive reabsorption from the gastrointestinal tract (Kudo et al. 2001; Vanden Heuvel et al. 1991b, 1991c). PFOS, PFHxS, and PFBS are excreted in feces following intravenous dosing of rats, suggesting that these perfluoroalkyls may also be secreted into bile (Chang et al. 2012; Johnson et al. 1984; Olsen et al. 2009; Sundström et al. 2012). The percentage of the dose excreted in the feces appears to vary with compound, 8–13% for PFOS, <0.5% for PFHxS, and 0.13–0.36% for PFBS. Renal clearances of PFOA from plasma in rats were approximately 0.032 mL/minute/kg body weight in male rats and 0.73 mL/minute/kg in female rats; plasma concentrations of PFOA during these measurements ranged from approximately 0.8 to 80 µg/mL (Kudo et al. 2002). In the latter study, approximately >95% of plasma PFOA was bound to high molecular weight protein and the glomerular filtration rate was approximately 10 mL/minute/kg; therefore, urinary excretion of PFOA was approximately 6% of the rate of glomerular filtration of PFOA in males and 146% in females. These estimates indicate that net renal tubular reabsorption of filtered PFOA occurred in male rats, whereas net renal tubular secretion of PFOA occurred in female rats (i.e., clearance of free PFOA in plasma > glomerular filtration rate). The pronounced sex difference in renal clearance of PFOA has been attributed to modulation of renal excretory transport of PFOA by testosterone and estradiol (Kudo et al. 2002; Vanden Heuvel et al. 1992a; see Section 3.1.5).

Rates of elimination of perfluoroalkyls vary substantially across chemical species and animal species, and show sex differences and age-dependencies within certain species. Table 3-5 summarizes estimates of the elimination $t_{1/2}$ for perfluoroalkyls in humans and experimental animals. In compiling the estimates presented in Table 3-5, preference was given to the terminal $t_{1/2}$ when multiple $t_{1/2}$ values were reported. The significance of the terminal $t_{1/2}$ is that it determines the time required for complete elimination of the perfluoroalkyl as well as the exposure duration required to achieve a steady state. Most of the $t_{1/2}$ values in Table 3-5 were estimated from analyses of data on declining serum concentrations of perfluoroalkyls after a single dose or following cessation of a period of repeated dosing. Estimates of the terminal $t_{1/2}$ based on serum concentrations can vary with the length of the observation period following the last dose and with the modeling approach used to estimate the $t_{1/2}$. Longer observation times are required to estimate the slowest phases of elimination. As a result, estimates of $t_{1/2}$ based on observation periods of 1–2 days can be much shorter than estimates for the same perfluoroalkyl based on observation periods of several weeks. Direct comparisons of $t_{1/2}$ values should be made with consideration of whether or not the observation periods were comparable. Differences in estimation methodology can also contribute to differences in $t_{1/2}$ values. Values reported in Table 3-5 are based on fitting data to single or multi-compartment models, or noncompartmental modeling of the data. While the terminal $t_{1/2}$ provides a metric for comparing times required for complete elimination and steady state, it does not always provide a measure of how rapidly the perfluoroalkyl is cleared from the body. A more useful metric for this is the systemic clearance (Cl_S), typically estimated from the absorbed dose (AD) and the area under the serum concentration curve (AUC_S):

$$Cl_S = \frac{AD}{AUC_S} \qquad \text{Eq. (3-3)}$$

Equation 3-3 will provide an accurate estimate of systemic clearance following an oral dose if the oral dose is completely absorbed. Accurate estimation of AUC_S also depends on fitness of the underlying model used to predict serum concentrations. Estimates of systemic clearance based on pharmacokinetics analyses of serum data from animal studies are presented in Table 3-6.

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFOA—Human					
Human (n=26), adult, M (n=24) F (n=2)	NA	NA	NA	3.8 years (95% CI 3.1–4.4, GM 3.5)	Olsen et al. 2007a
Human (n=20) 15–50 years, M	NA	NA	NA	2.8 years (95% CI 2.4–3.4)	Li et al. 2018
Human (n=30) 15–50 years, F	NA	NA	NA	2.4 years (95% CI 2.0–3.0)	Li et al. 2018
Human (n=66), >50 years, M, F	NA	NA	NA	2.6 years (SE 0.4, GM 1.2)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	2.1 years (SE 0.3, GM 1.5)	Zhang et al. 2013
Human (n=45), M, F	NA	NA	NA	3.9 years	Worley et al. 2017a
Human (n=5), 22±0.9, M	NA	NA	NA	2.3 years	Harada et al. 2005a
Human (n=5), 68±5, M	NA	NA	NA	2.6 years	Harada et al. 2005a
Human (n=5), 23±3, F	NA	NA	NA	3.5 years	Harada et al. 2005a
Human (n=5), 69±5, F	NA	NA	NA	2.9 years	Harada et al. 2005a
Human (n=200) 54±15, M, F	Oral	NA	NA	2.3 years (95% CI 2.1–2.4)	Bartell et al. 2010
Human (n=643), adult, M, F	Oral	NA	NA	2.9 years (<4 years) (95% CI 2.3–3.8) 10.1 years (>4 years)	Seals et al. 2011
Human (n=1,029), adult, M, F	Oral	NA	NA	8.5 years (<9 years) (95% CI 7.1–10.1)	Seals et al. 2011
Humans (n=17), adult, M, F	Oral	NA	NA	5.1 years (SD 1.7, GM 4.8)	Costa et al. 2009
Humans (n=6) adults, F	Inhalation	NA	NA	2.5 (range 1.8–3.1)	Gomis et a. 2016
PFOS—Human					
Human (n=26), adult, M (24) F (2)	NA	NA	NA	5.4 years (95% CI 3.9–6.9, GM 4.8)	Olsen et al. 2007a
Human (n≅1,000), >12–>80 years, M	NA	NA	NA	4.7 years	Wong et al. 2014

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Human (n≅1,000), >12–>80 years, F	NA	NA	NA	4.3 years (95% CI 4.1–4.5)	Wong et al. 2015
Human (n=66), >50 years, M, F	NA	NA	NA	27 years (SE 3.1, GM 18)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	6.2 years (SE 0.5, GM 5.8)	Zhang et al. 2013
Human (n=45), M, F	NA	NA	NA	3.3 years	Worley et al. 2017a
Human (n=20) 15–50 years M	NA	NA	NA	4.6 years (95% CI 3.7–6.1)	Li et al. 2018
Human (n=30) 15–50 years, F	NA	NA	NA	3.1 years (95% CI 2.7–3.7)	Li et al. 2018
Human (n=5), 22±0.9, M	NA	NA	NA	4.9 years	Harada et al. 2005a
Human (n=5), 68±5, M	NA	NA	NA	7.4 years	Harada et al. 2005a
Human (n=5), 23±3, F	NA	NA	NA	4.5 years	Harada et al. 2005a
Human (n=5), 69±5, F	NA	NA	NA	4.6 years	Harada et al. 2005a
PFHxS—Human					
Human (n=26), adult, M (24), F (2)	NA	NA	NA	8.5 years (95% CI 6.4–10.6, GM 7.3)	Olsen et al. 2007a
Human (n=20), ≤50 years, F	NA	NA	NA	7.7 years (SE 0.6, GM 7.1)	Zhang et al. 2013
Human (n=20) 15–50 years, M	NA	NA	NA	7.4 years (95% CI 6.0–9.7)	Li et al. 2018
Human (n=30) 15–50 years F	NA	NA	NA	4.7 years (95% CI 3.9–5.9)	Li et al. 2018
Human (n=45), M, F	NA	NA	NA	15.5 years	Worley et al. 2017a
Human (n=66), >50 years, M, F	NA	NA	NA	35 years (SE 3.9, GM 25)	Zhang et al. 2013
PFBA—Human					
Human (n=3), adult, M	NA	NA	NA	81 hours (SD 41)	Chang et al. 2008b
Human (n=9), adult, M (7), F (2)	NA	NA	NA	72 hours (SD 38)	Chang et al. 2008b
PFBS—Human					
Human (n=6), adult M (5), F(1)				665 hours (SD 266)	Olsen et al. 2009

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFNA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	4.3 years (SE 0.5, GM 3.2)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	2.5 years (SE 0.6, GM 1.7)	Zhang et al. 2013
PFDA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	12 years (SE 1.5, GM 7.1)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	4.5 years (SE 0.4, GM 4.0)	Zhang et al. 2013
PFUnA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	12 years (SE 2.0, GM 7.4)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	4.5 years (SE 0.5, GM 4.0)	Zhang et al. 2013
PFHpA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	1.2 years (SE 0.2, GM 0.82)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	1.5 years (SE 0.3, GM 1.0)	Zhang et al. 2013
PFOA—Nonhuman primate					
Cynomolgus monkey, adult, M	Oral	10 mg/kg/day	6 months	20.1 days	Butenhoff et al. 2004c
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	20.9 days (SD 12.5)	Butenhoff et al. 2004c
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	32.6 days (SD 8.0)	Butenhoff et al. 2004c
PFOS—Nonhuman primate					
Cynomolgus monkey, adult, M	Oral	0.15 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, M	Oral	0.75 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, F	Oral	0.15 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, F	Oral	0.75 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, M	IV	2 mg/kg	1 day	132 days (SE 7)	Chang et al. 2012
Cynomolgus monkey, adult, F	IV	2 mg/kg	1 day	110 days (SE 15)	Chang et al. 2012

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFHxA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	5.3 days (SD 2.5)	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	2.4 days (SD 1.7)	Chengelis et al. 2009a
PFHxS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	141 days (SE 30.)	Sundström et al. 2012
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	87 days (SE 27)	Sundström et al. 2012
PFBA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	40.3 hours (SD 2.4)	Chang et al. 2008b
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	41.0 hours (SD 4.7)	Chang et al. 2008b
PFBS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	15.0 hours (SD 9.8)	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	8.0 hours (SD 2.0)	Chengelis et al. 2009a
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	95.2 hours (SE 27.1)	Olsen et al. 2009
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	83.2 hours (SE 41.9)	Olsen et al. 2009
PFOA—Rat					
Rat (CR), adult, M	Oral	11.4 mg/kg	1 day	115 hours	Johnson and Ober 1980
Rat (Sprague-Dawley), adult, M	Oral	0.1 mg/kg	1 day	202 hours (SD 38)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1 mg/kg	1 day	138 hours (SD 32)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1 mg/kg	1 day	44 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	5 mg/kg	1 day	174 hours (SD 29)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	25 mg/kg	1 day	157 hours (SD 38)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1 mg/kg	1 day	185 hours (SD 19)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1 mg/kg	1 day	39 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	0.4 mg/kg	1 day	322 hours (SD 38)	Benskin et al. 2009

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Rat (Sprague-Dawley), adult, M	Oral	0.022 mg/kg/day	12 weeks	218 hours (95% CL 127–792)	De Silva et al. 2009
Rat (Wistar), adult, M	IV	21.5 mg/kg	1 day	136 hours (SD 24)	Kudo et al. 2002
Rat (Wistar), adult, M	IV	20.1 mg/kg	1 day	135 hours (SD 29)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, M	IP	3.9 mg/kg	1 day	216 hours (SE 30.9)	Vanden Heuvel et al. 1991c
Rat (Wistar), adult, M	IP	50 mg/kg	1 day	105 hours	Ylinen et al. 1990
Rat (Sprague-Dawley), adult, F	Oral	0.1 mg/kg	1 day	3.2 hours (SD 0.9)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1 mg/kg	1 day	3.5 hours (SD 1.1)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1 mg/kg	1 day	3.6 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	5 mg/kg	1 day	4.6 hours (SD 0.6)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	25 mg/kg	1 day	16.2 hours (SD 9.9)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1 mg/kg	1 day	2.8 hours (SD 0.5)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1 mg/kg	1 day	4.6 hours	Kim et al. 2016b
Rat (Wistar), adult, F	IV	21.5 mg/kg	1 day	1.9 hours (SD 0.7)	Kudo et al. 2002
Rat (Wistar), adult, F	IV	20.1 mg/kg	1 day	1.9 hours (SD 0.7)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, F	IP	3.9 mg/kg	1 day	2.9 hours (SE 0.2)	Vanden Heuvel et al. 1991c
Rat (Wistar), adult, F	IP	50 mg/kg	1 day	24 hours	Ylinen et al. 1990
PFOS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	4.2 mg/kg	1 day	179 hours	Johnson and Ober 1979
Rat (Sprague-Dawley), adult, M	Oral	0.27 mg/kg	1 day	809 hours	Benskin et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	0.023 mg/kg/day	12 weeks	1,968 hours (95% CL 1.584–2.568)	De Silva et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	2 mg/kg	1 day	1,495 hours (SE 50)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	Oral	2 mg/kg	1 day	635 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	15 mg/kg	1 day	1,707 hours (SE 270)	Chang et al. 2012

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Rat (Sprague-Dawley), adult, F	Oral	0.023 mg/kg/day	12 weeks	1,992 hours (95% CL 1,752–2,280)	De Silva et al. 2009
Rat (Sprague-Dawley), adult, F	Oral	2 mg/kg	1 day	919 hours (SE 56)	Chang et al. 2012
Rat (Sprague-Dawley), adult, F	Oral	2 mg/kg	1 day	564 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	15 mg/kg	1 day	989 hours (SE 48)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	IV	2 mg/kg	1 day	689 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	2 mg/kg	1 day	595 hours	Kim et al. 2016b
FOSA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	5.0 mg/kg	1 day	125 hours	Seacat and Luebker 2000
PFDA—Rat					
Rat (Sprague-Dawley), adult, M	IP	4.8 mg/kg	1 day	1,008 hours	Vanden Heuvel et al. 1991b
Rat (Wistar), adult, M	IV	25 mg/kg	1 day	958 hours (SD 207)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	25 mg/kg	1 day	1,406 hours (SD 140)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, F	IP	4.8 mg/kg	1 day	552 hours	Vanden Heuvel et al. 1991b
PFNA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	0.2 mg/kg	1 day	974 hours	Benskin et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	0.029 mg/kg/day	12 weeks	1,128 hours (95% CL 935–1,416)	De Silva et al. 2009
Rat (Sprague-Dawley), adult M	Oral	1, 3, or 10 mg/kg	1 day	734.4 hours	Tatum-Gibbs et al. 2011
Rat (Wistar), adult, M	IV	22.6 mg/kg	1 day	710 hours (SD 55)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, F	Oral	1, 3, or 10 mg/kg	1 day	33.6 hours	Tatum-Gibbs et al. 2011
Rat (Wistar), adult, F	IV	22.6 mg/kg	1 day	58.6 hours (SD 9.8)	Ohmori et al. 2003
PFHpA—Rat					
Rat (Wistar), adult, M	IV	17.7 mg/kg	1 day	2.4 hours (SD 1.2)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	17.7 mg/kg	1 day	1.2 hours (SD 0.2)	Ohmori et al. 2003

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFHxA—Rat					
Rat (Sprague-Dawley), adult, M	IV	10 mg/kg	1 day	1.0 hour	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	50 mg/kg	1 day	2.2 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	150 mg/kg	1 day	2.4 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	300 mg/kg	1 day	2.5 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	10 mg/kg	1 day	0.42 hour	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	Oral	50 mg/kg	1 day	2.6 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	Oral	150 mg/kg	1 day	2.2 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	Oral	300 mg/kg	1 day	2.1 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	2 mg/kg	1 day	1.7 hours (SD 0.6)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, M	Oral	10 mg/kg	1 day	0.5 hours (SD 0.1)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, F	Oral	2 mg/kg	1 day	1.5 hours (SD 0.2)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, F	Oral	10 mg/kg	1 day	0.7 hours (SD 0.3)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, M	Oral	50 mg/kg/day	26 days	2.0 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, M	Oral	150 mg/kg/day	26 days	2.1 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, M	Oral	300 mg/kg/day	26 days	2.9 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, F	Oral	50 mg/kg/day	26 days	1.9 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, F	Oral	150 mg/kg/day	26 days	2.2 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, F	Oral	300 mg/kg/day	26 days	3.0 hours	Kirkpatrick 2005
PFHxS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	0.030 mg/kg	1 day	382 hours	Benskin et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	4 mg/kg	1 day	645.6 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	4 mg/kg	1 day	41.28 hours	Kim et al. 2016b

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Rat (Sprague-Dawley), adult, M	IV	4 mg/kg	1 day	496.8 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	IV	10 mg/kg	1 day	688 hours (SE 14.4)	Sundström et al. 2012
Rat (Sprague-Dawley), adult, F	IV	4 mg/kg	1 day	21.12 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	10 mg/kg	1 day	39 hours (SE 1.9)	Sundström et al. 2012
PFBA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	30 mg/kg	1 day	9.22 hours (SE 0.75)	Chang et al. 2008b
Rat (Sprague-Dawley), adult, M	IV	30 mg/kg	1 day	6.38 hours (SE 0.53)	Chang et al. 2008b
Rat (Sprague-Dawley), adult, F	Oral	30 mg/kg	1 day	1.76 hours (SE 0.26)	Chang et al. 2008b
Rat (Sprague-Dawley), adult, F	IV	30 mg/kg	1 day	1.03 hours (SE 0.03)	Chang et al. 2008b
PFBS—Rat					
Rat (Sprague-Dawley), adult, M	IV	10 mg/kg	1 day	2.1 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), Rat (SD), adult, M	IV	30 mg/kg	1 day	4.51 hours (SE 2,22)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	30 mg/kg	1 day	4.68 hours (SE 0.07)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, F	IV	10 mg/kg	1 day	0.64 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	30 mg/kg	1 day	3.96 hours (SE 0.21)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, F	Oral	30 mg/kg	1 day	7.42 hours (SE 0.79)	Olsen et al. 2009
PFOS—Mouse					
Mouse (CD), adult, M	Oral	1 mg/kg	1 day	1,027 hours	Chang et al. 2012
Mouse (CD), adult, M	Oral	20 mg/kg	1 day	874 hours	Chang et al. 2012
Mouse (CD), adult, F	Oral	1 mg/kg	1 day	907 hours	Chang et al. 2012
Mouse (CD), adult, F	Oral	20 mg/kg	1 day	731 hours	Chang et al. 2012

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFHxS—Mouse					
Mouse (CD), adult, M	Oral	1 mg/kg	1 day	732 hours	Sundström et al. 2012
Mouse (CD), adult, M	Oral	20 mg/kg	1 day	671 hours	Sundström et al. 2012
Mouse (CD), adult, F	Oral	1 mg/kg	1 day	597 hours	Sundström et al. 2012
Mouse (CD), adult, F	Oral	20 mg/kg	1 day	643 hours	Sundström et al. 2012
PFNA—Mouse					
Mouse (CD-1), adult, M	Oral	1 or 10 mg/kg	1 day	823.2–1,653.6 hours	Tatum-Gibbs et al. 2011
Mouse (CD-1), adult, F	Oral	1 or 10 mg/kg	1 day	619.2–1,641.6 hours	Tatum-Gibbs et al. 2011
PFBA—Mouse					
Mouse (CD1), adult, M	Oral	10 mg/kg	1 day	13.34 hours (SE 4.55)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	30 mg/kg	1 day	16.3 hours (SE 7.2)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	100 mg/kg	1 day	5.22 hours (SE 2.27)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	10 mg/kg	1 day	2.87 hours (SE 0.30)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	30 mg/kg	1 day	3.08 hours (SE 0.26)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	100 mg/kg	1 day	2.79 hours (SE 0.3)	Chang et al. 2008b
PFOS—Rabbit					
Rabbit (New Zealand), adult, F	Oral	0.085 mg/kg/day	102 days	87 days (SD 31)	Tarazona et al. 2016

[a]Exposure durations of 1 day indicate that a single dose was administered.
[b]Reported half-lives are arithmetic means for the terminal elimination phase if multiple elimination phases were observed.

CI = confidence interval; CL = confidence limit; F = female; GM = geometric mean; IP = intraperitoneal; IV = intravenous; M = male; NA = not applicable; PFBA = perfluorobutanoic acid; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; FOSA = perfluorooctane sulfonamide; SD = standard deviation; SE = standard error

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
PFOA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	12.4 (SD 7.4)	Butenhoff et al. 2004c
Cynomolgus monkey, adult, F	IV	10	1 day	5.3 (SD 3.3)	Butenhoff et al. 2004c
PFOS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	2	1 day	1.10 (SE 0.06)	Chang et al. 2012
Cynomolgus monkey, adult, F	IV	2	1 day	1.65 (SE 0.04)	Chang et al. 2012
PFHxA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	569	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10	1 day	535	Chengelis et al. 2009a
PFHxS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	1.3 (SE 0.1)	Sundström et al. 2012
Cynomolgus monkey, adult, F	IV	10	1 day	1.9 (SE 0.4)	Sundström et al. 2012
PFBA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	2,371 (SE 293)	Chang et al. 2008a
Cynomolgus monkey, adult, F	IV	10	1 day	1,075 (SE 91)	Chang et al. 2008a
PFBS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	159	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10	1 day	238	Chengelis et al. 2009a
Cynomolgus monkey, adult, M	IV	10	1 day	12,264 (SE 3384)	Olsen et al. 2009
Cynomolgus monkey, adult, F	IV	10	1 day	8,832 (SE 2880)	Olsen et al. 2009
PFOA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	0.1	1 day	23.1 (SD 5.8)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1	1 day	20.9 (SD 3.8)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1	1 day	40.40 (SD 2.29)	Kim et al. 2016b

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
Rat (Sprague-Dawley), adult, M	Oral	5	1 day	20.4 (SD 5.0)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	25	1 day	27.1 (SD 7.4)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1	1 day	21.5 (SD 2.0)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1	1 day	47.39 (SD 3.40)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	0.1	1 day	778 (SD 144)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1	1 day	655 (SD 173)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1	1 day	645.12 (SD 43.44)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	5	1 day	1,164 (SD 118)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	25	1 day	842 (SD 166)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1	1 day	816 (SD 221)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1	1 day	612.84 (SD 32.54)	Kim et al. 2016b
Rat (Wistar), adult, M	IV	21.5	1 day	50.4 (SD 14.4)	Kudo et al. 2002
Rat (Wistar), adult, F	IV	21.5	1 day	2,233 (SD 805)	Kudo et al. 2002
Rat (Wistar), adult, M	IV	20.1	1 day	135 (SD 29)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	20.1	1 day	2,233 (SD 805)	Ohmori et al. 2003
PFOS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	2	1 day	7.33 (SD 0.55)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	2	1 day	11.3 (SE 0.56)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	Oral	15	1 day	4.9 (SE 0.52)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	IV	2	1 day	9.24 (SD 0.37)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	2	1 day	8.52 (SD 0.37)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	2	1 day	22.2 (SE 0.28)	Chang et al. 2012
Rat (Sprague-Dawley), adult, F	Oral	15	1 day	5.4 (SE 20)	Chang et al. 2012
Rat (Sprague-Dawley), adult, F	IV	2	1 day	9.82 (SD 0.21)	Kim et al. 2016b

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
PFDA—Rat					
Rat (Wistar), adult, M	IV	25	1 day	207 (SD 0.054)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	25	1 day	140 (SD 0.008)	Ohmori et al. 2003
PFNA—Rat					
Rat (Wistar), adult, M	IV	22.6	1 day	6.9 (SD 0.6)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	22.6	1 day	106 (SD 31)	Ohmori et al. 2003
PFHpA—Rat					
Rat (Wistar), adult, M	IV	17.7	1 day	1,604 (SD 558)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	17.7	1 day	3,071 (SD 781)	Ohmori et al. 2003
PFHxA—Rat					
Rat (Sprague-Dawley), adult, M	IV	10	1 day	2,784	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	10	1 day	18,600	Chengelis et al. 2009a
PFHxS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	4	1 day	7.15 (SD 0.06)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	IV	4	1 day	9.01 (SD 0.05)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	IV	10	1 day	6.7 (SE 0.06)	Sundström et al. 2012
Rat (Sprague-Dawley), adult, F	Oral	4	1 day	124.83 (SD 3.40)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	4	1 day	227.93 (SD 6.73)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	10	1 day	53.4 (SE 4.38)	Sundström et al. 2012
PFBA—Rat					
Rat (Sprague-Dawley), adult, M	IV	30	1 day	851 (SE 61)	Chang et al. 2008a
Rat (Sprague-Dawley), adult, F	IV	30	1 day	2,949 (SE 59)	Chang et al. 2008a
Rat (Sprague-Dawley), adult, M	Oral	30	1 day	494 (SE 29)	Chang et al. 2008a
Rat (Sprague-Dawley), adult, F	Oral	30	1 day	1,527 (SE 145)	Chang et al. 2008a
PFBS—Rat					
Rat (Sprague-Dawley), adult, M	IV	10	1 day	946	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	10	1 day	7,464	Chengelis et al. 2009a

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
Rat (Sprague-Dawley), adult, M	IV	30	1 day	2,856 (SE 816)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, F	IV	30	1 day	11,265 (SE 960)	Olsen et al. 2009
PFOA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.13	1 day	14.2 (SD 8.4)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.13	1 day	11.8 (SD 6.1)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.3	1 day	13.1 (SD 7.4)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.3	1 day	9.0 (SD 1.9)	Fujii et al. 2015a, 2015b
PFOS—Mouse					
Mouse (CD), adult, M	Oral	1	1 day	4.7	Chang et al. 2012
Mouse (CD), adult, M	Oral	20	1 day	4.7	Chang et al. 2012
Mouse (CD), adult, F	Oral	1	1 day	5.0	Chang et al. 2012
Mouse (CD), adult, F	Oral	20	1 day	6.0	Chang et al. 2012
PFHxS—Mouse					
Mouse (CD), adult, M	Oral	1	1 day	2.9	Sundström et al. 2012
Mouse (CD), adult, M	Oral	20	1 day	4.8	Sundström et al. 2012
Mouse (CD), adult, F	Oral	1	1 day	2.7	Sundström et al. 2012
Mouse (CD), adult, F	Oral	20	1 day	3.8	Sundström et al. 2012
PFNA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.14	1 day	3.9 (SD 1.9)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.14	1 day	5.1 (SD 2.3)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.4	1 day	4.0 (SD 1.7)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.4	1 day	2.4 (SD 1.0)	Fujii et al. 2015a, 2015b
PFDA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.16	1 day	2.2 (SD 0.9)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.16	1 day	2.8 (SD 1.2)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.6	1 day	3.9 (SD 1.8)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.6	1 day	2.2 (SD 1.1)	Fujii et al. 2015a, 2015b

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
PFUnA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.17	1 day	2.8 (SD 1.0)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.17	1 day	3.4 (SD 1.5)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.7	1 day	5.7 (SD 2.6)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.7	1 day	3.1 (SD 1.7)	Fujii et al. 2015a, 2015b
PFDoDA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.19	1 day	4.4 (SD 1.6)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.19	1 day	4.8 (SD 2.4)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.9	1 day	9.4 (SD 4.1)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.9	1 day	5.2 (SD 3.2)	Fujii et al. 2015a, 2015b
PFBA—Mouse					
Mouse (CD1), adult, M	Oral	10	1 day	280 (SE 72)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	30	1 day	296 (SE 640)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	100	1 day	784 (SE 112)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	10	1 day	564 (SE 24)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	30	1 day	696 (SE 32)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	100	1 day	1,336 (SE 64)	Chang et al. 2008b

[a]As reported in units of mL/day/kg or converted from mL/hour (x24), mL/hour (x24/body weight) or mL/minute (x60x24).

CI = confidence interval; F = female; IV = intravenous; M = male; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SD = standard deviation; SE = standard error

Elimination of Perfluoroalkyls in Humans. Elimination $t_{1/2}$ values for PFOA, PFOS, PFHxS, PFBA, and PFBS have been estimated in humans (Bartell et al. 2010; Costa et al. 2009; Chang et al. 2008a; Glynn et al. 2012; Harada et al. 2005a; Li et al. 2018; Olsen et al. 2007a, 2009; Seals et al. 2011; Spliethoff et al. 2008; Yeung et al. 2013; Wong et al. 2014, 2015; Worley et al. 2017a; Zhang et al. 2013). Estimates in humans are based on measurements of the decline in serum perfluoroalkyl concentrations following cessation or an abrupt decrease in exposure, or on measurements of renal plasma clearance

from serum in a general population sample from Japan (Harada et al. 2005a). The latter clearance estimates were converted to $t_{1/2}$ values, for display in Table 3-5 as follows (Equations 3-4 and 3-5):

$$k_e = \frac{Cl}{V} \qquad \text{Eq. (3-4)}$$

$$t_{1/2} = \frac{\ln(2)}{k_e} \qquad \text{Eq. (3-5)}$$

where k_e is the elimination rate constant (e.g., day^{-1}), Cl is the renal plasma clearance (e.g., mL plasma/day/kg), and V is the plasma volume (L/kg), which is assumed to be 4.3% of body weight (ICRP 1981). In general, these studies show that longer chain length is associated with slower elimination rates. For example, the elimination $t_{1/2}$ for PFBA was estimated to be 70–80 hours (Chang et al. 2008a), whereas the $t_{1/2}$ values for PFHxS, PFOS, and PFOA range from 2 to 35 years (Bartell et al. 2010; Harada et al. 2005a; Li et al. 2018; Olsen et al. 2007a; Seals et al. 2011; Worley et al. 2017a; Zhang et al. 2013). Longer $t_{1/2}$ values for PFOA have been reported with longer monitoring follow-up times, which allow the detection of slower elimination phases of multiphasic elimination kinetics (Seals et al. 2011). Perfluoroalkyl sulfonates are eliminated more slowly in humans than corresponding carboxylates of the same chain length (Zhang et al. 2013). Analytical methods typically used to measure serum perfluoroalkyls do not discriminate between linear and branched isomers and, as a result, these studies estimate elimination rates for the isomer mixture. A study that compared elimination rates of isomers of PFOA found that linear isomers tend to be eliminated more slowly than branched isomers (Zhang et al. 2013), consistent with results of studies conducted in rats (Benskin et al. 2009; De Silva et al. 2009).

An analysis of serum PFOS data from NHANES indicated that $t_{1/2}$ in females may be shorter (4.3 years) compared to males (4.7 years; Wong et al. 2014, 2015). The NHANES data are cross-sectional and, therefore, the estimates of $t_{1/2}$ required fitting the data to age patterns of PFOS intake. An improved fit to the data for females was achieved when estimated losses of PFOS in menstrual fluids were considered, suggesting that menstrual loss of PFOS may account for some, but not all, of the sex difference in the elimination rate (Verner and Longnecker 2015; Wong et al. 2015). Li et al. (2018) also found apparent sex differences in PFOS elimination in male and female residents in Sweden exposed to contaminated drinking water. The estimated $t_{1/2}$ for PFOS were 4.6 years in males and 3.1 years in females. Zhang et al. (2013) estimated serum $t_{1/2}$ for various age and sex strata in a population of 86 individuals. Serum $t_{1/2}$ for PFOS was lower for PFOS in younger females (≤50 years, $t_{1/2}$ = 6.2 years±0.3 SE, n=66) compared to males and older females ($t_{1/2}$ =27 years±3.1 SE, n=20). Zhang et al. (2013) attributed the difference in serum $t_{1/2}$ to clearance in menstrual fluids. However, the estimated serum $t_{1/2}$ of 27 years is much higher than values calculated from other studies; Zhang et al. (2013) noted that the serum $t_{1/2}$ should be

considered as an upper limit estimate. Estimated $t_{1/2}$ for PFOA was not different in younger females (2.1±0.3 SE, n=20) compared to males and older females (2.6±0.4 SE, n=66). Declines in serum PFOA concentrations were observed in populations following initiation of activated carbon filtration of public water supplies that had been contaminated with PFOA (Bartell et al. 2010). The estimated mean serum $t_{1/2}$ for a group of 200 adults followed for 1 year after filtration was initiated was 2.3 years (95% CI 2.1–2.4). Elimination rates were not different in males and females. Serum PFOA concentration ranged from 16 to 1,200 ng/mL. A larger follow-up study measured serum PFOA concentrations in two populations of former residents (n=1,672) of the same water districts (Seals et al. 2011). In one population (n=643), the serum $t_{1/2}$ increased with increasing elapsed time since leaving the water district. The $t_{1/2}$ values were 2.9 years (95% CI 2.3–3.8) for elapsed time of <4 years and 10.1 years for elapsed time of >4 years. In a second population with an elapsed time since residence of <9 years, the $t_{1/2}$ was 8.5 years (95% CI 7.1–10.1). Elimination rates (based on the annual percent decrease in serum concentrations) were faster in males (27%) compared to females (18%) for the first 4 years post-exposure; however, no difference was evident between sexes when elapsed time from exposure was >4 years.

Bartell (2012) and Russell et al. (2015) point out that most studies examining PFOA elimination half-lives fail to account for ongoing background exposure, which could result in an overestimation of elimination half-lives. Bartell (2012) estimated that the bias from background exposure could resulted in 1–26% overestimation of calculated PFOA half-lives and that greater overestimations can occur for half-lives based on longer follow-up times. Russell et al. (2015) estimated that the bias was greatest in populations with serum PFOA levels closest to background levels. In a re-analysis of the Olsen et al. (2007) occupational exposure data, Russell et al. (2015) estimated that overestimation was approximately 1.2% in workers with initial serum concentrations >500 ng/mL (100 times higher than NHANES general population data) and 13% for workers with lower initial serum PFOA levels. Restricting the elimination half-life calculation to workers with initial serum PFOA levels of >500 ng/mL would result in a half-life of 3.0 years (Russell et al. 2015), compared to 3.8 years calculated for the whole cohort (Olsen et al. 2007).

Analysis of kinetics of serum PFOS concentrations in retired U.S. fluorochemical production workers (24 males, 2 females) yielded a mean serum elimination $t_{1/2}$ estimate of 5.4 years (95% CI 3.9–6.9; geometric mean: 4.8 years, 95% CI 4.0–5.8) in subjects whose serum PFOS concentrations ranged from 37 to 3,490 ng/mL (Olsen et al. 2007a). Estimates for the two females in the same study were 4.9 and 6.8 years. Estimates based on renal clearance of PFOS from serum in subjects from the general population of Japan ranged from 2.9 to 7.4 years; these subjects had serum PFOS concentrations that

ranged from 4 to 49 ng/mL (Harada et al. 2005a). Estimates in males (7.4, 2.9 years) were similar to females (4.5, 4.6 years). This same study measured serum PFHxS concentrations in retired U.S. fluorochemical production workers (24 males, 2 females) and yielded a mean estimate of 8.5 years (95% CI 6.4–10.6; geometric mean: 7.3 years, 95% CI 5.8–9.2) for the serum elimination $t_{1/2}$ in subjects whose serum PFHxS concentrations ranged from 10 to 1,295 ng/mL (Olsen et al. 2007a). Estimates for the two females in the same study were 12.2 and 13.3 years.

The elimination rate of PFBA was estimated in fluorochemical workers who may have been exposed to various PFBA precursors (Chang et al. 2008a). In three male workers, the estimated mean $t_{1/2}$ based on serum PFBA kinetics was 81 hours (±41 SD). In a larger study of nine workers (seven males, two females), the mean $t_{1/2}$ was 72 hours (±38 SD). Estimates for the two female subjects were 56 and 118 hours. The combined mean value for the 12 estimates was 75 hours (±38 SD). Olsen et al. (2009) estimated serum $t_{1/2}$ of PFBS in six fluorochemical workers. The mean $t_{1/2}$ was 27.4 days (±11.1 SD). The group included a single female whose $t_{1/2}$ was 45.7 days. Based on these observations, PFBA (chain length 3) and PFBS (chain length 4) are eliminated substantially faster in humans than perfluoroalkyls having longer carbon chain lengths, such as PFHxS (chain length 6), PFOA (chain length 7), and PFOS (chain length 8).

Temporal trends in perfluoroalkyl serum concentrations have also been used to estimate population halving times (Glynn et al. 2012; Olsen et al. 2012; Spleithoff et al. 2008; Yeung et al. 2013). Population halving times are influenced by temporal trends in intakes and may therefore not accurately reflect clearance. Population halving times for PFOS ranged from 4 to 5 years (Olsen et al. 2012; Spleithoff et al. 2008; Yeung et al. 2013). Glynn et al. (2012) monitored serum perfluoroalkyls in a population of pregnant women (n=413) in Sweden over the period 1996–2010. Halving times were 22 years (95% CI 16–38) for PFOA and 8.2 years (95% CI 6.3–12) for PFOS.

Elimination of Perfluoroalkyls in Nonhuman Primates. Elimination $t_{1/2}$ values and systemic clearances for PFOA, PFOS, PFHxA, PFHxS, PFBA, and PFBS have been estimated in Cynomolgus monkeys (Buttenoff et al. 2004c; Chang et al. 2012; Chengelis et al. 2009a; Olsen et al. 2009; Seacat et al. 2002; Sundström et al. 2012). Estimated terminal $t_{1/2}$ values were 20–30 days for PFOA, 100–170 days for PFOS, 90–140 days for PFHxS, 40 hours for PFBA and 8–95 hours for PFBS. Elimination of perfluoroalkyls in monkeys is multiphasic and, as a result, estimates of the terminal $t_{1/2}$ can vary with the duration of the observation period and assumptions made in modeling elimination kinetics (Chang et al. 2012; Chengelis et al. 2009a; Olsen et al. 2009; Sundström et al. 2012). For example, the $t_{1/2}$ values for

PFBS were 8 and 15 hours in female and male monkeys, respectively, when monkeys were monitored for 48 hours following a single intravenous dose (Chengelis et al. 2009a), whereas the $t_{1/2}$ values were 95 and 83 hours in male and female monkeys, respectively, when the monitoring period was extended to 14 days and a three-compartment model was used to estimate the terminal $t_{1/2}$ (Olsen et al. 2009). Studies in monkeys confirm general trends observed in humans that perfluoroalkyl sulfonates are more slowly eliminated than perfluoroalkyl carboxylates and that elimination of longer-chain perfluoroalkyls occurs more slowly than short-chain perfluoroalkyls. Systemic clearances were lower for PFOS, PFHxS, and PFBS compared to the corresponding carboxylates, PFOA, PFHxA, and PFBA (Table 3-6). Systemic clearances were similar in male and female monkeys (Table 3-6).

Elimination of Perfluoroalkyls in Rats. Elimination $t_{1/2}$ values and systemic clearances for PFOA, PFOS, FOSA, PFDA, PFNA, PFHpA, PFHxA, PFHxS, PFBA, and PFBS have been estimated in rats (Benskin et al. 2009; Chang et al. 2008b, 2012; Chengelis et al. 2009a; De Silva et al. 2009; Johnson and Ober 1979; Kemper 2003; Kim et al. 2016b; Kudo et al. 2002; Ohmori et al. 2003; Olsen et al. 2009; Seacat and Luebker 2000; Sundström et al. 2012; Vanden Heuvel et al. 1991b, 1991c; Ylinen et al. 1990). Consistent with observations made in humans and Cynomolgus monkeys, perfluoroalkyl sulfonates are more slowly eliminated than perfluoroalkyl carboxylates and short-chain perfluoroalkyls (e.g., PFBA, PFBS) are eliminated faster in rats than long-chain perfluoroalkyls (e.g., PFOA, PFOS, PFHxA, PFHxS); Tables 3-5 and 3-6. Linear PFOA isomers tend to be eliminated more slowly than branched isomers (Benskin et al. 2009; De Silva et al. 2009).

Elimination of perfluoroalkyls exhibits pronounced sex differences in rats, with faster elimination in females than in males (Benskin et al. 2009; Chang et al. 2008b; Chengelis et al. 2009a; Kemper 2003; Kim et al. 2016b; Kudo et al. 2002; Ohmori et al. 2003; Sundström et al. 2012; Tatim-Gibbs et al. 2011; Vanden Heuvel et al. 1991c; Ylinen et al. 1990). Estimates of systemic clearance for PFOA in male rats ranged from 20 to 50 mL/day/kg, whereas estimates for female rats ranged from 600 to 2,200 mL/day/kg (Kemper 2003; Kudo et al. 2002; Ohmori et al. 2003). Systemic clearances of PFOA, PFOS, PFNA, PFHxA, PFHxS, PFBA, and PFBS are also higher in female rats compared to male rats (Table 3-6). Pronounced sex difference in elimination rates in rats (faster elimination in females) was observed in rats following 30-minute nose-only exposures to aerosols (MMAD=1.9–2.1 μm) of 1–25 mg ammonium PFOA/m^3 (Hinderliter et al. 2006a). Plasma PFOA concentrations were not detectable 12 hours after exposure of female rats, and were approximately 90% of peak plasma concentrations 24 hours after the exposure in male rats. The slower elimination of PFOA in male rats resulted in steady-state plasma concentrations within 3 weeks of repeated exposures (6 hours/day, 5 days/week) in male rats, whereas in

female rats, daily periodic oscillations of plasma concentrations from peak to below detection occurred on each day of exposure. Steady-state plasma concentrations in male rats were approximately 10 times that of daily peak concentrations in female rats.

Pronounced dose dependence appears in the $t_{1/2}$ estimates for PFOA in female rats. With increasing dose, plasma elimination kinetics in female rats converts from monophasic to biphasic. Following an oral dose of PFOA of 0.1, 1, 5, or 25 mg/kg, the terminal $t_{1/2}$ values in female rats were 3.2, 3.5, 4.6, or 16.2 hours, respectively; no apparent dose dependence was observed in male rats over the same dose range (Kemper 2003). Dose-dependent elimination of PFOA has been attributed to a capacity-limited renal tubular secretion of PFOA in female rats (see discussion below on *Mechanisms of Excretion*). The divergence in elimination kinetics between male and female rats appears to be age-dependent, with faster elimination becoming evident in female rats after 30 days of age, consistent with the timing of sexual maturation and involvement of sex hormones in the modulation of the renal excretion of PFOA in rats (Hinderliter et al. 2006b).

Elimination of Perfluoroalkyls in Mice. Elimination $t_{1/2}$ values and systemic clearances for PFOS, PFHxS, and PFBA have been estimated in mice (Chang et al. 2008a, 2012; Sundström et al. 2012). Consistent with studies conducted in rats and monkeys, PFBA is eliminated more rapidly in mice than PFOS and PFHxS. Systemic clearances ranged from 5 to 6 mL/day/kg for PFOS (Chang et al. 2012), from 3 to 5 mL/day/kg for PFHxS (Sundström et al. 2012), and from 300 to 1,300 mL/day/kg for PFBA (Chang et al. 2008a). Sex differences in elimination in mice were observed for PFBA, but not PFOS or PFHxS. Systemic clearances of PFBA in female mice were approximately 2 times that of males (Chang et al. 2008a). Systemic clearance of PFBA in male and female mice appeared to be dependent on dose. Systemic clearance following a single oral dose of 100 mg PFBA/kg was approximately 2 times higher than the systemic clearance following a dose of 10 or 30 mg PFBA/kg. Possible explanations for the apparent dependence of clearance on dose are dose-dependent bioavailability or that the one-compartment model used to estimate elimination rates and serum AUC did not adequately fit the serum kinetics observed at the higher dose (Chang et al. 2008a). The latter could occur if renal tubular reabsorption of PFBA or plasma protein binding of PFBA is saturable in mice. Systemic clearance rates for PFOA were similar in male mice (13.1 mL/kg/day) and in female mice (9.0 mL/kg/day) (Fuji et al. 2015a, 2015b).

Elimination of Perfluoroalkyls in Other Species. Sex differences in elimination of PFOA have also been observed in hamsters; unlike the rat, male hamsters excreted absorbed PFOA more rapidly than female hamsters. Following a single gavage dose of 10 mg/kg as ammonium [^{14}C]PFOA, cumulative excretion

of ^{14}C in urine at 24 hours post-dosing was 96.4% of the dose in female rats and 8.7% in male rats; 24.6% and 84.5% in female and male hamsters, respectively; 4.1% in male and female mice; and 90.5 and 80.2% in female and male rabbits, respectively (Hundley et al. 2006).

Mechanisms of Excretion. Urinary excretion of perfluoroalkyls involves glomerular filtration and renal tubular secretion and reabsorption (for PFOA, see Harada et al. 2005a; Kudo et al. 2002; Ohmori et al. 2003). Glomerular filtration of PFOA is limited by extensive binding of PFOA to albumin and other high molecular weight proteins in plasma (Han et al. 2003, 2005; Ohmori et al. 2003; Kerstner-Wood et al. 2003; Vanden Heuvel et al. 1992a, 1992b; Ylinen and Auriola 1990). Elimination of PFOA and other perfluoroalkyls shows pronounced sex differences in rats, with slower elimination in males for PFOA, PFOS, PFNA, PFHxA, PFHxS, PFBA, and PFBS (Chang et al. 2008a, 2012; Chengelis et al. 2009a; Kemper 2003; Kudo et al. 2002; Ohmori et al. 2003; Sundström et al. 2012). The sex difference in PFOA elimination in rats is dependent on testosterone (Hinderliter et al. 2006b; Kudo et al. 2002; Vanden Heuvel et al. 1992a). The significantly slower elimination of PFOA in adult male rats compared to female rats has been attributed to sex hormone modulation of organic anion transporters in kidney. At similar doses administered to male and female rats, PFOA undergoes net tubular reabsorption in male rats (i.e., urinary excretion rate < rate of glomerular filtration of PFOA) and net tubular secretion in female rats (i.e., urinary excretion rate > rate of glomerular filtration of PFOA) (Harada et al. 2005a; Kudo et al. 2002; Ohmori et al. 2003). In rats, several transporters have been shown to have affinity for C7–C9 perfluoroalkyl carboxylates. The transporters, OAT1 and OAT3, located on the basolateral membrane of the renal proximal tubule, appear to participate in secretion of C7–C9 perfluoroalkyl carboxylates into the tubular fluid (Nakagawa et al. 2008; Weaver et al. 2010). The transporters, OATP1a1 (rat), OAT4 (human), and URAT1 (human), located on the apical membrane, appear to mediate reabsorption of C8–C10 perfluoroalkyl carboxylates from the tubular fluid (Katakura et al. 2007; Nakagawa et al. 2009; Weaver et al. 2010; Yang et al. 2009, 2010). In rats and mice, expression of OAT1, OAT3, and OATP1a1 is controlled by male sex hormones and shows higher activities in males (Buist and Klaassen 2004; Gotoh et al. 2002; Kobayashi et al. 2002; Li et al. 2002; Lu et al. 1996; Lubojevic et al. 2004). The slower elimination of PFOA (and other long-chain perfluoroalkyl carboxylates) in male rats has been attributed to OATP1a1 (Weaver et al. 2010; Yang et al. 2009). Higher activity of OATP1a1 in male rats results in higher reabsorptive transport and lower rates of urinary excretion. However, saturation of this transporter could result in an increase in urinary elimination of perfluoroalkyls due to decreased tubular reabsorption. This is consistent with the apparent plateau in plasma concentration with increasing dose observed in cancer patients treated with PFOA (Convertino et al. 2018). Affinities of OATP1a1 (rat), OAT4 (human), and URAT1 (human) are highest for C7–C10 perfluoroalkyl carboxylates (Weaver et al.

2010; Yang et al. 2009, 2010). Affinity of rat OATP1a1 is strongly correlated with total clearance in rats (r^2=0.98; Yang et al. 2009).

Although sex differences for elimination of perfluoroalkyls have been detected in laboratory animals, human monitoring studies have not consistently detected sex differences in elimination $t_{1/2}$ of perfluoroalkyls; this may reflect limitations in the studies, including numbers and age of subjects (Bartell et al. 2010; Seals et al. 2011; Wong et al. 2014, 2015; Zhang et al. 2013). Menstruation may contribute to faster elimination of PFOS in women (Wong et al. 2014, 2015; Zhang et al. 2013). The effect of menstruation or other variables related to menstruation appear to contribute to faster elimination in younger (≤50 years) women compared to men and older women (Zhang et al. 2013). Two studies have found evidence for elimination of PFOS being affected by menstruation (Wong et al. 2014, 2015; Zhang et al. 2013). The estimated $t_{1/2}$ for PFOA was not different in younger females compared to males and older females. Mechanisms by which menstruation could affect PFOS clearance are not understood. Bulk elimination of blood would be expected to affect serum clearance of both PFOS and PFOA; therefore, other mechanisms must contribute that discriminate between perfluoroalkyl species. A better metric than serum $t_{1/2}$ for evaluating sex differences in elimination for this would be systemic or renal clearance of the perfluoroalkyl. Harada et al. (2005a) measured renal clearance in a small sample of young adults (five males and five females, age 22–23 years) and found that renal clearance was not different in males and females. Zhang et al. (2013) estimated renal clearance of PFOA and PFOS in a population of younger females (≤50 years, n=20), older females (>50 years), younger males (≤50 years), and older males (>50 years) and did not find significant sex or age differences. Studies that measured systemic clearance in monkeys also have not found significant sex differences in systemic clearance of PFOA (Buttenoff et al. 2004c) or PFOS (Chang et al. 2012).

Studies conducted in rats have shown that PFDA, PFNA, PFOA, PFOS, and PFHxA are secreted in bile and undergo extensive reabsorption from the gastrointestinal tract (Johnson et al. 1984; Kudo et al. 2001; Vanden Heuvel et al. 1991b, 1991c). Biliary secretion rates of PFOA are similar in male and female rats when renal excretion is blocked by ligation of the kidneys (Vanden Heuvel et al. 1991a, 1991b). This lack of sex influence on biliary secretion (compared to the sex influence on renal clearance) may reflect a relative sex insensitivity of OAT2 (or other organic anion transporter) expression in liver, compared to kidney; the latter is approximately 7–8 times higher in adult female rats compared to male rats (Kudo et al. 2002).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

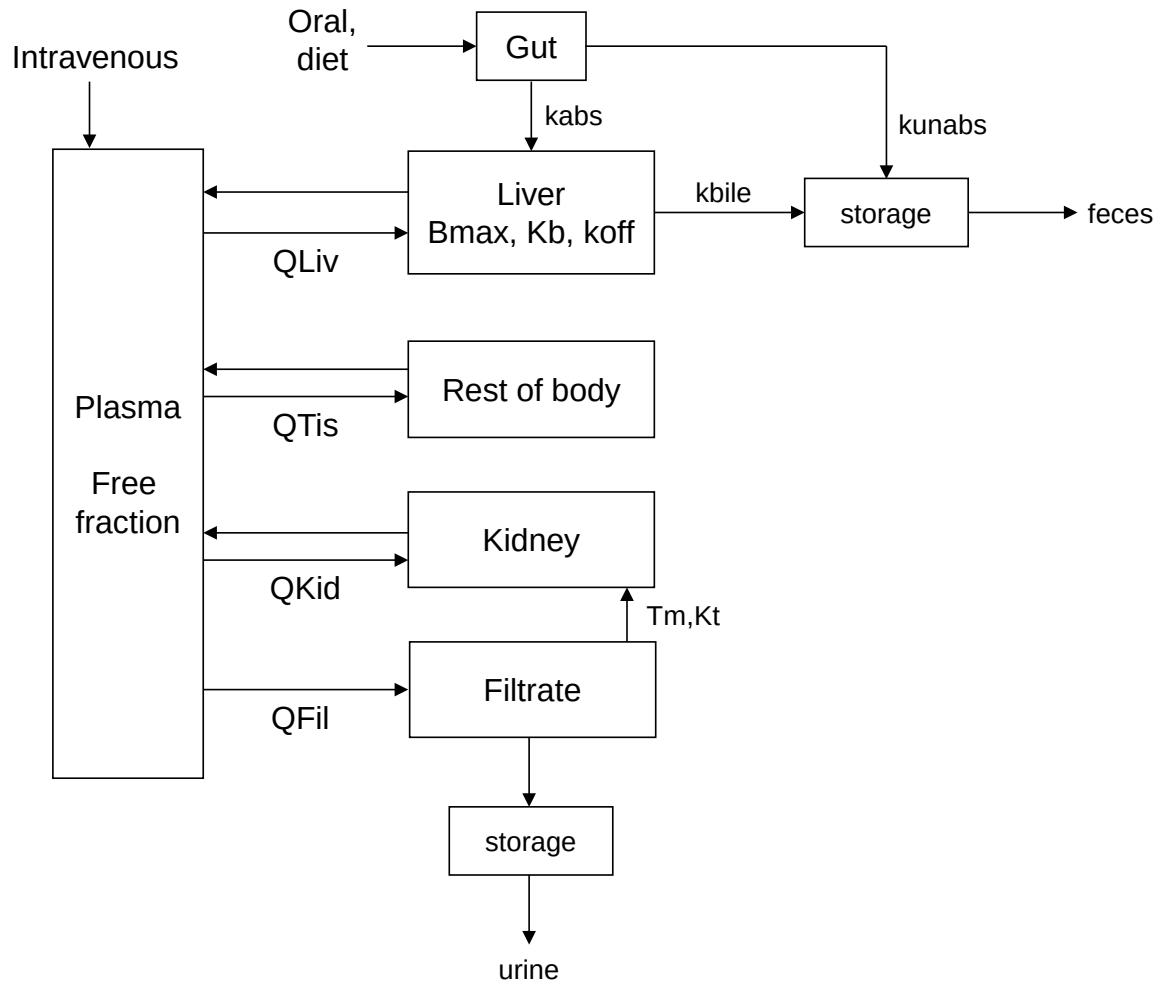
Several PBPK models of PFOA and PFOS have been reported. These include a human model for PFOA and PFOS (Fàbrega et al. 2014, 2016; Loccisano et al. 2011; Worley et al. 2017b), models for PFOA and PFOS in monkeys (Loccisano et al. 2011), models for PFOA and PFOS in rats (Harris and Barton 2008; Loccisano et al. 2012a, 2012b; Tan et al. 2008; Worley and Fisher 2015a. 2015b), and a model for PFOA in mice (Rodriguez et al. 2009). Models of PFOA and PFOS kinetics during gestation and lactation in rats and mice also have been reported (Loccisano et al. 2012a, 2012b; Rodriguez et al. 2009). Various empirical and compartmental models have also been reported (Hoffman et al. 2011; Lorber and Egeghy 2011; Lou et al. 2009; Thompson et al. 2010; Verner et al. 2016; Wambaugh et al. 2013; Wu et al. 2009). Tardiff et al. (2009) utilized a human pharmacokinetic model to estimate an average daily oral dose corresponding to a Reference Dose for PFOA plasma concentration in humans. Cheng and Ng (2017) developed a permeability-limited PBPK model for PFOA in male rats that could be used for *in vitro* to *in vivo* extrapolation. Kim et al. (2018) developed a PBPK model for PFHxS in rats and humans. PBPK models were not identified for other perfluoroalkyls examined in this profile. Given the toxicokinetic differences between compounds, the PFOA, PFOS, and PFHxS PBPK models may not be appropriate for other compounds.

3.1.5.1 Loccisano et al. (2012a, 2012b) Rat Models

Loccisano et al. (2012a) developed a model for simulating the kinetics of PFOA and PFOS in male and female rats. The model was based, in part, on a multi-compartmental model developed by Tan et al. (2008; Andersen et al. 2006). The female rat model (Loccisano et al. 2012a) was subsequently extended to include gestation and lactation (Loccisano et al. 2012b). The general structures of the models are depicted in Figures 3-3, 3-4, and 3-5. Complete lists of parameters and parameter values and the bases

for parameter values and evaluations of model predictions in comparison to observations are described in Loccisano et al. (2012a, 2012b).

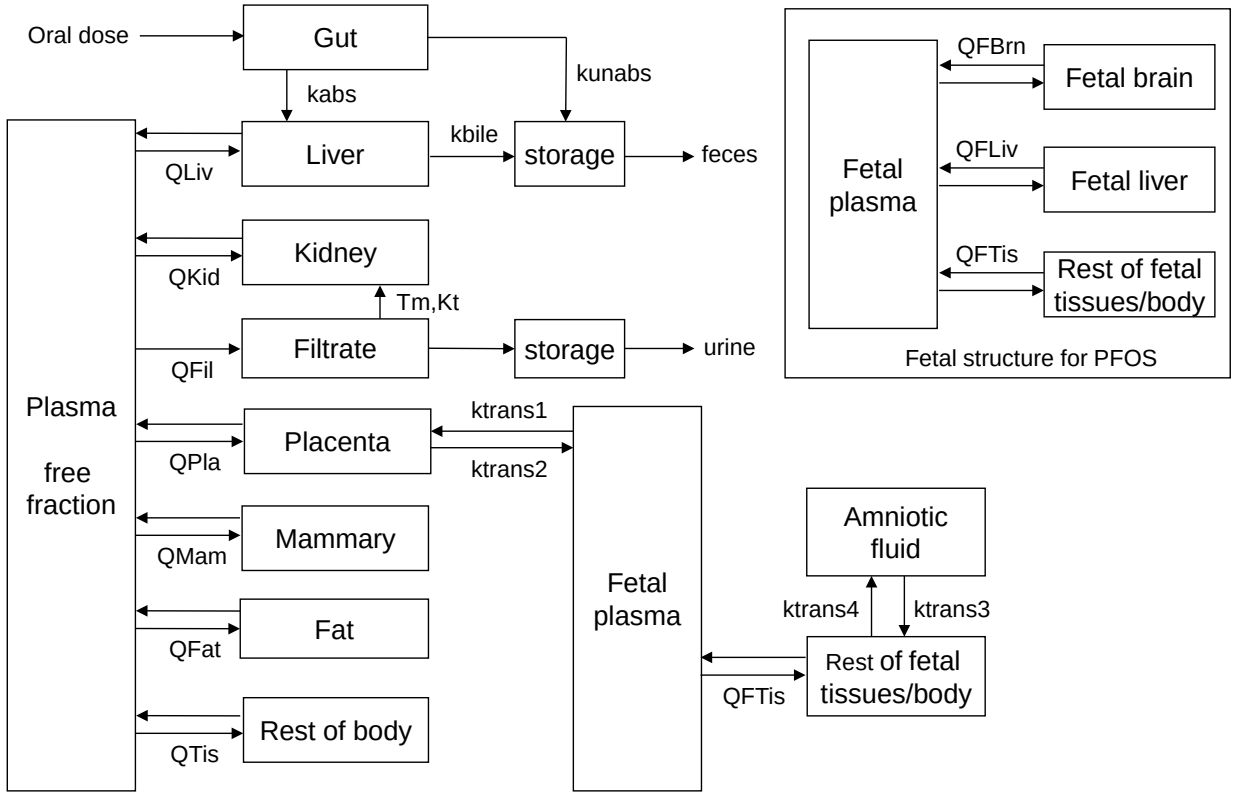
Figure 3-3. Structure of PBPK Model of PFOA and PFOS in the Rat



Bmax = liver binding capacity; kabs = first-order absorption rate constant; Kb = liver binding affinity constant; kbile = biliary excretion rate constant; Koff = liver binding dissociation constant; Kt = affinity constant; kunabs = rate of unabsorbed dose to appear in feces; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFil = clearance from plasma to glomerular filtrate; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QTis = blood flow in and out of tissues; Tm = transporter maximum

Source: Loccisano et al. 2012a (reproduced with permission of Elsevier Inc. in the format reuse in a government report via Copyright Clearance Center; Reproductive Toxicology by Reproductive Toxicology Center; Washington, DC)

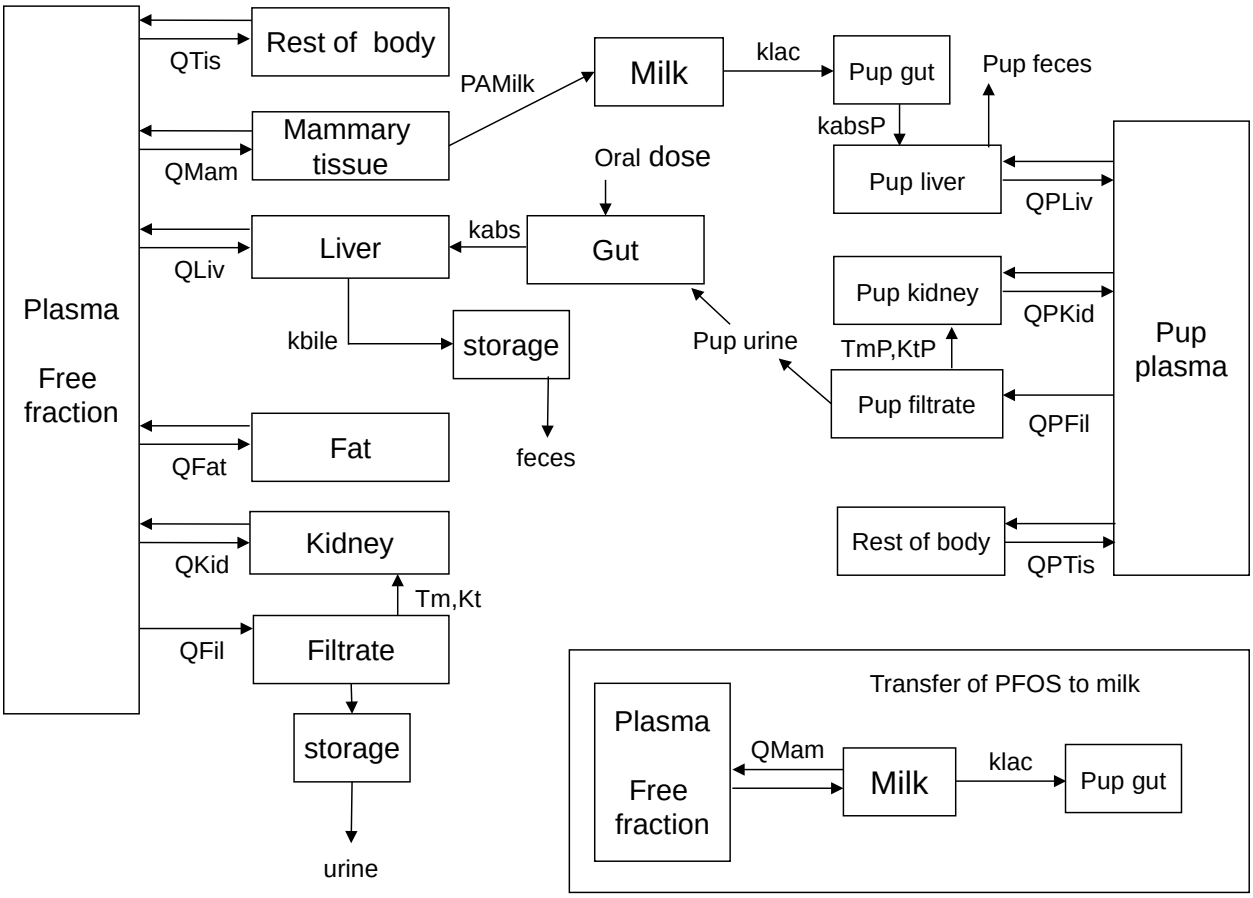
Figure 3-4. PBPK Model Structure for Simulating PFOA and PFOS Exposure During Gestation in the Rat (Dam, Left; Fetus, Right)



kabs = first-order absorption rate constant; kbile = biliary excretion rate constant; Kt = affinity constant; ktrans1/ktrans 2 = transfer between placenta and fetal plasma; ktrans3/ktrans4 = transfer between amniotic fluid and rest of the body; kunabs = rate of unabsorbed dose to appear in feces; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFat = blood flow in and out of fat; QFBrn = blood flow in and out of fetal brain; QFil = clearance from plasma to glomerular filtrate; QFLiv = blood flow in and out of fetal liver; QFTis = blood flow in and out of fetal tissue; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QMam = blood flow in and out of mammary tissue; QPla = blood flow in and out of placenta; QTis = blood flow in and out of tissues; Tm = transporter maximum

Source: Loccisano et al. 2012b (reproduced with permission of Elsevier Inc. in the format reuse in a government report via Copyright Clearance Center; Reproductive Toxicology by Reproductive Toxicology Center; Washington, DC)

Figure 3-5. PBPK Model Structure for Simulating PFOA/PFOS Exposure During Lactation in the Rat (Dam, Left; Pup, Right)



kabs = first-order absorption rate constant; kabsP = pup first-order absorption rate constant; kbile = biliary excretion rate constant; klac = transfer to pup through milk; Kt = affinity constant; KtP = pup affinity constant; PAMilk = transfer from mammary tissue to liver; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFat = blood flow in and out of fat; QFil = clearance from plasma to glomerular filtrate; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QMam = blood flow in and out of mammary tissue; QPFil = clearance from pup plasma to glomerular filtrate; QPKid = blood flow in and out of pup kidney; QPLiv = blood flow in and out of pup liver; QPTis = blood flow in and out of pup tissue; QTis = blood flow in and out of tissues; Tm = transporter maximum; TmP = pup transporter maximum

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The basic (i.e., adult nonpregnant rat) model includes compartments representing plasma (including a bound and free fraction), kidney and renal glomerular filtrate, liver, and a lumped compartment representing all other tissues. Two storage compartments are included in the model: one receives perfluoroalkyl from the gastrointestinal tract (unabsorbed) and liver (bile) and the other receives perfluoroalkyl from the glomerular filtrate. The storage compartments were included in the model to simulate time delays between elimination from plasma and appearance of perfluoroalkyl in feces or urine. Absorption from the gastrointestinal tract is simulated as the balance between first-order absorption and fecal excretion of unabsorbed chemical. Absorbed PFOA and PFOS are assumed to be delivered to the liver where saturable binding of PFOS (but not PFOA) to liver proteins occurs. Saturable binding of PFOS in liver was included to simulate the relatively long retention times of PFOS in liver that have been observed in rats. Exchanges between PFOA or PFOS in liver (free fraction), kidney, and other tissues with the free pool in plasma are assumed to be flow-limited (governed by blood flow) with equilibrium determined by the tissue:blood partition coefficient. PFOA and PFOS in plasma are simulated as instantaneous distributions into free and bound fractions. Extensive binding of PFOA and PFOS to plasma proteins has been demonstrated in various animal species including rats (see Section 3.1.2). For PFOA, the free fraction is assigned a constant of 4.5% in females and 0.6% in males. These values were optimized to fit observed kinetics of PFOA in plasma and urine of rats following intravenous and oral exposures (Loccisano et al. 2012a). Adequate fit to observed PFOS plasma kinetics following single doses of PFOS required introducing a time-dependence in binding of PFOS to protein (Loccisano et al. 2012a; Tan et al. 2008). The free fraction for PFOS in plasma decreases from an initial value (after dosing) of 2.2% to a minimum of 0.1% with a $t_{1/2}$ for the change of approximately 14 hours in a 0.25-kg rat ($k=0.035$ hours^{-1}/kg$^{-0.25}$). The relatively short $t_{1/2}$ for the change limits the effects of the time-dependent plasma kinetics over the first 1–2 days of dosing (including peak concentrations) and has no effect on longer-term kinetics or steady state. Although the time-dependence of the free fraction in plasma was needed to simulate short-term plasma PFOS kinetics in rats, the physiological mechanism for a dependence of plasma binding on the time following dosing (i.e., not on concentration of PFOS in plasma or some other dose surrogate) has not been established. Elimination of absorbed chemical occurs by biliary excretion and urinary excretion. Transfer from liver to feces (representing excretion following biliary transfer) is represented as a first-order process acting on the free fraction in liver. Excretion in urine is simulated as the balance between transfer from the free fraction to the glomerular filtrate and renal tubular reabsorption, which removes PFOA and PFOS from the glomerular filtrate and returns it to kidney tissue. Renal tubular reabsorption is simulated as a capacity-limited process with parameters T_m (µg/hour/kg body weight), representing the maximum rate of transport, and K_T (µg/L), representing affinity for the transporter (the concentration in the glomerular filtrate at which reabsorptive transport rate

is half of maximum). This representation of renal tubular reabsorption is used to simulate observed sex differences in elimination of PFOA from plasma, which have been attributed to higher reabsorptive capacity in male rats (see Section 3.1.4). Values for the maximum and affinity parameters for PFOA result in higher reabsorptive clearances from the glomerular filtrate (T_m/K_T=4.1) in male rats compared to female rats (T_m/K_T=0.045), and correspondingly lower urinary clearance of PFOA from plasma in male rats. Reabsorption parameters for PFOS are the same in both sexes and result in reabsorptive clearances that are approximately twice that of PFOA in female rats (T_m/K_T=7.2).

The basic rat model was extended to simulate gestation with inclusion of additional compartments representing adipose and mammary tissue in the dam, placenta, and fetus (Figure 3-4); Loccisano et al. 2012b). Transfer of PFOA and PFOS to the fetus is simulated as a flow-limited transfer to the placenta, with first-order exchange between the placenta and the free fraction in fetal plasma. The free fraction in fetal plasma is simulated as a constant fraction for PFOA and PFOS (i.e., no dependence on time as in the adult). Within the fetus, PFOA in the free fraction of plasma exchanges with a single lumped compartment representing the fetal body, which exchanges with PFOA in amniotic fluid. The fetal PFOS model subdivides fetal tissue into brain, liver, and a lumped compartment for other tissues, all of which undergo flow-limited exchanges with the free fraction of PFOS in fetal plasma. Binding of PFOA and PFOS in fetal liver is assumed to be negligible. Differences in the structure of the fetal models for PFOA and PFOS reflect the differences in the availability of data for estimating parameter values for the various compartments (e.g., perfluoroalkyl concentrations in amniotic fluid, liver).

The lactation model extends the dam portion of the gestational model to include milk and pup (Figure 3-5; Loccisano et al. 2012b). Transfer of PFOA to milk occurs through the mammary gland with flow-limited exchange between plasma and mammary tissue and diffusion into milk from mammary tissue. The model also includes transfer from the pup to the dam, which occurs during maternal stimulation of the neonatal pup to induce elimination and during pup grooming. Data on PFOS in mammary tissue of rodents were not available to establish parameters for a mammary tissue compartment; therefore, the mammary tissue compartment was left out of the PFOS model, and transfer of PFOS to milk is simulated as diffusion directly from plasma. The pup model includes compartments representing the free fraction in plasma, liver, kidney, glomerular filtrate, and a lumped compartment representing all other pup tissues. This structure is essentially identical to the nonpregnant rat model (Loccisano et al. 2012a) with a few differences. Absorption from the gastrointestinal tract is assumed to be complete in pups, and binding in pup liver is assumed to be negligible in pups. There are no storage compartments for biliary or glomerular filtrate perfluoroalkyl in the pup model. Sex differences in renal

tubular reabsorption of PFOA are assumed to develop in response to sexual maturation and, therefore, are not present during lactation (i.e., parameter values are allometrically scaled to pup body weight from the male rat values). Reabsorptive transport parameters for PFOS are allometrically scaled from the lactating dam. The liver/plasma partition coefficient for PFOS in the pups was set lower than that in the dam, based on observations in rats. All other parameters for PFOA and PFOS in the pup were the same or allometrically scaled from values for the dam.

Optimization of parameter values and evaluations of the rat models are described in Loccisano et al. (2012a, 2012b). Data sets utilized in developing and evaluating the nonpregnant rat models included single-dose intravenous and gavage studies and short-term feeding studies (Johnson and Ober 1979; Kemper 2003; Kudo et al. 2007; Perkins et al. 2004). Data used in development and evaluation of the gestation and lactation models included data from gestational and/or lactational exposure studies in rats (Chang et al. 2009; Hinderliter et al. 2005; Kuklenyik et al. 2004; Luebker et al. 2002, 2005a, 2005b; Thibodeaux et al. 2003).

Applications for Dosimetry Extrapolation and Risk Assessment. The wealth of data on pharmacokinetics of PFOA and PFOS in rats allowed an extensive evaluation of the rat models for predicting plasma urinary and liver PFOA and PFOS following single intravenous or single and repeated oral dosing. Inclusion of renal tubular reabsorption parameters in the model provided accurate simulations of sex differences in elimination rates of PFOA from plasma and excretion in urine, and differences in rates of elimination of PFOA and PFOS. The gestation model successfully predicted fetal plasma and liver PFOA and PFOS at the end (or near the end) of pregnancy. Consistent with observations, the model predicts higher fetal plasma concentrations and lower fetal liver concentrations of PFOS compared to maternal, and lower internal exposure (plasma concentrations) to PFOA in the fetus compared to maternal (fetal liver data were not available for PFOA). The lactation model successfully predicted PFOA and PFOS in pup plasma following dosing of the dam. Predicted plasma concentrations of PFOA in nursing pups were approximately 10–50% lower than maternal concentrations, whereas maternal and pup concentrations of PFOS were similar. The model could be used to estimate liver doses and corresponding plasma profiles resulting from single or repeated dosing of adult male or female rats, and maternal-fetal and maternal-pup transfer of PFOA and PFOS. The rat model was evaluated with data from a 14-week oral dosing study and has not been tested for longer exposures. Harris and Barton (2008) developed a PBPK model for PFOS in the rat and found that time adjustments that increased renal clearance and decreased the liver-plasma partition coefficient as a function of time and dose improved predictions of plasma and liver PFOS in adult rats exposed for a period of 105 weeks. Although the

Harris and Barton (2008) model is very different from the Loccisano et al. (2012a) model, these results suggest the possibility that clearance of PFOS may be age- and/or dose-dependent in rats. This may reflect age- or dose-related changes in kidney function, including tubular reabsorption or secretion of PFOS.

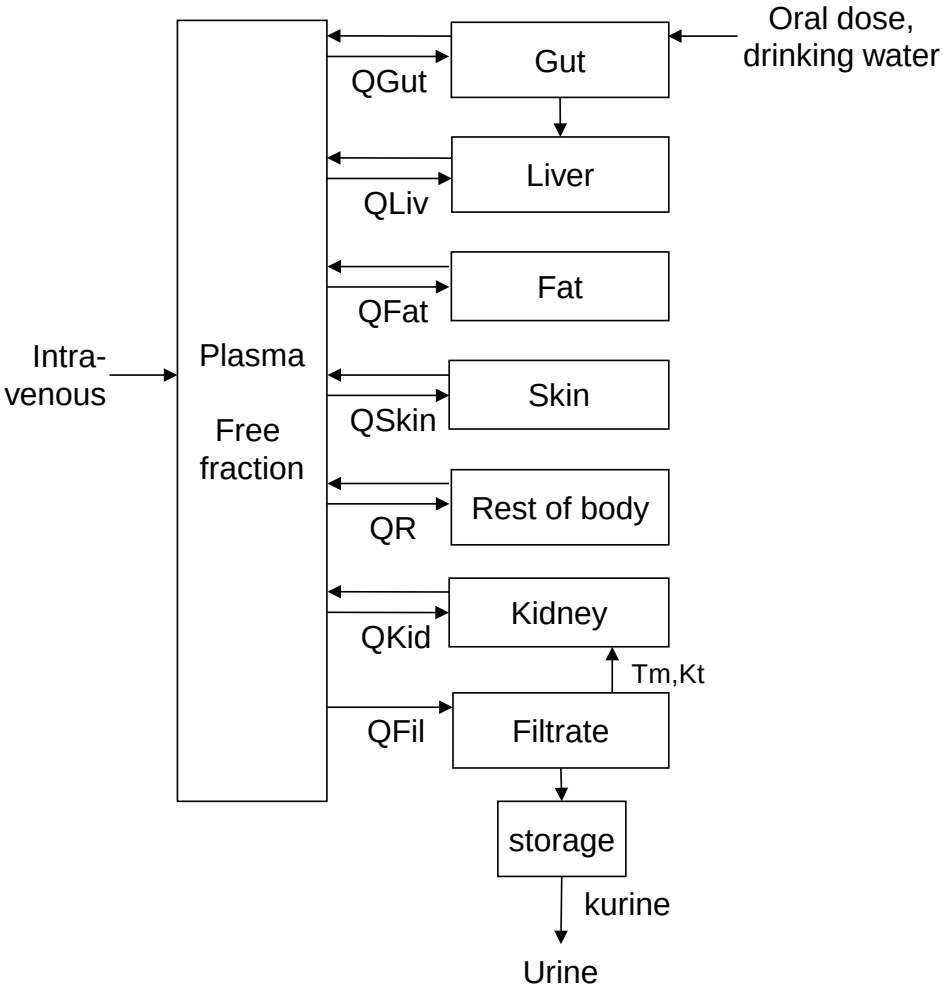
3.1.5.2 Loccisano et al. (2011, 2013) Monkey and Human Models

Loccisano et al. (2011) developed a model for simulating the kinetics of PFOA and PFOS in monkeys and humans. The human model described in Loccisano et al. (2011) was subsequently extended to include simulations of pregnancy and lactation (Loccisano et al. 2013). The monkey model was based, in part, on a multi-compartmental model developed by Tan et al. (2008; Andersen et al. 2006) for simulating the kinetics of plasma and urinary PFOA in monkeys. The structures of the monkey and human models are identical (Figure 3-6) and are very similar to the structure of the rat model (Loccisano et al. 2012a), with inclusion of compartments representing fat and skin, and absence of a storage compartment for biliary transfer. Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Loccisano et al. (2011).

Parameters in the monkey and human models differ in several ways from the rat model. The free fraction in plasma is represented as a constant for both PFOA and PFOS; time-dependency for PFOS in the rat model is absent in the monkey and human models. The parameters for renal tubular reabsorption of PFOA and PFOS are the same for males and females. This is consistent with the absence of evidence for a sex difference in elimination kinetics in monkeys (Butenhoff et al. 2002, 2004a; Seacat et al. 2002).

Values for the affinity constant (K_T) and maximum (T_m) for tubular reabsorption were optimized to plasma concentration kinetics in monkeys. The value for K_T in monkeys was used in the human model. The value for T_m for PFOA in humans was set to yield a plasma elimination $t_{1/2}$ of 2.3 or 3.8 years. The latter two values were derived from estimates of the serum $t_{1/2}$ in populations exposed to PFOA in drinking water (2.3 years; Bartell et al. 2010) or in retired fluorochemical workers (3.8 years; Olsen et al. 2007a). The value for T_m for PFOS in humans was set to yield a plasma elimination $t_{1/2}$ of 5.4 years, based on observations in retired fluorochemical workers (Olsen et al. 2007a). Binding of PFOA and PFOS in the liver was assumed to be negligible in monkeys and humans. Tissue-plasma partition coefficients used in both models were derived from observations in rodents and were the same in the monkey and human models.

Figure 3-6. Structure of PBPK Model for PFOA and PFOS in Monkeys and Humans



Kt = half-saturation constant; kurine = urinary elimination rate; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFat = blood flow in and out of fat; Qfil = clearance from plasma to glomerular filtrate; QGut = blood flow in and out of gut; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QR = blood flow in and out of rest of body; QSkin = blood flow in and out of skin; Tm = transport maximum

Source: Loccisano et al. 2011 (reproduced with permission of Academic Press in the format reuse in a government report via Copyright Clearance Center; Regulatory Toxicology and Pharmacology: RTP by International Society of Regulatory Toxicology and Pharmacology)

Optimization of parameter values and evaluation of the monkey and human models are described in Loccisano et al. (2011). Data sets utilized in developing and evaluating the monkey model included single-dose intravenous and oral studies and repeated-dose oral studies conducted in Cynomolgus monkeys (Butenhoff et al. 2004c; Noker and Gorman 2003; Seacat et al. 2002). Data used in evaluating the human model consisted of serum measurements in people who experienced environmental exposures (Emmett et al. 2006a; Hölzer et al. 2008; Steenland et al. 2009b), adult Red Cross donors (Olsen et al. 2003b, 2008), and retired fluorochemical workers (Olsen et al. 2007a). In general, PFOA and PFOS intakes and exposure durations were not known with certainty in these populations and, as a result, these data do not yield confident evaluations of the ability of the human model to predict intake-plasma level relationships. Follow-up monitoring after a cessation or decrease in exposure can provide data that allow evaluation of the ability of the model to accurately simulate elimination kinetics. Predicted declines in serum PFOA concentrations encompassed observed group mean declines when the T_m for renal tubular reabsorption was set to yield an elimination $t_{1/2}$ of 2.3 or 3.8 years. Group mean declines in serum PFOS were predicted reasonably well for some populations, but not all populations, when the T_m for renal tubular reabsorption was optimized to yield an elimination $t_{1/2}$ of 5.4 years.

The human pregnancy model includes additional compartments representing the free fractions in plasma, amniotic fluid, and a lumped compartment for fetal tissue (Loccisano et al. 2013). The same conceptual approach was used in the rat pregnancy model (Loccisano et al. 2012b, Figure 3-4). Rate constants for placental transfer were initially those from the rat model, adjusted to yield predicated maternal/fetal plasma ratios that agreed with observed maternal/fetal ratios in cord blood (Apelberg et al. 2007b; Fei et al. 2007; Midasch et al. 2007; Washino et al. 2009). Transfers from amniotic fluid to fetus were the same as those used in the rat model, as there were no data on which to base estimates for humans. The lactation model included additional compartments for mammary milk and a lumped compartment representing the infant. Transfer of PFOA to milk is simulated as flow-limited exchange between plasma and milk, governed by mammary tissue blood flow and a milk/plasma partition coefficient. This structure obviated the need to simulate mammary tissue kinetics, for which there were no data in humans. The milk/plasma partition coefficient was calibrated to yield predictions of observed milk/plasma ratios (Fromme et al. 2010; Kärrman et al. 2007). Transfer from maternal milk to infants is the product of the milk concentration and milk production rate (assumed to be equal to sucking rate). The pregnancy model was evaluated by comparing predicted maternal/fetal plasma ratios for PFOA and PFOS with observations from various human monitoring studies (Fei et al. 2007; Fromme et al. 2010; Hanssen et al. 2010; Inoue et al. 2004; Kim et al. 2011; Midasch et al. 2007; Monroy et al. 2008; Tittlemier et al. 2004). The lactation model was evaluated by comparing predicted maternal plasma/milk ratios for PFOA and PFOS

with observations from various human monitoring studies (Fromme et al. 2009; Kärrman et al. 2007; Liu et al. 2011). In general, most model predictions were within plus or minus 2-fold of observations.

Applications for Dosimetry Extrapolation and Risk Assessment. The model predicts plasma concentrations and tissue levels of PFOA and PFOS following intravenous or oral dosing. A skin compartment is included in the model, which may serve for simulating absorption and distribution following deposition onto the skin surface; however, the dermal absorption model was not evaluated in Loccisano et al. (2011). The human model was calibrated to predict $t_{1/2}$ values estimated for human populations (e.g., 2.3 or 3.8 years for PFOA, 5.4 years for PFOS). As a result, comparisons made between observed and predicted serum concentrations evaluate whether or not the populations actually exhibit the $t_{1/2}$ to which the model was calibrated, and not the validity of the model to predict the internal distribution of PFOA or PFOS. It is not currently possible to assess with confidence whether the human model can accurately predict doses to liver or any other tissues. Fábrega et al. (2014) applied the human adult model to estimate plasma concentrations and tissue levels of PFOA and PFOS in human autopsy samples. Exposure inputs to the model were intakes of PFOA and PFOS estimated from public water supply concentrations in the local area where the subjects had resided (Catalonia, Spain) and concentrations in local market basket foods (Domingo et al. 2012a, 2012b). The human model predicted levels of PFOA in plasma and liver that were approximately 10- and 5-fold higher, respectively, than observed. Predicted plasma levels of PFOS were approximately 2-fold higher than observed, and predicted levels of PFOS in kidney were approximately 25% of observed. Fábrega et al. (2014) explored alternative values for tissue/plasma partition coefficients, determined from human autopsy issues (Maestri et al. 2006). The adjusted partition coefficients improved predictions of observed tissue PFOA and PFOS levels. Although the model could be applied to predicting plasma concentrations of PFOA and PFOS or intakes associated with specific plasma concentrations (e.g., oral MRLs), it is not clear what advantages the model offers over simpler empirical or compartmental models similarly calibrated to predict the serum $t_{1/2}$. The monkey model has been more thoroughly evaluated for predicting plasma and urinary kinetics of PFOA and PFOS. This was possible because of the availability of more extensive experimental data on plasma and urine PFOA and PFOS following intravenous and oral (single and repeated) dosing in male and female monkeys. Nevertheless, data on internal distribution were not available to allow evaluation of how well the monkey model predicts doses to the liver or other tissues. Predictions of plasma PFOA and PFOS concentrations from the monkey (and human) model were highly sensitive to values assigned to the maximum rate for tubular reabsorption (T_m) and other parameters that govern urinary elimination of PFOA and PFOS (e.g., free fraction in plasma and glomerular filtration rate; Loccisano et al. 2011). Optimization of the monkey models relied heavily on adjusting these same parameters and, for the human

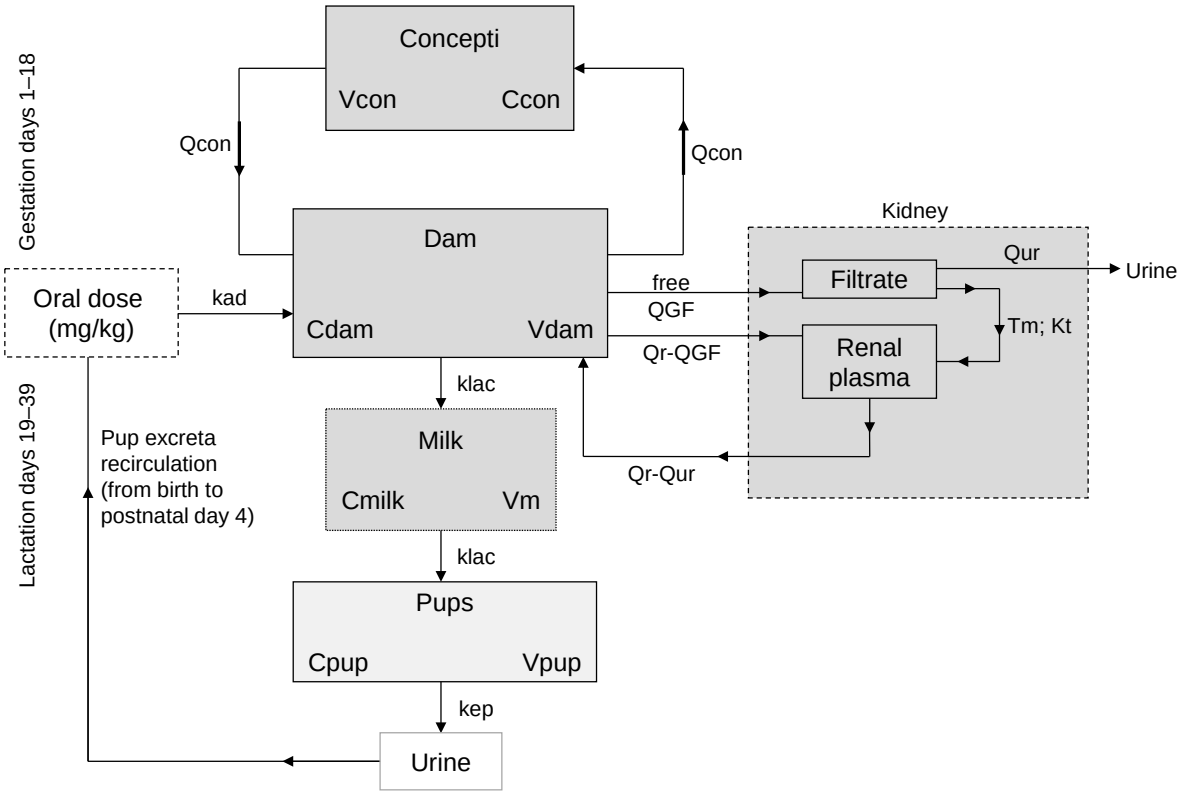
model, the target plasma elimination $t_{1/2}$ was achieved solely by adjusting T_m. Thus, despite the complexity of the models, their potential to accurately predict plasma elimination kinetics and, therefore, steady-state plasma concentrations and associated oral intakes, depends largely on how well they predict plasma clearance. If plasma clearance and the free-fraction in plasma can be reliably predicted empirically for the animal species of interest, then far simpler compartmental models can be used for dosimetry extrapolation of steady-state free plasma concentrations.

3.1.5.3 Rodriguez et al. (2009) Mouse Model

Rodriguez et al. (2009) developed a model for simulating the maternal-fetal and maternal-pup kinetics of PFOA in mice. The general structure of the model is depicted in Figure 3-7. Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Rodriguez et al. (2009). The maternal, fetal, and pup systems are simulated as single well-mixed compartments. Absorption from the gastrointestinal tract is simulated as first-order with complete absorption of the ingested dose. Elimination of absorbed PFOA from the maternal system is simulated as the balance between glomerular filtration and renal tubular reabsorption. The latter is represented as a saturable process with parameters T_m and K_T. Transfer to the fetus is flow-limited and governed by a fetus/maternal partition coefficient and placental blood flow. Transfer from the maternal system to the pup by lactation is simulated as first-order governed by a lactation transfer rate constant. Elimination of PFOA from the pup is first-order to urine. Data sets utilized in developing and evaluating the mouse model included oral gestational dosing studies.

Applications for Dosimetry Extrapolation and Risk Assessment. The model predicted observed concentrations of PFOA in maternal, fetal, and pup serum following oral gestational exposures to mice (Abbott et al. 2007; Lau et al. 2006; White et al. 2007). Residuals for predictions are presented, which provide a quantitative measure of how well the model predicted observations (Rodriguez et al. 2009). Similar to the rat, the mouse model predicts higher internal exposure (serum PFOA concentrations) in the maternal system compared to the fetus. It also predicts accelerated loss of PFOA from the maternal system during lactation. The model simulates the maternal, fetal, and pup systems as single compartments. Although this serves for simulating plasma concentrations (the main objective of the modeling effort), it does not allow for simulation of tissue levels of PFOA in the maternal system, fetus, or pup.

Figure 3-7. Renal Resorption Pharmacokinetic Model of Gestation and Lactation used in the Analysis of CD-1 Mice



Ccon = concentration in concepti; Cdam = concentration in dam; Cmilk = concentration in milk; Cpup = concentration in pup; kad = first-order absorption rate; kep = urinary excretion rate; klac = transfer rate via milk; Kt = half-saturation constant; Qcon = blood flow to and from placenta; QGF = glomerular filtrate; Qr = renal plasma flow; Qur = urine flow; Tm = transport maximum; Vcon = volume in concepti; Vdam = volume in dam; Vmilk = volume in milk; Vpup = volume in pup

Source: Rodriguez et al. 2009 (reproduced with permission of Elsevier Inc. in the format reuse in a government report via Copyright Clearance Center; Reproductive Toxicology by Reproductive Toxicology Center; Washington, DC)

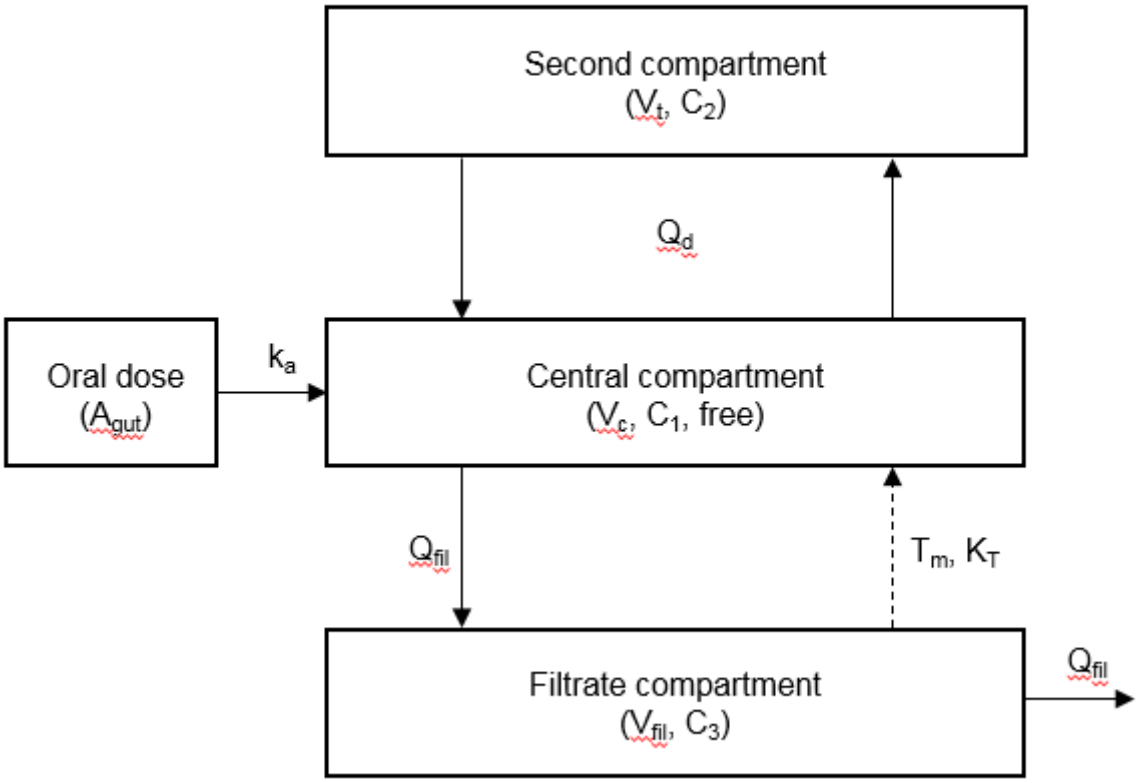
3.1.5.4. Wambaugh et al. 2013 (Andersen et al. 2006) Model

The Wambaugh et al. (2013) model is a three-compartment model based on the three-compartmental monkey model of Andersen et al. (2006). The structure of the two models are identical (Figure 3-8). Parameter values for the Wambaugh et al. (2013) model are presented in Table 3-7. The model includes a central compartment, a secondary distribution compartment, and a renal glomerular filtrate compartment. The central compartment (C1), which includes plasma, receives PFOA or PFOS from oral dosing (first-order k_a, $hour^{-1}$) and exchanges perfluoroalkyl with the secondary compartment (C2, which lumps all other tissues and distribution volumes into a single compartment) and with the glomerular filtrate (C3). A fraction of the perfluoroalkyl in C1 is free (Free) and available for exchange with C2 and C3. Exchanges between C1 and C2 are first order (k_{12}, k_{21}, $hour^{-1}$) with k_{21} assigned a value equal to the $R_{V2/V1}$, where $R_{V2/V1}$ is the ratio of the volumes of the two compartments (V_2/V_1). Transfer of perfluoroalkyl into the glomerular filtrate is first order and governed by the glomerular filtration rate (Q_{filc}, L/hour). Transfer for perfluoroalkyl from the glomerular filtrate to C1 (representing renal tubular reabsorption) is capacity limited (T_{maxc}, µmol/hr; K_T, µM). Perfluoroalkyl that is not reabsorbed is excreted.

Parameter values for the various species and strains were estimated from experimental pharmacokinetic data for each species and strain using Bayesian Markov Chain Monte Carlo (MCMC) analysis. Studies that provided data used to estimate parameter values are listed in Wambaugh et al. (2013). The parameter values shown in Table 3-7 are the mean values and posterior distributions (95% credible interval) from the MCMC analyses.

Applications for Dosimetry Extrapolation and Risk Assessment. Wambaugh et al. (2013) applied the model to predicting internal doses (mean and maximum serum concentrations and plasma AUC) for Benchmark Dose Software (BMDS) modeling and for comparing internal dosimetry from *in vivo* toxicity studies to estimates of potency (AC_{50}, maximum Efficacy) from *in vitro* studies. EPA applied the Wambaugh et al. (2013) model to deriving chronic oral reference doses (RfDs) for PFOA and PFOS (EPA 2016e, 2016f). The model was used to predict internal doses (time-integrated plasma PFOA or PFOS concentrations) achieved in toxicity studies conducted in various laboratory animal models (CD-1 mouse, C57Bl/6 mouse, Sprague-Dawley rat, Cynomolgus monkey). Plasma concentrations were then extrapolated to equivalent steady-state concentrations in humans using a model of first-order elimination of PFOA and PFOS from plasma. The same approach was used to derive MRLs for PFOA and PFOS (see Appendix A).

Figure 3-8. Andersen et al. (2006) Pharmacokinetic Model with Oral Absorption



A_{gut} is the amount of chemical in the gut; k_a is the first-order rate constant for absorption from the gut; Q_{fil} is the flow through the filtrate compartment; C_1, C_2, and C_3 are the chemical concentrations in the central, second, and filtrate compartments, respectively; V_c, V_t, and V_{fil} are the volumes of distribution of the central, second, and filtrate compartments; free is the free fraction of compound in the central compartment; Q_d is the flow between the central and second compartments; the saturable resorption process from the filtrate back into the central compartment is modeled with Michaelis-Menten kinetics, with a maximum rate $T_{maximum}$ and a half-maximum concentration K_T.

Reprinted from Wambaugh et al. (2013) by permission of Oxford University Press

Table 3-7. Estimated and Assumed Pharmacokinetic Parameters for the Modified Andersen et al. (2006) Model for PFOA and PFOS

		Parameter (units)										
Reference	Species	BW (kg)	Cardiac output[a] (L/hour/kg$^{0.74}$)	K_a (hour^{-1})	V_{cc} (L/kg)	k_{12} (hour^{-1})	$R_{V2:V1}$ (unitless)	T_{maxc} (µmole/hour)	K_T (µM)	Free (unitless)	Q_{filc} (L/hour)	V_{filc} (L/kg)
						PFOA[b]						
Lou et al. (2009)	Mouse: CD1 (F)	0.02	8.68	290 (0.6–73,000)	0.18 (0.16–2.0)	0.021 (3.1x10^{-10} to 3.8x10^{4})	1.07 (0.26–5.84)	4.91 (1.75–2.96)	0.037 (0.0057–0.17)	0.011 (0.0026–0.051	0.077 (0.015–0.58)	9.7x10^{-4} (3.34x10^{-9}–7.21)
Dewitt et al. (unpublished)	Mouse: C57Bl/6 (F)	0.02	8.68	340 (0.53–69,000)	0.17 (0.13–2.3)	0.35 (0.058–52)	53 (11–97)	2.7 (0.95–22)	0.12 (0.033–0.24)	0.034 (0.014–0.17)	0.017 (0.010–0.081)	7.6x10^{-5} (2.7x10^{-10}–6.4)
Kemper (2003)	Rat: Sprague-Dawley (F)	0.20 (0.16–0.23)[c]	12.39	1.7 (1.1–3.1)	0.14 (0.11–0.17)	0.098 (0.039–0.27)	9.2 (3.4–28)	1.1 (0.25–9.6)	1.1 (0.27–4.5)	0.086 (0.031–0.23)	0.039 (0.014–0.13)	2.6x10^{-5} (2.9x10^{-10}–28)
Kemper (2003)	Rat: Sprague-Dawley (M)	0.24 (0.21–0.28)[c]	12.39	1.1 (0.83–1.3)	0.15 (0.13–0.16)	0.028 (0.0096–0.08)	8.4 (3.1–23)	190 (5.5–50,000)	0.092 (3.4x10^{-4}–1.6)	0.08 (0.03–0.22)	0.22 (0.011–58)	0.0082 (1.3x10^{-8}–7.6)
Butenhoff et al. (2004b)	Monkey: Cynomolgus (M/F)	7 (m), 4.5 (f)	19.8	230 (0.27–73,000)	0.4 (0.29–0.55)	0.0011 (2.4x10^{-10} to 3.5x104)	0.98 (0.25–3.8)	3.9 (0.65–9,700)	0.043 (4.3x10^{-5}–0.29	0.01 (0.0026–0.038)	0.15 (0.02–24)	0.0021 (3.3x10^{-9}–6.9)
		Parameter (units)										
Reference	Species	BW[d] (kg)	Cardiac output[e] (L/hour/kg$^{0.74}$)	K_a (hour^{-1})	V_{cc} (L/kg)	k_{12} (hour^{-1})	$R_{V2:V1}$ (unitless)	T_{maxc} (µmol/hour)	K_T (µM)	Free (unitless)	Q_{filc} (L/hour)	V_{filc} (L/kg)
						PFOS						
Chang et al. (2012)	Mouse: CD1 (F)	0.02	8.68	1.16 (0.617–42,400)	0.264 (0.24–0.286)	0.0093 (2.63e-10–38,900)	1.01 (0.251–4.06)	57.9 (0.671–32,000)	0.0109 (1.44x10^{-5}–1.45)	0.00963 (0.00238–0.0372)	0.439 (0.0125–307)	0.00142 (4.4x10^{-10}–6.2)
Chang et al. (2012)	Mouse: CD1 (M)	0.02	8.68	433.4 (0.51–803.8	0.292 (0.268–0.317	2,976 (2.8e-10–4.2e4)	1.29 (0.24–4.09)	1.1e4 (2.1–7.9e4)	381 (2.6x10^{-5}–2,900)	0.012 (0.0024–0.038)	27.59 (0.012–283)	0.51 (3.5x10^{-10}–6.09)
Chang et al. (2012)	Rat: Sprague-Dawley (F)	0.203	12.39	4.65 (3.02–1,980)	0.535 (0.49–0.581)	0.0124 (3.1e-10–46 800)	0.957 (0.238–3.62)	1,930 (4.11–83,400)	9.49 (0.00626–11,100)	0.00807 (0.00203–0.0291)	0.0666 (0.0107–8.95)	0.0185 (8.2x10^{-7}–7.34)
Chang et al. (2012)	Rat: Sprague-Dawley (M)	0.222	12.39	0.836 (0.522–1.51)	0.637 (0.593–0.68)	0.00524 (2.86e-10–43,200)	1.04 (0.256–4.01)	1.34e-06 (1.65e-10–44)	2.45 (4.88x10^{-10}–60 300)	0.00193 (0.000954–0.00249)	0.0122 (0.0101–0.025)	0.000194 (1.48x10^{-9}–5.51)
Seacat et al. (2002) and Chang et al. (2012)	Monkey: Cynomolgus (M/F)	3.42	19.8	132 (0.225–72,100)	0.303 (0.289–0.314)	0.00292 (2.59e-10–34,500)	1.03 (0.256–4.05)	15.5 (0.764–4,680)	0.00594 (2.34 x10^{-5}–0.0941)	0.0101 (0.00265–0.04)	0.198 (0.012–50.5)	0.0534 (1.1x10^{-7}–8.52)

[a]Cardiac outputs obtained from Davies and Morris (1993).
[b]Means and posterior distributions from the Bayesian Markov Chain Monte Carlo (MCMC) analysis (95% credible interval in parentheses) are reported.
[c]Estimated average body weight (BW) for species used except with Kemper (2003) study where individual rat weights were available and assumed to be constant.
[d]Average BW for species: individual-specific BWs.
[e]Cardiac outputs obtained from Davies and Morris (1993).

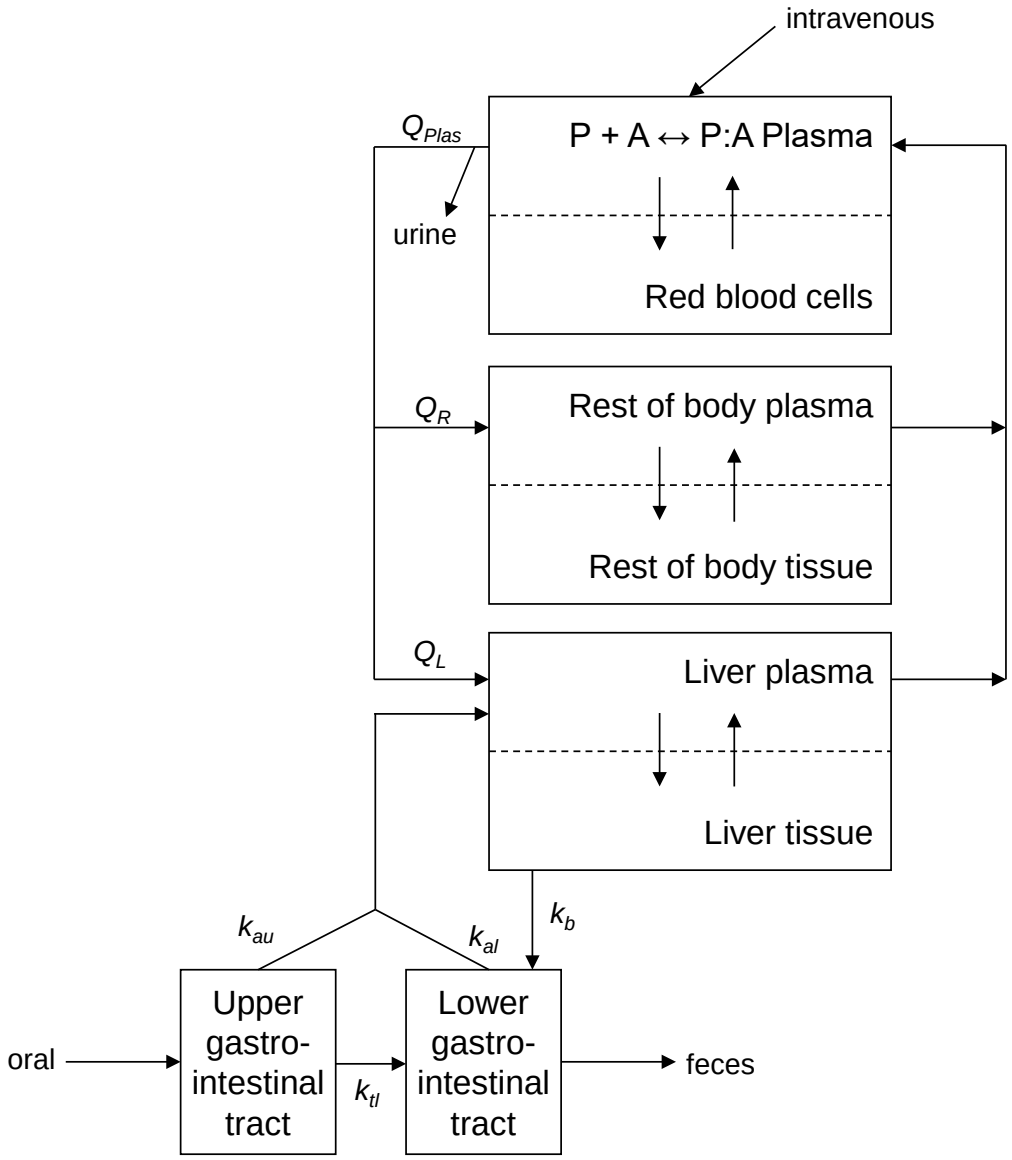
Source: Wambaugh et al. (2013)

3.1.5.5 Harris and Barton (2008) Rat Model

Harris and Barton (2008) developed a model for simulating PFOS kinetics in adult rats. The general structure of the model is depicted in Figure 3-9. Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Harris and Barton (2008). The model includes systemic compartments representing blood (including a bound and free fraction of plasma and red blood cells), liver, and a lumped compartment representing all other tissues. The gastrointestinal tract is simulated as separate compartments representing the upper and lower tracts. Absorption occurs from both the upper and lower tracts, with distinct first-order rate constants assigned to each. Biliary PFOS is transferred from liver to the lower tract. Absorbed PFOS is delivered to the liver where it enters plasma to be distributed to other tissues. Exchanges between PFOS in plasma and all tissues are assumed to be diffusion-limited, with the free pool in plasma participating in the exchange with red blood cells, and the total plasma pool exchanging with liver and all other tissues. Binding of PFOA to plasma albumin is assumed to be saturable, with a dissociation constant 10^{-7} M and a maximum capacity 4.1x 10^{-4} M. This is implemented by assigning bound PFOA to a subcompartment of plasma in which PFOA enters (binds) or exits (unbinds) at rates governed by binding *on* and *off* rates, respectively, that yield a dissociation constant of 10^{-7} M. Elimination of absorbed chemical occurs by biliary excretion and urinary excretion. Transfer from liver to the lower gastrointestinal tract (representing excretion following biliary transfer) is represented as a first-order process acting on the total amount of PFOS in liver. PFOA is transferred to urine from the free fraction of plasma at a rate governed by a urinary clearance parameter, which is assigned a value of 28% of renal plasma flow.

In evaluating performance of the model for simulating PFOS concentrations in a chronic rat feeding study, Harris and Barton (2008) found that the model predicted plasma and liver concentrations measured at 4 and 16 weeks, but over-predicted both at 104 weeks. Performance of the model was improved by having renal clearance increase and the liver/plasma partition coefficient decrease as a function of time (i.e., study duration). These results suggest the possibility that clearance of PFOS may be dependent on age and/or a metric of dose (e.g., cumulative internal dose). This may reflect age- or dose-related changes in kidney function, including tubular reabsorption or secretion of PFOS.

Figure 3-9. Conceptual Representation of a Physiologically Based Pharmacokinetic Model for PFOS Exposure in Rats



k_{al} = rate of absorption from the lower gastrointestinal tract; k_{au} = rate of absorption from the upper gastrointestinal tract; k_b = maximum rate of biliary elimination; k_{tl} = rate of transfer from upper-lower gastrointestinal tract; P:A = PFOS-bound albumin in plasma; PFOS = perfluorooctane sulfonic acid Q_L = plasma flow rate to the liver; Q_{Plas} = plasma flow rate by the heart; Q_R = plasma flow rate to the rest of body

Source: Harris and Barton 2008 (reproduced with permission of Elsevier Ireland Ltd. in the format reuse in a government report via Copyright Clearance Center; Toxicology Letters by European Societies of Toxicology)

Applications for Dosimetry Extrapolation and Risk Assessment. The model simulates kinetics of PFOS following oral or intravenous dosing in adult rats and includes several features that are different from other PBPK models of perfluoroalkyls. The Harris and Barton (2008) model includes a red cell compartment that allows predictions of whole-blood concentrations. The utility of this feature remains to be determined, since PFOS does not appreciably concentrate in red blood cells and PFOS (and other perfluoroalkyls) is typically monitored in the central compartment with measurements of plasma or serum concentrations. The model assumes that the total concentration of PFOS (not just the free concentration) in plasma is available for distribution to liver and other tissues, whereas other models assume that only the free pool in plasma exchanges with tissues. The practical consequence of this difference may not be significant in terms of the toxicokinetics of PFOS if the tissue/plasma partition coefficients in the various models were estimated based on the relevant perfluoroalkyl pool in plasma. However, without basing distribution kinetics on the free concentration, it is not possible for concentration-dependent free fraction to be modeled. The model assumes time-dependence in the liver uptake and urinary excretion of PFOS, which were needed to improve predictions of plasma and liver concentrations of PFOS during chronic exposures. Other rat models (Loccisano et al. 2012a) have not been similarly evaluated. A mechanistic understanding of the time-dependent changes in PFOS kinetics will be important for applications of these models for dosimetry extrapolation across exposure durations.

3.1.5.6 Worley and Fisher (2015a, 2015b) Rat Model

Worley and Fisher (2015a, 2015b) expanded the Loccisano et al. (2012a) adult rat model to include simulation of renal proximal tubule apical (tubule-lumen) and basolateral (tubule-plasma) PFOA transport. This configuration allowed the use of data from *in vitro* studies of kinetics of specific transporters thought to be involved in proximal tubular transport of PFOA in the parametrization of the model. The kidney compartment was expanded to include compartments representing the proximal tubule lumen (glomerular filtrate) and proximal tubule cells. In the model, transfer of PFOA to the tubule lumen is governed by the glomerular filtration rate, represented by a clearance parameter (L/hour/kg kidney). PFOA in the tubule lumen can undergo first-order transfer to urine or saturable transport into the tubule cell (Km, Vmax). PFOA in the tubule exchanges with PFOA in plasma by three mechanisms: saturable transport from plasma into the cell (Km, Vmax), first-order transport from the cell to plasma (kefflux), or bidirectional diffusion between the cell and plasma (kdif). Parameter values (Km, Vmax) for apical and basolateral transport of PFOA were derived from *in vitro* estimates for OATP1a1 (apical) and OAT1 and OAT3 (basolateral) (Nakagawa et al. 2008; Weaver et al. 2010; Yamada et al. 2007). These estimates were scaled to kidney proximal tubule cell mass (Hsu et al. 2014) and the mass-scaled estimates

of Vmax were adjusted with relative activity factors, which were calibrated to *in vivo* observations of plasma PFOA elimination kinetics in rats (Kemper 2003). Values for kefflux (proximal tubule cell to kidney plasma) and kdif (diffusion between kidney plasma and the tubule cell) were also calibrated with *in vivo* data (Kemper 2003; Kudo et al. 2007).

Calibration of the relative activity factor for apical and basolateral membrane transport of PFOA to serum observations made in male and female rats resulted in lower values for activity of both transporters in females compared to males. This resulted in the model predicting lower rates of reabsorptive transfer of filtered PFOA to plasma, and higher renal and systemic (plasma) clearance in females compared to males. Because proximal tubule transporters were assumed to be saturable, the model predicts an increase in clearance with increasing PFOA dose, with larger increases in clearance at lower doses in females compared to males. The model simulated the observed dose-dependent increase in serum clearance (decreasing serum $t_{1/2}$) and higher serum clearance of PFOA (lower $t_{1/2}$) in female rats compared to males (Kemper 2003).

3.1.5.7 Worley et al. (2017b) Human Model

Worley et al. (2017b) scaled and calibrated the Worley and Fisher (2015a, 2015b) rat model to simulate PFOA kinetics in humans exposed to PFOA in drinking water. Physiological parameters were allometrically scaled to the human. Tissue-plasma partition coefficients were derived from human autopsy data (kidney, liver) or studies of distribution of PFOA in rats (Fabrega et al. 2014; Kudo et al. 2007; Perez et al. 2013). Parameter values (Km, Vmax) for apical and basolateral transport of PFOA were derived from *in vitro* estimates for OAT4 (apical) and OAT1 and OAT3 (basolateral) (Nakagawa et al. 2008; Weaver et al. 2010; Yang et al. 2010; Yamada et al. 2007). These estimates were scaled to kidney proximal tubule cell mass (Hsu et al. 2014) and the mass-scaled estimates of Vmax were adjusted with relative activity factors. Parameters that control apical and basolateral transfers of PFOA in the proximal tubule and absorption in the gastrointestinal tract were calibrated against data on serum PFOA concentrations measured in people who drank water from a municipal water supply (Worley et al. 2017b). Model parameter values were adjusted to achieve agreement with geometric mean serum PFOA concentrations measured at two times separated by 6 years. The model was evaluated by comparing predicted and observed serum PFOA concentrations in populations exposed to PFOA in drinking water (Bartell et al. 2010; Emmett et al. 2006b; Steenland et al. 2009a, 2009b). A sensitivity analysis of the model identified that following biokinetic parameters that had standardized sensitivity coefficients >0.1: parameters controlling proximal tubule transport and urinary excretion, plasma-liver partition coefficient,

biliary excretion and protein binding. These parameters, along with drinking water consumption, were assigned probability distributions to conduct a Monte Carlo analysis of predicted serum PFOA predictions associated with exposures to PFOA in drinking water. The probabilistic model simulated interindividual variability in serum PFOA concentrations observed in exposed populations (Bartell et al. 2010; Emmett et al. 2006b; Steenland et al. 2009a, 2009b). These results suggest that that biokinetic variability, as well as exposure variability, may contribute to variability in serum PFOA concentrations observed in populations.

3.1.5.8 Fàbrega et al. (2014, 2016) Human Model

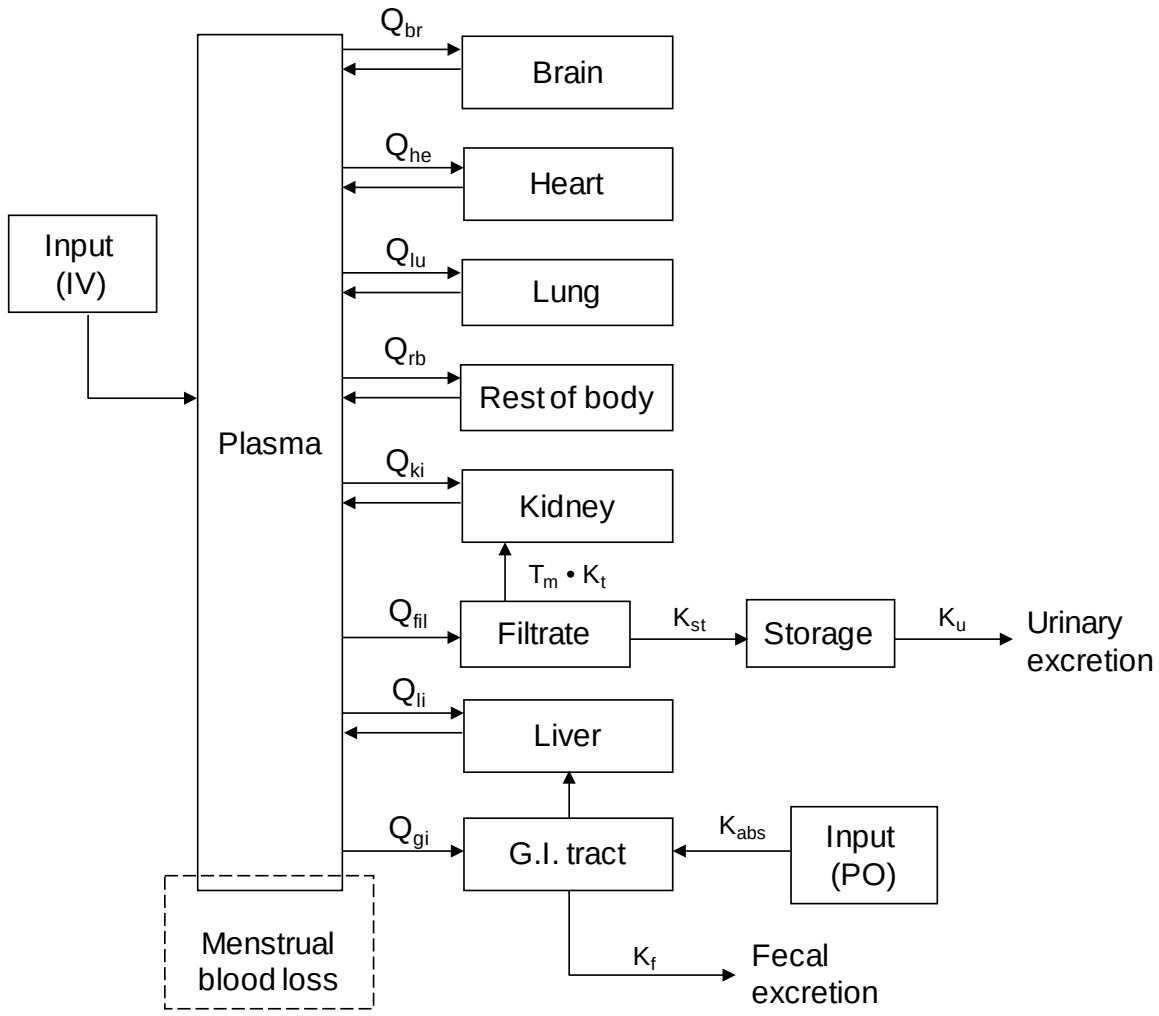
Fàbrega et al. (2014, 2016) modified the Loccisano et al. (2011, 2013) human models for PFOA and PFOS with inclusion of brain and lung compartments and removal of the skin compartment. Tissue-plasma partition coefficients were re-estimated using data from human cadavers (Maestri et al. 2006) in place of estimates based on rat data (Loccisano et al. 2011). The major differences in the partition coefficients for PFOA were lower values for liver in humans (1.03) compared to rats (2.20), higher values for fat in humans (0.47) compared to rats (0.04), and inclusion of partition coefficients for brain (0.17) and lung (1.27). For PFOS, the major differences in the partition coefficients were lower values for liver in humans (2.67) compared to rats (3.72) and higher values for fat in humans (0.33) compared to rats (0.14). Values for parameters that control urinary excretion (Tm and Km for reabsorptive transport from glomerular filtrate to kidney tissue) were recalibrated based on plasma concentration data (Ericson et al. 2007). Fàbrega et al. (2014) compared predictions to observed concentrations of PFOA and PFOS in cadaver samples (from Tarragona County, Spain) for constant intakes of 0.11 µg/day for PFOA or 0.13µg/day for PFOS. Better agreement with observations was achieved with partition coefficients based on cadaver data. Fàbrega et al. (2016) performed a quantitative uncertainty analysis of predictions of tissue PFOA and PFOS concentrations by assigning lognormal probability distributions to renal transport parameters, the unbound fraction in plasma, and intake. Probability distributions for PFOA and PFOS intakes were based on data from Domingo et al. (2012a, 2012b). Distributions for biokinetic parameters were established to achieve a coefficient of variation of 0.3 (Allen et al. 1996; Brochot et al. 2007; Sweeney et al. 2001). Observations of tissue PFOA and PFOS were within uncertainty bounds on predictions.

3.1.5.9 Kim et al. (2018) Rat and Human Model

Kim et al. (2018) developed a model for simulating the kinetics of PFHxS in rats and humans. The structures of the rat and human models are identical (Figure 3-10). Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Kim et al. (2018). The model includes compartments representing plasma (including a bound and free fraction), brain, gastrointestinal tract, heart, lung, kidney and renal glomerular filtrate, liver, and a lumped compartment representing all other tissues. A storage compartment receives PFHxS from the glomerular filtrate and is included in the model to simulate the time delay between elimination from plasma and appearance of PFHxS urine. Absorption from the gastrointestinal tract is simulated as the balance between first-order absorption and fecal excretion of unabsorbed PFHxS. Absorbed PFHxS is assumed to be delivered to the liver. Exchanges between PFHxS in tissues with the free pool in plasma are assumed to be flow-limited (governed by blood flow) with equilibrium determined by the tissue:plasma partition coefficient. Partition coefficients were estimated from the tissue:plasma concentration ratios measured in female and male rats 14 days after a single intravenous dose of PFHxS (0.5–10 mg/kg). Values for each sex were significantly different for brain, lung, liver, spleen, gastrointestinal tract, adipose, and skeletal muscle; in each case, male>female. The highest partition coefficient was in male liver (approximately 0.13), with the value for female being approximately half of the male value. PFHxS in plasma is simulated as instantaneous distributions into free and bound fractions. The free fraction was estimated from ultrafiltration studies of rat and human plasma. The free fraction was assigned a constant of 0.069% in female and 0.076% in male rats.

Elimination of absorbed PFHxS in the rat model occurs by fecal and urinary excretion. Fecal excretion of absorbed PFHxS is represented as flow-limited transfer from plasma to the gastrointestinal tract and first order transfer from the gastrointestinal tract to feces. Excretion in urine is simulated as the balance between transfer from the free fraction of plasma to the glomerular filtrate and renal tubular reabsorption, which removes PFHxS from the glomerular filtrate and returns it to kidney tissue. Renal tubular reabsorption is simulated as a capacity-limited process with parameters T_m (µg/hour), representing the maximum rate of transport, and K_T (µg/L), representing affinity for the transporter (the concentration in the glomerular filtrate at which reabsorptive transport rate is half of maximum). This representation of renal tubular reabsorption is used to simulate observed sex differences in elimination of PFHxS from

Figure 3-10. Structure of the PBPK Model for PFHxS in Rats and Humans*



*PFHxS can be resorbed into the kidney with transporter maximum (T_m) and transporter affinity constant (K_t). Ks indicates a rate constant; K_{st}, the rate constant to the storage compartment; K_u, the urinary elimination rate constant; K_f, the transfer rate constant from the G.I. tract to fecal elimination; and K_{abs}, the oral absorption rate constant. Q_s refers to the blood flows between plasma and tissues, except for Q_{fil}, which is a clearance from the plasma to the filtrate compartment. Menstrual blood loss (dotted square) is only applicable to female humans.

G.I. = gastrointestinal; IV = intravenous; PBPK = physiologically based pharmacokinetic; PFHxS = perfluorohexane sulfonic acid; PO = *per os*

Source: Reprinted by permission from Springer Nature, Kim et al. 2018

plasma, which have been attributed to higher reabsorptive capacity in male rats (see Section 3.1.4). Values for the maximum and affinity parameters for PFHxS result in higher reabsorptive clearances from the glomerular filtrate (T_m/K_T=5.2) in male rats compared to female rats (T_m/K_T=0.057), and correspondingly lower urinary clearance of PFHxS from plasma in male rats. Values for T_m and K_t in humans were assumed to be the same as those in rats. Tissue volumes and blood flows were assigned values based on various sources (Davies and Morris 1993; Igari et al. 1983). Glomerular filtrate volume and flow were assigned values from Loccisano et al. (2012a).

The rat model was calibrated and evaluated against data on plasma and tissue levels of PFHxS measured following a single intravenous (0.5–10 mg/kg) or gavage dose (1 or 4 mg/kg) of PFHxS (Kim et al. 2018). Temporal profiles of plasma PFHxS and cumulative urinary excretion following intravenous or oral dosing were within ±1 SD of observations. Predicted cumulative urinary excretion of PFHxS reproduced the observed sex differences in urinary excretion with slower excretion and higher plasma levels in males compared to females. Terminal levels of PFHxS in heart, kidney, liver, and lung predicted for 14 days following oral dosing were within the range of observed values.

The human model was developed from the rat model with the following attributes:

- Human tissue volumes and blood flows were assigned values based on various sources (Davies and Morris 1993; Igari et al. 1983).
- Glomerular filtrate volume and flow were assigned values from Loccisano et al. (2011).
- Values for the free fraction in human plasma were 0.023% in females and 0.025% in males, based on results from ultrafiltration studies.
- Sex-specific values for renal tubular reabsorption parameters, T_m and K_t, were assumed to be the same in rats and humans.
- First order rate constants were scaled by 0.25 power of body weight ($BW^{0.25}$).
- Loss of PFHxS in menstrual blood was included in the human female model. This is represented as a direct loss of loss of 42.5 mL blood (25 mL plasma) per month (Verner and Longnecker 2015).

Kim et al. (2018) does not report an evaluation of the human model.

Applications for Dosimetry Extrapolation and Risk Assessment. The rat and human models were applied interspecies dosimetry extrapolation of a rat NOAEL for PFHxS (1 mg/kg/day). The rationale for the rat NOAEL is described in Kim et al. (2018). The dosimetry extrapolation was applied to the rat

model to predict a steady-state plasma concentration of PFHxS corresponding to a chronic oral dose of 1 mg/kg/day (value not reported). The equivalent human dose was predicted from the human PBPK model as the daily dose required to achieve the same steady state concentration in the human.

3.1.6 Animal-to-Human Extrapolations

Interspecies differences in the toxicokinetics of perfluoroalkyls and possible differences in the mechanisms of toxicity have been found. The elimination rate for PFOA in female rats is approximately 45 times faster than in male rat, 150 times faster than in Cynomolgus monkeys, and approximately 5,000–9,000 times faster than in humans (Bartell et al. 2010; Butenhoff et al. 2004c; Kemper 2003; Olsen et al. 2007a). Elimination of PFOS in male rats is approximately 3 times faster than in Cynomolgus monkeys and approximately 40 times faster than in humans (Chang et al. 2012; De Silva et al. 2009; Olsen et al. 2007a; Seacat et al. 2002). These large differences in elimination rates imply that similar external PFOA or PFOS dosages (i.e., mg/kg/day) in rats, monkeys, or humans would be expected to result in substantially different steady-state internal doses (i.e., body burdens, serum concentrations) of these compounds in each species. In addition, exposure durations required to achieve steady state would be expected to be much longer in humans than in monkeys or rats. Assuming a terminal elimination $t_{1/2}$ of 1,400 days for PFOA in humans (Olsen et al. 2007a), a constant rate of intake for 17 years would be required to achieve 95% of steady state. Steady state (i.e., 95%) would be achieved in approximately 110 days in monkeys ($t_{1/2}$=25 days, Butenhoff et al. 2004c), 30 days in male rats ($t_{1/2}$=7 days; Kemper 2003), and 1 day in female rats ($t_{1/2}$=0.2 days; Kemper 2003). Using an internal dose metric such as serum perfluoroalkyl concentration and PBPK models that can account for these differences in elimination rates can decrease the uncertainty in extrapolating from animals to humans.

The mode of action for most health outcomes associated with perfluoroalkyl exposure has not been fully characterized in humans or laboratory animals. Some perfluoroalkyl-induced effects observed in rats and mice appear to be mediated through the PPARα-dependent and -independent mechanisms (see Section 2.20 for additional information). Interpretation of the relevance of the effects observed in laboratory animals is complicated since it is generally agreed that humans and nonhuman primates are refractory, or at least less responsive than rodents, to PPARα-mediated effects (Corton et al. 2014; Klaunig et al. 2003; Maloney and Waxman 1999). While studies in mice have identified specific effects that require PPARα activation, for example, postnatal viability (Abbott et al. 2007) and some immunological effects (Yang et al. 2002b), other effects such as hepatomegaly and antigen-specific antibody response (DeWitt et al. 2016) were reported to be PPARα-independent (Yang et al.

2002b). Therefore, further studies are needed to expand the knowledge regarding PPARα-dependent and -independent effects that would allow selection of an appropriate animal model for perfluoroalkyls toxicity. In the absence of data to the contrary, ATSDR assumes that the health effects observed in laboratory animals are relevant to humans. The exception is some of the hepatic effects observed in rodents; increases in liver weight and hepatocellular hypertrophy observed in rats and mice were considered adaptive and not relevant to humans (see Section 2.9 for details).

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to perfluoroalkyls are discussed in Section 5.7, Populations with Potentially High Exposures.

The possible association between serum perfluoroalkyl levels in children and health effects has been examined in participants of the C8 Health Project and in the general population. The studies examined a number of health effects including alterations in serum lipid levels, adverse renal outcomes, neurodevelopmental alterations, and reproductive development. Immunotoxicity has been examined in children in several general population studies. Additionally, a large number of studies have examined the possible association of elevated serum perfluoroalkyl levels and adverse birth outcomes.

Similar to adults, associations between serum PFOA and PFOS and serum cholesterol levels were observed in a study of over 12,000 children (Frisbee et al. 2010); an increased risk of high cholesterol was also observed in children with higher serum PFOA and PFOS levels. A smaller study of children (n=43)

living in the Mid-Ohio Valley did not find associations between serum PFOA levels and hematology parameters, total cholesterol and liver enzymes, indices of kidney function, or serum TSH levels (Emmett et al. 2006b). Another study of highly-exposed residents did not find any associations between serum PFOA levels in children aged 6–12 years and IQ, reading and math skills, language, memory, learning, or attention (Stein et al. 2013). Similarly, no association between serum PFOA, PFOS, or PFNA levels in children 5–18 years old and the likelihood of ADHD diagnosis was observed in a study of highly-exposed residents, although the study did find an increased risk associated with higher PFHxS levels (Stein and Savitz 2011). A general population study that utilized the NHANES data found an association between serum PFOA, PFOS, and PFHxS levels and the risk of ADHD diagnosis (as reported by the parent) (Hoffman et al. 2010). Another smaller-scale study found associations between serum PFOS, PFNA, PFDA, PFHxS, and FOSA and impulsivity; no association with PFOA was found (Gump et al. 2011). A study of children 8–18 years of age participating in the C8 studies found reduced odds of reaching puberty at higher serum PFOA levels (Lopez-Espinosa et al. 2011); however, the biological significance of the short delay (4–5 months) is not known.

Several studies have evaluated immunotoxicity in children and adolescents. These studies have found impaired antibody responses associated with serum PFOA, PFOS, PFHxS, and PFDA (Grandjean et al. 2012, 2017; Granum et al. 2013; Mogensen et al. 2015a; Stein et al. 2016a). An increased asthma diagnosis was also associated with serum PFOA levels (Dong et al. 2013; Humblet et al. 2014; Zhu et al. 2016). Marginal evidence of an association with asthma diagnosis was also found for PFOS, PFHxS, PFNA, PFDA, PFBS, and PFDoDA (Dong et al. 2013; Zhu et al. 2016), although some studies found no associations for these compounds (Humblet et al. 2014; Smit et al. 2015; Stein et al. 2016a).

Hines et al. (2009) showed that *in utero* exposure (GDs 1–17) to low levels of PFOA (0.01–0.3 mg/kg/day) resulted in increases in body weight gain in 10–40-week-old mice; by 18 months of age, the body weights in these mice were similar to controls. Increases in serum insulin and leptin levels were also observed in the mice exposed to 0.01 and 0.1 mg/kg/day. The study also compared body weight and body composition of *in utero* exposed mice (exposed on GDs 1–17) and adult exposed mice (exposed for 17 days starting at 8 weeks of age) and found that *in utero* exposure to 1 mg/kg/day resulted in significantly higher body weight, brown fat weight, and white fat weight; this was not observed in mice exposed to 5 mg/kg/day. The results of the study suggest that gestational exposure to low doses of PFOA may result in increased susceptibility to PFOA toxicity.

A number of studies of highly exposed residents and the general population have examined the potential associations between serum perfluoroalkyl levels and alterations in birth weight. Decreases in birth weight have been found to be associated with higher PFOA (Fei et al. 2007; Lee et al. 2013; Maisonet et al. 2012; Savitz et al. 2012b) or PFOS levels (Maisonet et al. 2012), but not with lower levels of perfluoroalkyls (Fei et al. 2007; Hamm et al. 2010; Inoue et al. 2004; Kim et al. 2011; Monroy et al. 2008; Washino et al. 2009; Whitworth et al. 2012b). The decreases in birth weight were small (<20 g or 0.7 ounces per 1 ng/mL). Additionally, no increases in the risk of low birth weight infants were found in highly exposed populations (Darrow et al. 2013; Nolan et al. 2009; Savitz et al. 2012b; Stein et al. 2009). No apparent alterations in the risk of birth defects were found in C8 Health Studies (Darrow et al. 2013; Savitz et al. 2012b; Stein et al. 2009) or in another study of these communities (Nolan et al. 2009).

The developmental toxicity of PFOA and PFOS has been investigated in a number of rat and mouse studies. The observed effects include PFOA- and PFOS-induced increases in prenatal losses and decreases in pup survival, decreases in pup body weight, and neurodevelopmental toxicity (Abbott et al. 2007; Albrecht et al. 2013; Case et al. 2001; Chen et al. 2012b; Era et al. 2009; Fuentes et al. 2006, 2007a, 2007b; Grasty et al. 2003; Hu et al. 2010; Johansson et al. 2008; Lau et al. 2003, 2006; Luebker et al. 2005a, 2005b; Onishchenko et al. 2011; Thibodeaux et al. 2003; White et al. 2007, 2009, 2011; Wolf et al. 2007; Xia et al. 2011; Yahia et al. 2008, 2010). Additionally, delays in mammary gland development were observed in mice exposed to PFOA (Macon et al. 2011; White et al. 2007, 2009, 2011). A limited number of developmental endpoints have been examined in rats and mice exposed to PFDA, PFHxS, or PFBA (Butenhoff et al. 2009a; Das et al. 2008; Harris and Birnbaum 1989; Johansson et al. 2008; Viberg et al. 2013). A more in-depth discussion of the developmental toxicity of perfluoroalkyls in animals is included in Section 2.17.

PFOA and PFOS, as well as other perfluoroalkyls, are valid biomarkers of exposure to these compounds in children, as they are in adults. No relevant studies were located regarding interactions of perfluoroalkyls with other chemicals in children or adults.

No studies examining increased susceptibility to the toxicity of perfluoroalkyls were identified. The available epidemiological data identify several potential targets of toxicity of perfluoroalkyls, and individuals with pre-existing conditions may be unusually susceptible. For example, it appears that exposure to PFOA or PFOS can result in increases in serum lipid levels, particularly cholesterol levels. Thus, an increase in serum cholesterol may result in a greater health impact in individuals with high levels of cholesterol or with other existing cardiovascular risk factors. Associations have been found between

PFOA and PFOS levels and an increased risk of hypertension/pre-eclampsia in pregnant women. The liver has been shown to be a sensitive target in a number of animal species and there is some indication that it is also a target in humans. Therefore, individuals with compromised liver function may represent a susceptible population. Likewise, individuals with a compromised immune system may have an increased risk of perfluoroalkyl-induced immunotoxicity.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to perfluoroalkyls are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for perfluoroalkyls from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by perfluoroalkyls are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the

biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Measurement of serum or whole-blood perfluoroalkyl concentrations is the standard accepted biomarker of perfluoroalkyl exposure in humans. Perfluoroalkyls have been detected in the serum of workers, residents living near perfluoroalkyl facilities, and the general population. As part of NHANES, CDC has been measuring serum levels of perfluoroalkyls in the U.S. general population since 1999. Of the 12 perfluoroalkyls examined in this toxicological profile, blood concentrations of 7 compounds (PFOA, PFOS, PFDA, PFHxS, PFNA, and PFUnA) were detected in enough subjects to allow for estimation of the geometric mean. As compared to the general population, serum PFOA and PFOS levels are much higher in individuals with occupational exposure to these compounds (Olsen et al. 2003a; Sakr et al. 2007a) and PFOA levels are much higher in individuals living near a PFOA manufacturing facility (Emmett et al. 2006a; Steenland et al. 2009a), suggesting that serum levels are a good biomarker of exposure. Due to the long half-life of some perfluoroalkyls, particularly PFOA and PFOS, elevated serum levels may not be indicative of recent exposure. Although elevated serum levels are likely to be indicative of exposure to the parent compound, their presence in blood can also indicate exposure to other perfluoroalkyls. For example, PFOS can be derived from metabolism of FOSA (Olsen et al. 2005; Seacat and Luebker 2000). PFOA can be derived from metabolism of 8-2 fluorotelomer alcohol (Fasano et al. 2006; Henderson and Smith 2007; Kudo et al. 2005; Nabb et al. 2007). Exposure of mice to 8–2 telomer alcohol also generated PFNA as a metabolite (Kudo et al. 2005). Most epidemiological studies measured serum perfluoroalkyl levels as a biomarker of exposure. In general, these studies provided a one-time serum perfluoroalkyl level, but lacked information on actual environmental exposure concentrations or doses, route of exposure, and exposure duration. The differences in elimination half-lives between perfluoroalkyls also confounds the interpretation of one-time measurements; the relative concentration of the perfluoroalkyls measured in serum may not be reflective of the actual mixture to which the individual was exposed.

Two studies have also evaluated the use of perfluoroalkyl levels in hair as a biomarker of exposure. In rats administered PFOA, PFOS, or PFNA in the drinking water for 90 days, significant correlations between hair perfluoroalkyl levels and serum and tissue (liver, heart, lung, kidney) levels were found, suggesting that hair perfluoroalkyl levels may be a reliable biomarker of exposure (Gao et al. 2015). A study in humans (Alves et al. 2015) has also found detectable levels of PFBA, PFHxA, PFOA, PFBS, and

PFHxS in hair samples, but PFHpA, PFNA, and PFOS were not detected in hair samples. The study did not evaluate the potential relationship between serum perfluoroalkyl levels and hair levels, which does not allow for an assessment of whether hair is a viable biomarker of exposure.

Urinary perfluoroalkyl levels have also been evaluated as a biomarker of exposure (Worley et al. 2017a). A study of highly exposed residents measured urinary PFOA, PFOS, PFNA, and PFHxS levels. With the exception of PFOA, the proportion of values below the detection limit was too high to calculate mean or median values. The study found a strong linear correlation between serum PFOA levels and urinary PFOA levels in men and a nonsignificant weak correlation between serum and urinary PFOA levels in women.

3.3.2 Biomarkers of Effect

There are no specific biomarkers of effect caused by perfluoroalkyls.

3.4 INTERACTIONS WITH OTHER CHEMICALS

There are limited data on the interactions of perfluoroalkyls with other chemicals. Particularly absent are studies examining toxicological and toxicokinetic interactions of a perfluoroalkyl with other perfluoroalkyls. Olestra decreased the absorption of PFOA from the gastrointestinal tract of mice (Jandacek et al. 2010). No additional information was located regarding interactions among chemicals of this class or between perfluoroalkyls and other chemicals. Both PFOA and PFOS (and many other diverse chemicals) can activate the PPARα, as well as other PPARs to a lesser extent (Takacs and Abbott 2007; Vanden Heuvel et al. 2006). Therefore, it is not unreasonable to speculate that interactions at the receptor level might occur; however, there are no experimental data to support or rule out this presumption. PPARα-independent mechanism are also involved in the toxicity of perfluoroalkyls and interactions between compounds are also likely to influence these mechanisms.

Attachment 6

An official website of the United States government
Here's how you know

Drinking Water Health Advisories for PFOA and PFOS

Additional PFOA and PFOS Information

- General about PFOA, PFOS and Other PFAS <https://epa.gov/pfas>
- PFOA and PFOS UCMR data <https://epa.gov/dwucmr>
- Laboratory Method 537 Q&A <https://epa.gov/node/204271>
- EPA programs
  - TSCA related <https://epa.gov/assessing-and-managing-chemicals-under-tsca/and-polyfluoroalkyl-substances-pfass-under-tsca>
  - Research Activities <https://epa.gov/chemical-research/perfluorinated-chemical-pfc-research>
- Federal partners
  - ATSDR EXIT <https://www.atsdr.cdc.gov/pfas/>
- En español: Avisos de salud sobre el PFOA y PFOS en el agua potable <http://espanol.epa.gov/espanol/avisos-de-salud-sobre-el-pfoa-y-pfos-en-el-agua-potable>

Health Advisories

EPA has established health advisories for PFOA and PFOS based on the agency's assessment of the latest peer-reviewed science to provide drinking water system operators, and state, tribal and local officials who have the primary responsibility for overseeing these systems, with information on the health risks of these chemicals, so they can take the appropriate actions to protect their

residents. EPA is committed to supporting states and public water systems as they determine the appropriate steps to reduce exposure to PFOA and PFOS in drinking water. As science on health effects of these chemicals evolves, EPA will continue to evaluate new evidence.

To provide Americans, including the most sensitive populations, with a margin of protection from a lifetime of exposure to PFOA and PFOS from drinking water, EPA has established the health advisory levels at 70 parts per trillion.

What's a health advisory?

Health advisories provide information on contaminants that can cause human health effects and are known or anticipated to occur in drinking water. EPA's health advisories are non-enforceable and non-regulatory and provide technical information to states agencies and other public health officials on health effects, analytical methodologies, and treatment technologies associated with drinking water contamination. EPA's health advisory level for PFOA and PFOS offers a margin of protection for all Americans throughout their life from adverse health effects resulting from exposure to PFOA and PFOS in drinking water.

What health effects are the basis for the health advisories?

EPA's health advisories are based on the best available peer-reviewed studies of the effects of PFOA and PFOS on laboratory animals (rats and mice) and were also informed by epidemiological studies of human populations that have been exposed to perfluoroalkyl substances (PFASs). These studies indicate that exposure to PFOA and PFOS over certain levels may result in adverse health effects, including developmental effects to fetuses during pregnancy or to breastfed infants (e.g., low birth weight, accelerated puberty, skeletal variations), cancer (e.g., testicular, kidney), liver effects (e.g., tissue damage), immune effects (e.g., antibody production and immunity), thyroid effects and other effects (e.g., cholesterol changes). There is limited information identifying health effects from inhalation or dermal exposures to PFOA or PFOS in humans and animals. To learn more about the underlying studies for the health advisories, see EPA's Health Effects Support Documents for PFOA and PFOS. <https://epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos>

- **Read more questions and answers** <https://epa.gov/node/251099>

Basic Information

- Fact Sheet on PFOA and PFOS Drinking Water Health Advisories (November 2016) <https://epa.gov/node/149925/>
    - HOJA INFORMATIVA Presencia de PFOA y PFOS en el agua potable Avisos de salud <https://espanol.epa.gov/espanol/hoja-informativa-presencia-de-pfoa-y-pfos-en-el-agua-potable-avisos-de-salud>
- EPA memorandum "Clarification about the Appropriate Application of the PFOA and PFOS Drinking Water Health Advisories <https://epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos>" (November 2016)

Technical Information

- FR Notice on the Health Advisories for PFOA and PFOS (May 25, 2016) EXIT <https://www.gpo.gov/fdsys/pkg/fr-2016-05-25/pdf/2016-12361.pdf>
- 2016 PFOA Health Advisory <https://epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos>
- 2016 PFOA Health Effects Support Document <https://epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos>
- 2016 PFOS Health Advisory <https://epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos>
- 2016 PFOS Health Effects Support Document <https://epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos>
- 2016 EPA Response to Peer Review Comments <https://epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos>

Provisional Health Advisories and Draft Health Effects Documents

Technical documents

- 2009 Provisional Health Advisory <https://epa.gov/sdwa/provisional-health-advisories-perfluorooctanoic-acid-pfoa-and-perfluorooctane-sulfonate-pfos>
- 2014 Draft Health Effects Document for Perfluorooctanoic Acid (PFOA) EXIT
- 2014 Draft Health Effects Document for Perfluorooctane Sulfonate (PFOS) EXIT
- Peer Reviewer Summary Report: External Peer Review of EPA’s Draft Health Effects Documents for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) EXIT

Peer Review

- Contractor Led Peer Review <https://epa.gov/ground-water-and-drinking-water/>

- Ground Water and Drinking Water Home <https://www.epa.gov/ground-water-and-drinking-water>
- Basic Information <https://www.epa.gov/ground-water-and-drinking-water/drinking-water-your-home>
- Private Wells <https://www.epa.gov/privatewells>
- Consumer Confidence Reports <https://www.epa.gov/ccr>
- Regulatory Requirements <https://www.epa.gov/dwreginfo>
- Standards and Regulations <https://epa.gov/sdwa>
- All Drinking Water Topics <https://epa.gov/ground-water-and-drinking-water/drinking-water-topics>
- Safe Drinking Water Information System
- For Students and Teachers <https://www.epa.gov/ground-water-and-drinking-water/drinking-water-activities-kids-and-teachers>

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Attachment 7

OPEN ACCESS

ORIGINAL ARTICLE

Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water

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ABSTRACT

Background Municipal drinking water contaminated with perfluorinated alkyl acids had been distributed to one-third of households in Ronneby, Sweden. The source was firefighting foam used in a nearby airfield since the mid-1980s. Clean water was provided from 16 December 2013.

Objective To determine the rates of decline in serum perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), and their corresponding half-lives.

Methods Up to seven blood samples were collected between June 2014 and September 2016 from 106 participants (age 4–84 years, 53% female).

Results Median initial serum concentrations were PFHxS, 277 ng/mL (range 12–1660); PFOS, 345 ng/mL (range 24–1500); and PFOA, 18 ng/mL (range 2.4–92). The covariate-adjusted average rates of decrease in serum were PFHxS, 13% per year (95% CI 12% to 15%); PFOS, 20% per year (95% CI 19% to 22%); and PFOA, 26% per year (95% CI 24% to 28%). The observed data are consistent with a first-order elimination model. The mean estimated half-life was 5.3 years (95% CI 4.6 to 6.0) for PFHxS, 3.4 years (95% CI 3.1 to 3.7) for PFOS and 2.7 years (95% CI 2.5 to 2.9) for PFOA. The interindividual variation of half-life was around threefold when comparing the 5th and 95th percentiles. There was a marked sex difference with more rapid elimination in women for PFHxS and PFOS, but only marginally for PFOA.

Conclusions The estimated half-life for PFHxS was considerably longer than for PFOS and PFOA. For PFHxS and PFOS, the average half-life is shorter than the previously published estimates. For PFOA the half-life is in line with the range of published estimates.

What this paper adds

- Limited information on the elimination of perfluorinated alkyl acids in humans after end of exposure has suggested half-lives of several years.
- This study provides refined estimates of half-lives of perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) from a highly exposed general population after end of exposure. There is substantial interindividual variability and slower excretion for men than women, for PFHxS and PFOS.
- Future research to understand the determinants of elimination is needed in order to guide risk assessment and regulatory measures for perfluorinated chemicals.

INTRODUCTION

Perfluorinated and polyfluorinated substances (PFASs) comprise a group of many different synthetic substances that have been produced and widely used for approximately 50 years. They are found in industrial applications and household products mainly due to their properties of withstanding heat, oil, dirt and water. PFASs are also used as surfactants in firefighting foam of the aqueous film forming foam (AFFF) type.[1]

In the general population, the dominating sources of exposure are through diet and consumer products.[2] However, during the past decade it has become apparent that localised PFAS contamination to surface and groundwater occurs around military and civilian firefighting training facilities, where large quantities of AFFF foams have been used. These substances are further disseminated by means of groundwater flows, and may also reach drinking water wells.

PFASs are excreted via urine and faeces. In animals half-lives (T½) for PFASs vary markedly between species and are usually much shorter than in humans, with elimination half-life counted in hours or days.[3] Reabsorption by organic anion transporters (OATs) in the kidneys and extensive uptake from enterohepatic circulation for PFASs are believed to be more active processes in humans, slowing down the excretion of these substances. In observational studies, based on observations in individuals followed over time, T½ between 2 and 3 years was reported for perfluorooctanoic acid (PFOA), while longer half-lives for perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS), 4 and 7 years, respectively, have been observed (table 1). Time-trend general population studies during periods of observed decay have reported half-lives in similar ranges.

However, it should be noted that the interindividual variation in elimination of PFASs can be substantial in both high and low exposure ranges, as observed in retired fluorochemical workers and after drinking water exposure.[4 5] Observational data and pharmacokinetical modelling indicate that PFAS half-life is likely to be shorter in women, explained partly by menstrual blood losses, but there may also be other sex-specific elimination mechanisms.[6] Except for perfluorobutane sulfonic

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Table 1 Half-lives for PFOS, PFHxS and PFOA in longitudinally followed humans

Reference	Setting	Model	Subjects	Initial PFAS level (ng/mL, serum)	Half-life (years)		
					PFOS	PFHxS	PFOA
Olsen *et al*[4]	Retired fluorochemical workers, followed 5 years Repeated samplings with batch-wise analysis	First-order elimination First and last sample	22 men, 2 women Age 55–75	Median (range) PFOS: 626 (145–3490) PFHxS: 193 (16–1295) PFOA: 408 (72–5100)	Median 4.6, range 2.4–21.7 GM 4.8 95% CI 4.0 to 5.8	Median 7.1, range 2.2–27.0 GM 7.3 95% CI 5.8 to 9.2	Median 3.4, range 1.5–9.1 GM 3.5 95% CI 3.0 to 4.1
Brede *et al*[5]	Drinking water exposure to PFOA, follow-up 2 years after installation of charcoal filters	First-order elimination First and last sample	20 children 22 mothers 23 men	Median (range) PFOS: ≈9 (2.6–33.3) PFHxS: ≈2.0 (<0.1–2.7) PFOA: ≈24 (6.4–77.5)	(Relative reduction 2006–2008, 22% in women, 25% in men)	(Relative reduction 2006–2008, 30% in women, 14% in men)	GM 3.26 years (range 1.03–14.67) (relative reduction 2006–2008, 39% in women, 25% in men)
Bartell *et al*[12]	Drinking water exposure to PFOA, follow-up after installation of charcoal filter Repeated sampling, follow-up after 1 year	First-order elimination Mixed models, five samples per person	100 men 100 women Age 53±15	Mean, SD PFOA 180±209	–	–	Average 2.3 95% CI 2.1 to 2.4 No sex-dependence
Gomis *et al*[13]	Ski waxers, followed after marked reduction of occupational exposure		4 men	Range 250–1050	–	–	Median 2.4, range 2.0–2.8
Worley *et al*[11]	Drinking water exposure to PFAS, emanating from contaminated sewage sludge applied to agricultural fields; follow-up after 6 years	One-compartment pharmacokinetic model First and last sample	First sample: 63 men, 90 women Average 52 Last sample: 22 men, 23 women	GM PFHxS: 6.4 PFOS: 39.8 PFOA: 16.3	Average 3.3	Average 15.5	Average 3.9

PFHxS, perfluorohexane sulfonate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Table 2 Perfluorinated and polyfluorinated substance levels (ng/L) in outgoing drinking water from the two waterworks in Ronneby, Sweden, on 10 December 2013

	Brantafors	Kärragården
Perfluoropentanoic acid	38	10
Perfluorohexanoic acid	320	3.6
Perfluoroheptanoic acid	32	1.4
Perfluorooctanoic acid	100	1.0
Perfluorononanoic acid	<1	<1
Perfluorodecanoic acid	<1	<1
Perfluoroundecanoic acid	<10	<10
Perfluorododecanoic acid	<10	<10
Perfluorobutane sulfonic acid	130	<2.6
Perfluorohexane sulfonic acid	1700	4.6
Perfluoroheptane sulfonic acid	60	<1
Perfluorooctane sulfonic acid	8000	27

acid, which has a much shorter half-life, around 1 month,[7] there are no human data after end of exposure for other PFASs.

Ronneby: a case study from Sweden

In autumn 2013 a survey of groundwater quality in Blekinge county in southern Sweden showed alarmingly high levels of PFASs in groundwater from a glaciofluvial water reservoir, the Bredåkra delta, which has a military and civil airfield located in its centre. Extended water sampling revealed very high levels of PFASs in outgoing drinking water from Brantafors, one of the two municipal waterworks in Ronneby, a municipality with 28 000 inhabitants (table 2). This waterworks provided drinking water to one-third of the households in Ronneby. The contaminated waterworks was closed on 16 December 2013, and clean water was promptly provided by Kärragården, the second waterworks in the municipality. After a few days no elevated levels of PFASs could be detected in the distribution network. Brantafors waterworks was reopened in May 2014, supplied with new coal filters and using water only from wells with low PFASs levels, but the trial was ended in October 2014. During this trial the levels of PFASs (sum of 11) were closely monitored, reaching at most 40 ng/L (ie, well below 90 ng/L, the present Swedish recommended action level).

It was soon confirmed that the fire drill site at the nearby military airport localised within the aquifer area had leached PFASs to the environment. Despite considerable efforts from the Armed Forces, it has not been possible to reconstruct the detailed historical use of AFFF at the airfield, but the best estimate as to the start of the use of these foams is the mid-1980s. Very little information on past or current PFAS content in the foams used at the facility was available, only that PFOS-containing foams were not purchased since 2004. For a general overview of AFFFs, see refs 1 and 8.

Extensive biomonitoring in the municipality population started in June 2014, approximately 6 months after end of exposure through drinking water, by open invitations and free of cost. Subjects living and working in the contaminated as well as in the uncontaminated district were invited. During the period 2014–2016 a total of 3418 persons from Ronneby participated. Considerable efforts were made to recruit persons with little exposure to the contaminated water, in order to ensure a broad range of serum PFASs levels for further research on health effects. A reference group of 242 subjects from a nearby unexposed municipality (Karlshamn) was also examined in 2016.

METHODS

Study group

From among the first participants in the screening programme, volunteers were invited to participate in the half-life study until the target of 100 subjects, evenly split by gender, was achieved. The panel study group (n=106) with a large age span, 4–83 years at baseline, was established in June 2014. The proportion of women was 53%. There were 20 men aged 15–50, and 30 women (menstruating ages). The participants have donated blood regularly, initially every third month, then with longer intervals. Analysis of PFASs in serum is performed after each sampling round and the individual results are immediately reported back to the participants.

We here report findings from the first seven sampling rounds (in June 2014, October 2014, January 2015, April 2015, September 2015, March 2016 and September 2016). The median number of samples per person was 6. Continued sampling twice a year is planned for several years to come.

Chemical analysis

Plasma concentrations of PFHxS, PFOS and PFOA were analysed at the Department of Occupational and Environmental Medicine in Lund, Sweden, using liquid chromatography-tandem mass spectrometry (LC/MS/MS). The samples were analysed according to a modified method[9] and determined as the total, non-isomer-specific compounds. The aliquots of 25 µL serum were added with 75 µL of water. A solution containing labelled internal standards were added and the proteins were precipitated using acetonitrile followed by vigorous shaking for 30 min. The samples were then centrifuged and 1 µL of the supernatant was analysed using an LC (UFLCXR, Shimadzu Corporation, Kyoto, Japan) connected to the MS/MS (QTRAP 5500, AB Sciex, Foster City, California, USA). Limits of detection determined as the concentrations corresponding to three times the SD of the responses in chemical blanks were 0.5 ng/mL for PFHxS and PFOS, and 0.4 ng/mL for PFOA. Coefficients of variation of quality control (QC) samples at 100 ng/mL were 6% for PFHxS and PFOS, and 8% for PFOA. The analyses of PFOS and PFOA are part of a quality control programme between analytical laboratories coordinated by Professor Hans Drexler, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, Germany.

Modelling of half-life

A linear mixed-effect model was used to assess predictors of subject-specific serum PFAS concentrations over time, from which we derive excretion rate and serum elimination half-lives of each PFAS. The following mixed model was used to fit the panel data:

$$\ln C_{ij} = \alpha_i + t_{ij}k_i + X_i\beta + \varepsilon_{ij},$$

where C_{ij} is the serum PFAS concentrations for individual i and sampling round j, α_i is the subject-specific intercept, t_{ij} is the time elapsed between the clean water was provided and the blood sample collection, k_i is the subject-specific slope, X_i is a vector of fixed covariates for individual i, including age, gender and body mass index (BMI), β is the fixed effect coefficient and ε_{ij} is the random error term. The subject-specific intercept α_i, the subject-specific slope k_i and the random error term ε_{ij} were modelled as random with normal distribution; others were treated as fixed effects.

The slope (k_i) is the excretion rate constant, and the mean value of k_i derived from the model was converted to half-life (ln2/mean(k_i)). The values of k_i were predicted using the best linear unbiased prediction method.[10] To examine the variability of the half-life, the predicted k_i values were converted to half-lives. A small number of observations were however excluded, with negative values (apparently increasing serum levels) or extremely high half-life (with minimal k_i). Summary half-life values have been presented as either a mean half-life (calculated from the mean elimination rate constant k) or as median half-life (the median value of the individually modelled half-life values). The 95% CI for mean(k_i) from the regression was used to derive the CI for the half-life, by converting as for the mean.

The analyses were repeated for the age group 15–50 (at the start), stratified by gender. An interaction term for gender and excretion rate constant was used to test the significance of a sex difference in excretion rate.

The general background exposure was not subtracted when modelling the half-life, since the PFAS levels of the last sample for all the individuals were far above what is expected in the background.

Table 3 Summary statistics of PFAS concentrations (ng/mL) in 106 participants in a panel study 6 months after end of exposure through contaminated drinking water (baseline investigation)

PFAS	Group	Participants (n)	Mean±SD	(Min, median, max)
PFHxS	Panel study	106	353±260	(12.3, 277, 1660)
	Main Ronneby	3418	228±232	(<0.5*, 152, 1790)
	Karlshamn reference	242	1.91±5.27	(<0.5*, 0.84, 60.1)
PFOS	Panel study	106	387±259	(24.1, 345, 1500)
	Main Ronneby	3418	245±234	(0.58, 176, 1870)
	Karlshamn reference	242	5.68±6.19	(<0.5*, 4.21, 55.3)
PFOA	Panel study	106	21.1±14.7	(2.38, 17.5, 92)
	Main Ronneby	3418	13.7±12.0	(<0.4*, 10.4, 91.9)
	Karlshamn reference	242	1.77±0.81	(<0.4*, 1.59, 4.98)

*Limit of detection.
PFAS, perfluorinated and polyfluorinated substance; PFHxS, perfluorohexane sulfonate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

RESULTS

Serum levels at baseline

The median serum level of PFHxS was 180 times higher in the investigated Ronneby population compared with the referents from a neighbouring municipality, 42 times higher for PFOS and 6 times higher for PFOA (table 3). In the main Ronneby study group 98% of the 3418 participants had PFHxS levels over the 90th centile (2.58 ng/mL) of PFHxS levels observed in the Karlshamn group. A similar pattern was seen for PFOS, where 90% of the main Ronneby group had levels in excess of the 90th centile (9.85 ng/mL) in the Karlshamn group, and PFOA, where 85% of the main Ronneby group had levels in excess of the 90th centile (2.91 ng/mL).

The participants in the panel study initially had serum levels of PFHxS, PFOS and PFOA that were somewhat higher than in the main Ronneby study population. This difference reflected the fact that the main population, but not the panel group, included persons living in the non-exposed area of Ronneby. The baseline serum levels in the panel study group ranged from 12.3 to 1660 ng/mL for PFHxS, 24.1 to 1500 ng/mL for PFOS, and 2.38 to 92 ng/mL for PFOA (table 3).

Table 4 Excretion rate and half-lives for serum PFAS concentrations in 106 participants in a panel study after end of exposure through contaminated drinking water

	All		Men aged 15–50		Women aged 15–50		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	p*
Excretion rate constant (per year)†							
PFHxS	0.13	0.12 to 0.15	0.09	0.07 to 0.11	0.15	0.12 to 0.18	0.008
PFOS	0.20	0.19 to 0.22	0.15	0.11 to 0.18	0.22	0.19 to 0.26	0.004
PFOA	0.26	0.24 to 0.28	0.25	0.19 to 0.26	0.29	0.23 to 0.34	0.29
Half-life (years)‡							
PFHxS	5.3	4.6 to 6.0	7.4	6.0 to 9.7	4.7	3.9 to 5.9	0.008
PFOS	3.4	3.1 to 3.7	4.6	3.7 to 6.1	3.1	2.7 to 3.7	0.004
PFOA	2.7	2.5 to 2.9	2.8	2.4 to 3.4	2.4	2.0 to 3.0	0.29

The subgroup aged 15–50 includes 20 men and 30 women.
*p Values for the difference between genders in the model for excretion rate.
†The estimates in the table are adjusted for age, gender and body mass index in a mixed-effects model.
‡Half-life values are all calculated from excretion rate constant.
PFAS, perfluorinated and polyfluorinated substance; PFHxS, perfluorohexane sulfonate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Age at baseline had a strong effect on serum PFHxS, PFOS and PFOA concentrations with average increases of 1.5%, 1.4% and 1.1% per year of age, respectively. Gender and BMI were not associated with any of the PFAS at baseline.

Decline of serum levels during follow-up

The average decreases in serum level were for PFHxS 25%, for PFOS 35% and for PFOA 38% from June 2014 to September 2016.

Table 4 shows the results for each excretion rate constant and the corresponding half-life in models for each PFAS. The mean excretion rate constant for PFHxS was 0.13, which is the annual change in log concentration, equivalent to the concentration of PFHxS in serum reducing by 13% per year since clear water was provided. This excretion rate constant is equivalent to a mean half-life of 5.3 (95% CI 4.6 to 6.0) years. For PFOS, the annual decrease was 20%, and the mean half-life was 3.4 years (95% CI 3.1 to 3.7 years). The average decrease in PFOA was 26% of its previous value each year, corresponding to a mean half-life of 2.7 years (95% CI 2.5 to 2.9 years).

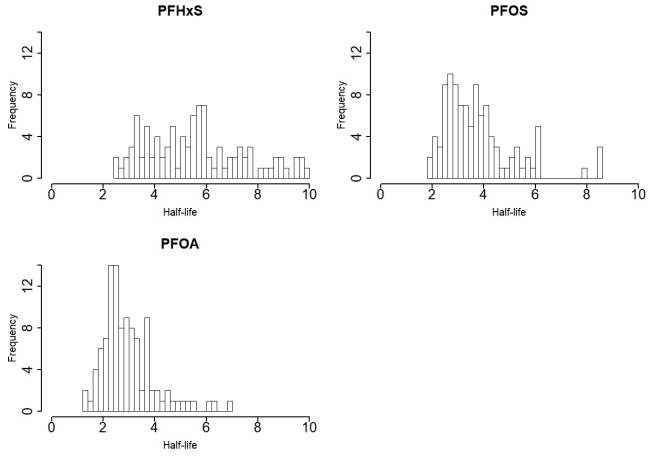

Figure 1 The interindividual variation of half-lives for perfluorinated and polyfluorinated substances in 106 participants in a panel study after end of exposure through contaminated drinking water, excluding outliers. PFHxS, perfluorohexane sulfonate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

The distributions of half-lives are shown in figure 1, after exclusion of outliers for the fitted estimated half-life as follows: one with a negative half-life (n=1 for PFHxS) and nine over 10 years (n=8 for PFHxS, n=1 for PFOS). The median value of the remaining half-lives for PFHxS was 5.5 years (5%–95% range: 3.0–9.2 years). For PFOS, the median half-life was 3.5 years (5%–95% range: 2.2–6.2 years). For PFOA, the median half-life for PFOA was 2.7 years (5%–95% range: 1.8–5.1 years).

Women aged 15–50 had a considerably shorter mean half-life for PFHxS compared with men (table 3), with men 1.6-fold longer. For PFOS the pattern was similar, with men 1.5-fold longer. For PFOA the difference was small. The distributions of half-lives in women and men aged 15–50 are illustrated in online supplementary figure S1.

DISCUSSION

Among 106 persons observed between 6 and 33 months after end of exposure to PFAS-contaminated drinking water, the shortest half-life was observed for PFOA with a mean of 2.7 years. The half-life for PFHxS was twice as long, 5.3 years, and for PFOS the mean was 3.4 years. These results are somewhat shorter than the prior results for PFOS and PFHxS, based on observations in 24 retired fluorocarbon workers, to our knowledge the only other study that hitherto has reported apparent half-lives for PFOS and PFHxS after end of exposure that was substantially higher than the general population background.[4] The retired workers, all but two men, were older than our population, had higher serum levels of PFOA and PFOS, and were followed for a longer period, 5 years. For PFHxS, the apparent half-life has been estimated to be 15.5 years in a recent study from a community with residential exposure to PFAS.[11] The PFHxS levels in serum in that study were much lower than in our study, that is, 6.4 ng/mL vs 152 ng/mL. Furthermore, their population still had ongoing exposure, and a pharmacokinetic modelling approach based only on water intake was used to account for ongoing exposure. In our study, the background exposure was not subtracted when modelling half-life since the exposure levels in the general population from all sources were negligible compared with earlier drinking water intake in the study population. Our estimate of apparent half-life, which was obtained after a documented abrupt end of the dominating source of exposure, is thus a reliable estimate of the actual half-life of PFHxS.

Our estimate of apparent half-life for PFOA is in the range of values reported from five studies with averages ranging from 2.3 to 3.94 years, observed in fluorocarbon workers[4]; studies in populations living in PFOA-polluted areas around production plants, followed for 1–2 years after provision of clean drinking water[5 12]; occupationally exposed ski waxers[13]; and a study in a community exposed residentially to PFAS.[11] For PFOS, the population half-life has been estimated to be 4.3 years from studies in US blood donors reflecting general population reduction in exposure.[14] After an abrupt end of a dominating source of exposure, as in Ronneby, the finding of a shorter apparent half-life is as expected.

The interindividual variation in half-life was substantial, with a threefold difference between the 5th and 95th percentiles in each of the three PFAS, plus a few extreme outliers with extremely long half-lives. Large interindividual differences were also observed in retired fluorocarbon workers[4] and in the general population after end of drinking water exposure.[5 12] The variability between individuals, and between men and women, has not yet been adequately explained.

Blood loss due to menstruation accounts partly for a shorter elimination half-life in women, and was estimated to account for 30% of the discrepancy in elimination of PFOS between men and women.[6] In this respect, the marked gender difference in elimination of PFHxS, as observed in our study and by Brede *et al*[5] but not for PFOA (our study and Bartell *et al*[12]), with PFOS in between, is intriguing. Elimination pathways that are sex-specific and substance-specific appear to exist.

Reabsorption by OATs in the kidneys and extensive uptake from enterohepatic circulation for PFOS and PFOA are active processes that may differ between individuals, but also between different PFASs. An increased renal PFAS elimination at high doses indicates a capacity-limited, saturable renal resorption process via high-efficiency OATs,[15 16] which may have sex-different expression.[17] Moreover, in a PFOA-exposed US population, the excretion rate was related to polymorphisms (single-nucleotide polymorphisms; SNPs) in tubular transporter proteins.[18] Faecal elimination is little studied in humans, with the exception of some case reports that indicate that cholestyramine, a lipid-lowering pharmaceutical, may enhance elimination.[19]

In addition to differences between individuals as to excretion capacity, recent data using paired human serum and urine samples for estimation of T½ have indicated marked differences between excretion of PFASs with different chain-length and isomers.[20] It is likely that linear isomers are preferentially retained,[21] but observational longitudinal human data on the excretion of linear versus branched chain isomers are absent. Thus, variation of T½ between populations and between individuals using total PFOS, PFOA and PFHxS determinations (as in this study) may in part reflect body burdens with different isomer composition.

Such differences are likely to be found in humans, given the varying production methods of PFAS over time. Synthesis of PFAS is by electrochemical fluorination or fluorotelomerisation. Electrochemical fluorination was used from the 1950s until the early 2000s and yielded branched and linear isomers. By contrast, fluorotelomerisation, which was later introduced, produces almost exclusively linear compounds.[22] The firefighting foams used over time have differed in composition, but there may also be varying fate of different PFAS structural isomers during soil and groundwater transportation. Thus, it is of importance to include determination of both linear and branched isomers of PFOS, PFHxS and PFOA in order to understand differences in observed half-lives. Refined estimates of the half-lives of PFAS compounds and the important denominators of variance are needed to reconstruct historical exposure for epidemiological studies as well as to project future exposures for risk assessment.

Out of the hundreds of PFAS compounds now available, only PFOS is internationally regulated according to the Stockholm Convention, and PFOA is on the candidate list. The human data on PFHxS uptake and elimination are hitherto very limited. The present observations confirm the long persistence of this compound after end of external exposure—a rough extrapolation based on the mean half-life indicates that 10-year-old children from the contaminated water district cannot expect to attain the same PFHxS levels as their peers in the neighbouring town of Karlshamn until the age of 60–70. Thus, even after prompt end of external exposure, AFFF contamination of drinking water can result in very high exposure levels in a life-course perspective in local general populations. Hence, the need for precautionary regulations for classes of PFASs is imperative.

Limitation

The main limitation of the present first analysis is that the serum samples were analysed during a 2-year period and each individual's samples were not analysed in the same batch. All samples were analysed at the same laboratory with the same methods and work-up procedure. However, there is a need to reanalyse all samples from each individual in the same batch to reduce laboratory variation, especially when determinants for variation in half-lives are investigated. This is planned as a next step in our studies.

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Contributors YL carried out the statistical analyses, interpreted the results and wrote the manuscript. TF interpreted the results and wrote the manuscript. DM reviewed drafts of the manuscript and provided valuable comments for the manuscript. KS participated in the study design, communicated with study participants and managed the data, reviewed the drafts, and provided valuable comments. CHL is responsible for the chemical analysis in the study, reviewed the drafts and provided valuable comments for the manuscript. PT collected the serum samples, communicated with study participants and reviewed the draft. KJ is the principal investigator, led the conceptual and methodological design of the study, and wrote the manuscript. All authors have read and approved the final version of the manuscript.

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Patient consent Obtained.

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Attachment 8

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Accumulation of perfluoroalkyl substances in human tissues

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ABSTRACT

Perfluoroalkyl substances (PFASs) are environmental pollutants with an important bioaccumulation potential. However, their metabolism and distribution in humans are not well studied. In this study, the concentrations of 21 PFASs were analyzed in 99 samples of autopsy tissues (brain, liver, lung, bone, and kidney) from subjects who had been living in Tarragona (Catalonia, Spain). The samples were analyzed by solvent extraction and online purification by turbulent flow and liquid chromatography coupled to tandem mass spectrometry. The occurrence of PFASs was confirmed in all human tissues. Although PFASs accumulation followed particular trends depending on the specific tissue, some similarities were found. In kidney and lung, perfluorobutanoic acid was the most frequent compound, and at highest concentrations (median values: 263 and 807 ng/g in kidney and lung, respectively). In liver and brain, perfluorohexanoic acid showed the maximum levels (median: 68.3 and 141 ng/g, respectively), while perfluorooctanoic acid was the most contributively in bone (median: 20.9 ng/g). Lung tissues accumulated the highest concentration of PFASs. However, perfluorooctane sulfonic acid and perfluorooctanoic acid were more prevalent in liver and bone, respectively. To the best of our knowledge, the accumulation of different PFASs in samples of various human tissues from the same subjects is here reported for the very first time. The current results may be of high importance for the validation of physiologically based pharmacokinetic models, which are being developed for humans. However, further studies on the distribution of the same compounds in the human body are still required.

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1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a large group of surface-active organic compounds. Because of their chemical and thermal stability, as well as their hydrophobic and lipophobic nature, they have been used for over 50 years in a number of industrial and commercial applications (Zhao et al., 2012). PFASs are highly resistant to breakdown. Therefore, they are persistent in the environment, being able to accumulate in living organisms and biomagnified through the trophic web (Loi et al., 2011; Powley et al., 2008). Moreover, there is a growing concern related to their potentially harmful effects on human health (Vieira et al., 2013). Due to these reasons, the U.S. industry undertook voluntary actions to phase out production of perfluorooctane sulfonic acid (PFOS) between 2000 and 2002, and in 2007 the United States Environmental Protection Agency (US EPA) published the Significant New Use Rules (SNURs) to restrict the production of PFOS and related substances (Lindstrom et al., 2011). Moreover, in 2006, the major PFAS producers committed the Stewardship Program to phase out the global emissions and products containing perfluorooctanoic acid (PFOA) for 2015. Despite these measures, hundreds of other different PFASs are currently being produced and used. Thus, although the production of PFOA is being phased out by the companies participating in the Voluntary Stewardship Program, environmental contamination and human exposure from PFOA and higher homologue chemicals (e.g. PFNA, PFDA, etc.) are anticipated to continue for the foreseeable future due to a number of reasons: its persistence, their formation from precursor compounds, and the potential for continued production by other manufacturers in the U.S. and/or overseas (Lindstrom et al., 2011).

In 2008, the European Food Safety Authority (EFSA, 2008) established a series of Tolerable Daily Intakes (TDIs) values for PFOS and PFOA at 150 and 1500 ng/kg/day, respectively. PFOS was subsequently included as a persistent organic pollutant (POP) under the Stockholm Convention (UNEP 2010). In 2009, the US EPA Office of Water established the provisional health advisory values for PFOS and PFOA at 200 and 400 ng/L, respectively. It must be highlighted that, although TDIs and the water provisional health advisory were calculated in different basis, in both cases short-term exposure was considered as the relevant period of exposure. This was consistent with PFOA and PFOS toxicity data, which in turn rely upon subchronic exposure experimental values. However, long-term exposures must be considered for the accurate assessment of their potential risk on human health, taking into account that their

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presence has been reported in drinking water, ambient air, and food (Domingo et al., 2012a,b; Ericson Jogsten et al., 2012; Ericson et al., 2008, 2009; Post et al., 2009, 2012).

PFASs have been related to different toxicological effects on mammals. In mice, the neonatal exposure to PFOS and PFOA has been linked up to changes in proteins of importance for the neuronal growth and synaptogenesis in the brain developing (Johansson et al., 2009), as well as with neurobehavioral defects and changes in the cholinergic system (Johansson et al., 2008). In addition, perfluorohexanesulphonate (PFHxS) has been related to irreversible neurotoxic effects in neonatal mice, showing a similar behavior to that of other POPs, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and bisphenol A (Viberg et al., 2013). A recent study in human suggested that higher PFOA serum levels might be associated with testicular, kidney, prostate, and ovarian cancers, and non-Hodgkin lymphoma, according to the concentrations of residents in 6 areas with contaminated drinking water supplies (Vieira et al., 2013).

In the human body, the polar hydrophobic nature of fluorine-containing compounds can lead to increased affinity for proteins (Jones et al., 2003; Luebker et al., 2002; Vanden Heuvel et al., 1992; Weiss et al., 2009). A number of PFASs have been detected in human serum, cord blood and breast milk (Domingo et al., 2012a; Ericson et al., 2007; Fromme et al., 2010; Haug et al., 2009a,b; Llorca et al., 2010). As other bioacumulative halogenated contaminants (e.g., polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and PCBs), PFASs can have long persistence in the body. However, they do not tend to accumulate in fat tissue. According to outcomes of animal studies, PFOA and PFOS are mostly excreted through the urine (Cui et al., 2010), but limited observations in humans suggest that only one-fifth of the total body clearance is renal (Harada et al., 2005). The elimination half-life of PFOA in humans was roughly estimated to be 3.5 years, while that of PFOS was approximately 4.8 years (Olsen et al., 2007), according to data from retired workers. Post et al. (2012) recently reviewed studies reporting the elimination half-life values between 2.3 and 3.3 years, following an exposure to contaminated drinking water (Post et al., 2012). Information about sources, environmental fate and toxicokinetics of PFOS and PFOA is largely available, while estimation values in the half-lives of PFBS, PFHxS and PFBA (Chang et al., 2008; Lau et al., 2007). In contrast, data on most of the PFASs currently in use, continues to be very limited. It has been hypothesized that the possible harmful effects associated to PFASs accumulation are of special concern during early stages of life (Maisonet et al., 2012; Post et al., 2012; Schecter et al., 2012). However, their accumulation and distribution in the different human tissues are still poorly understood. The potential accumulation of PFASs with different chain lengths is an issue of great importance for exposure assessment and risk characterization studies. Most current investigations on human accumulation have focused on the occurrence in blood and breast milk, while very few studies have reported levels in other tissues. Kärrman et al. (2009) determined the concentrations of six PFASs in liver samples collected post-mortem in Spain. Mean concentrations of 27 and 1 ng/g of PFOS and PFOA, respectively, were found. In turn, Maestri et al. (2006) found levels of 14 ng/g of PFOS and 3 ng/g of PFOA in a pooled liver samples corresponding to seven subjects from northern Italy, while Olsen et al. (2003) reported mean PFOS and PFOA concentrations of 19 and 47 ng/g, respectively, in 30 subjects from USA. Finally, Pirali et al. (2009) detected PFOA and PFOS in thyroid tissue (median levels: 2 and 5.3 ng/g, respectively), concluding that those compounds are not actively concentrated in the thyroid.

The main objectives of the present study were the following: 1) to optimize and validate an on-line analytical approach based on turbulent flow chromatography coupled to tandem mass spectrometry (TFC-LC-MS/MS) for determining PFASs in various human tissues; 2) to measure the levels of 21 PFASs in these human tissues in order to elucidate their distribution and accumulation in the human body. The method optimized for the tissue analysis was carefully selected to accomplish the minimum sample size requirements and to reduce sample manipulation. The analytical procedure was validated for different kinds of tissues, and applied for the determination of selected compounds in liver, lung, brain, bone, and kidney samples collected post-mortem from 20 subjects. PFASs values were correlated with the concentrations of some heavy metals (unpublished results) in the same tissue samples, as well as with the levels of PCDD/Fs in adipose tissue from 15 of the same individuals (Nadal et al., 2009). To the best of our knowledge, these are the first data reporting the accumulation of a notable number of PFASs in human tissues, as well as comparing the body burden of these pollutants with that of other environmental contaminants (metals and PCDD/Fs).

2. Materials and methods

2.1. Chemicals and standards

Standard solutions were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). The standard analytes used in this study were: i) PFAC-MXB [98% purity in methanol] containing perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrA), perfluorotetradecanoic acid (PFTeA, perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutanesulphonate (PFBS), perfluorohexanesulphonate (PFHxS), perfluorooctanesulphonate (PFOS) and perflurodecanesulphonate (PFDS); ii) FTA [98% purity in isopropanol] including perfluorohexyl ethanoic acid (FHEA), perfluorooctyl ethanoic acid FOEA, and perfluorodecyl ethanoic acid FDEA; iii) perfluorooctane sulfonamide (PFOSA) [98% pure in methanol]. Identification and quantification were performed using the following internal standards: i) MPFAC-MXA [>98%] containing [$^{13}C_4$]-perfluorobutanoic acid (MPFBA ($^{13}C_4$)), ion [$^{18}O_2$]-perfluorohexanesulfonate (MPFHxS ($^{18}O_2$)), [$^{13}C_2$]-perfluorohexanoic acid (MPFHxA ($^{13}C_2$)), ion [$^{13}C_4$]-perfluorooctanesulfonate (MPFOS ($^{13}C_4$)), [$^{13}C_4$]-perfluorooctanoic acid (MPFOA ($^{13}C_4$)), [$^{13}C_5$]-perfluorononanoic acid (MPFNA ($^{13}C_5$)), [$^{13}C_2$]-perfluorododecanoic acid (MPFDoA ($^{13}C_2$)), [$^{13}C_2$]-perfluorodecanoic acid (MPFDA ($^{13}C_2$)), [$^{13}C_2$]-perfluoroundecanoic acid (MPFUdA ($^{13}C_2$)); ii) MFTA-MXA [>98%] [$^{13}C_2$]-perfluorohexylethanoic acid (MFHEA($^{13}C_2$)), [$^{13}C_2$]-perfluorooctylethanoic acid (MFOEA($^{13}C_2$)), [$^{13}C_2$]-perfluorodecylethanoic acid (MFDEA ($^{13}C_2$)) and iii) [$^{13}C_8$]-perfluorooctanesulfonamide (MPFOSA ($^{13}C_8$)).

Water, methanol, acetonitrile, CHROMASOLV®Plus for HPLC grade, ammonium acetate salt ($AcNH_4$: MW, 77.08; 98%), and formic acid (HFo) were obtained from Sigma-Aldrich (Steinheim, Germany). To remove possible cross contamination, polypropylene (PP) insert vials and inert taps were used.

2.2. Sampling and pre-treatment

Samples from liver, kidney, brain, lung, and bone (rib) were collected in 2008 from 20 subjects who had been living in different areas of Tarragona County (Catalonia, Spain) at least for the last 10 years. Causes of death were varied, including multiple trauma, subdural hematoma, ischemic heart disease, accident or self-injury. Autopsies and extraction of samples were carried out during the first 24 h after the time of death. Additional data from the subjects, such as age (mean: 56; range: 28–83) and smoking habits information, were collected (Table S1; Supporting Information). Tissue samples were stored at −20 °C before analysis. The study protocol was reviewed and approved by the Ethical Committee for Human Studies of the School of Medicine, Universitat Rovira i Virgili, Reus/Tarragona, Spain.

Sample pre-treatment was based on a previously published protocol (Llorca et al., 2010). Briefly, 1 g of each sample was weighed and transferred into a 15 mL PP tube. Then, 2 mL of water were added, and the mixture was shaken. Homogenates were fortified with surrogate

internal standards (to obtain a concentration of each internal standard of 10 μg/L), being digested with 5 mL of sodium hydroxide (20 mM in methanol) during 4 h at 125 rpm on an orbital shaker table at room temperature. After digestion, samples were centrifuged at 4000 rpm, and 20 μL of supernatant were directly injected into the turbulent flow chromatography system.

2.3. Analysis

A turbulent flow chromatograph Aria TLX-1 system (Thermo Fisher Scientific, Franklin, MA, USA) comprised of a PAL auto sampler (CTC Analytics, Zwingen, Switzerland), two mixing binary pumps (eluting pump and loading pump), and a three-valve switching device unit with six-port valve. The entire system was controlled via Aria software, version 1.6. The on-line enrichment was achieved using a Hypersil GOLD aQ column (2.1 × 20 mm, 12 μm particle size from Thermo Fisher Scientific, Franklin, MA, USA). The analytical column used for the chromatographic separation was a Hypersil GOLD PFP (50 × 3) (Thermo Fisher Scientific, Franklin, MA). The sample was loaded into enrichment columns using ultrapure water acidified at pH 4.5 with formic acid. After the enrichment step, the analytes were transferred to the analytical column for their chromatographic separation. The gradient used is shown in Table S2 (Supporting Information).

After separation, the detection of the selected analytes was accomplished by using a triple quadrupole mass spectrometer Thermo Scientific TSQ Vantage (Thermo Fisher Scientific, San Jose, CA), equipped with a Turbo Ion Spray source. All the analyses were performed operating in the negative electrospray ionization (ESI (−)) mode. Acquisition was performed in selected reaction monitoring mode (SRM) to obtain enough identification points (IP) for confirmation of each analyte (European Commission Decision 2002/657/EC). The main m/z transitions are summarized in Table S3 (Supporting Information). For analyte identification, the following conditions had to be met: i) analyte retention time in the sample must be in agreement with analyte retention time in the calibration curve; ii) two m/z transition were confirmed for every analyte; iii) ratio between the two transitions in the sample compared to ratio in the calibration curve should be in agreement to [calibration curve average ± SD (calibration curve)]. Table S4 (Supporting Information) provides the method limit of detection (MLOD) and the method limit of quantification (MLOQ) of the selected compounds in the five analyzed human tissues.

2.4. Quality assurance and quality control

To eliminate sources of contamination from the analytical system, all the polytetrafluoroethylene (PTFE) tubing was replaced by polyether ether ketone (PEEK) connections. In addition, an extra analytical column (C8 50 × 3 Thermo Scientific) was directly placed upstream of the injector to trap the instrumental sources of analytes, and therefore, to minimize the background signal and inter-run variability of all analytes. Blanks, consisting on initial conditions of mobile phase, were analyzed every 5 sample injections. For assessment of matrix interference in the analysis, matrix-matched calibration curves, and blank samples, were introduced in each run of analysis.

Spiking experiments were performed with blank animal (pig) matrices of brain, lung, liver, bone and kidney fortified at three different concentration levels (6, 12 and 24 ng/g of tissue). To assess the initial concentrations of PFASs, these samples were analyzed prior to fortification, being in all cases below the MLOD. The method was validated according to the criteria described by the EC Decision 2002/657/EC. The following parameters were established: instrumental selectivity and methodology limits of detection and quantification (ILOD, MLOD, ILOQ and MLOQ, respectively), linearity, recoveries, and precision expressed as intraday and inter-day repeatability.

2.5. Multivariate analysis

Before executing the multivariate data analysis, non-detected values were assumed to be equal to one-half of the method limit of detection (ND = 1/2 MLOD). The whole data set from the 5 human tissues was analyzed both individually and by using a column-wise 99 × 20 matrix augmentation strategy (Navarro et al., 2006). Auto scaling was chosen as pre-treatment method. With this procedure, the mean of the column elements was subtracted from individual elements and divided by their column standard deviation. Consequently, each column has zero mean and unit variance (Brodnjak-Vončina et al., 2002; Massart et al., 1998). Auto scaling can be applied either to the individual matrices corresponding to each tissue before matrix augmentation, or once they have been arranged in the column-wise augmented data matrix. The former system identifies differences in the tissues, while the latter detects differences among individual samples.

Data were also subjected to Principal Component Analysis (PCA). This is a data reduction technique aimed at explaining most of the variance in the data by transforming a set of correlated measured variables into a new set of uncorrelated Principal Components (PCs), which preserve the relationships present in the original data (Rovira et al., 2011a). The main goal of this multivariate statistical technique is to extract useful information and provide an easier visualization of the existent relationships between objects and variables determined in large or complex data set (Rovira et al., 2011b). PCA can be easily extended to the simultaneous analysis of multiple correlated data sets. In the present study, PCA was conducted to assess the possible distribution of the different compounds in the tissues studied, as well as to assess any possible correlation between age and smoking habits of the subjects and their PFASs accumulations. PCA modelling was conducted using the PLS Toolbox (Eigenvector Research, Manson WA, USA) appropriate functions under the MATLAB computer and visualization environment (The Mathworks, Natick, MA, USA). Finally, a hierarchical cluster analysis was conducted to confirm some of the conclusions obtained by the PCA. Data were also treated by normalization. The dissimilitude matrix was conducted by the Euclidean distance, while the Ward method was chosen for the aggrupation approach. This part of the multivariate analysis was conducted using the XLSTAT module version 2012.042.

3. Results and discussion

The concentrations of detected PFASs in human samples of brain, liver, lung, bone and kidney are depicted in Fig. 1. The complete set of results of each one of the 99 analyzed samples is given in Table S5 (Supporting Information), while a summary of median and range values is presented in Table 1. All samples showed detectable values of at least two of the investigated compounds. Although PFASs accumulation followed different trends depending on the specific tissue, some similarities were observed between liver and brain, on one hand, and between kidney and lung, on the other hand. In liver, PFHxA, PFOS and FHEA were the most prevalent compounds, with median concentrations of 68.3, 41.9 and 16.7 ng/g, respectively. PFOS, one of the most toxic PFASs, was present in 90% of the samples, while PFOA could be quantified in 45% of the samples (median: 4.0 ng/g). In brain, PFHxA was the main compound, being detected in all the samples at concentrations ranging from 10.1 to 486 ng/g. The contributions of PFNA (median: 13.5 ng/g) and PFDA (median: 12.4 ng/g) were also relatively important in brain samples. In contrast, PFOS was only quantified in 20% of the samples (median: 1.9 ng/g), whereas PFOA was not detected in any of them. In general terms, lung was the tissue showing the highest accumulation of PFASs. PFBA and PHFxA were the compounds presenting the highest median concentrations (807 and 207 ng/g, respectively). Only two lung samples showed PFOS levels under the limit of detection, with a median value of 28.4 ng/g. Although the percentage of samples with detected values of PFOA fell down to 45%, the contribution of PFOA to the total PFASs in lung was quite important, in comparison to

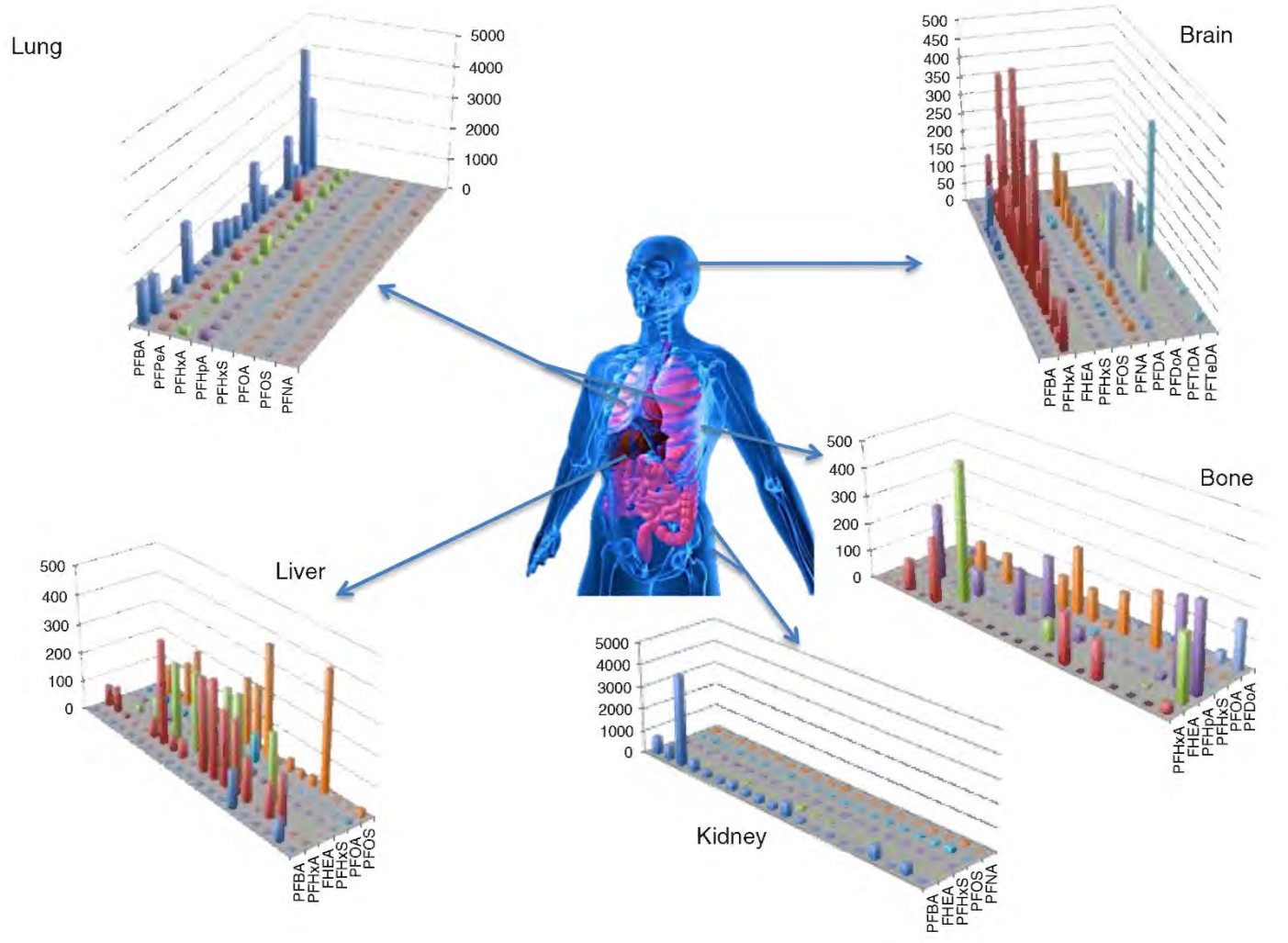

Fig. 1. Concentrations of various PFASs (in ng/g) in 5 human tissues from 20 residents of Tarragona (Catalonia, Spain).

other tissues and analytes. PFBA was also the predominant compound in all kidney samples, whose median concentration was 263 ng/g. PFDoDA and PFDA were also detected in kidney samples, but at much lower concentrations (median: 91.4 and 90.2 ng/g, respectively). High concentrations of PFOS were also found in kidney (median: 55.0 ng/g), while the presence of PFOA was minor. In contrast to lung, bone was identified as the tissue with the lowest burdens of PFASs. Furthermore, the PFAS profile was substantially different from those of the remaining tissues, as PFOA was, far the major contributor to the total concentration of PFASs (median: 20.9 ng/g). In turn, PFOS was not detected in any of the bone samples (Table 1). In summary, the profiles of PFASs accumulation in the different tissues reflected some common trends. Thus, PFHxA showed the highest concentrations in brain and liver, while PFBA presented the maximum median levels in kidney and lung, with PFOA as the predominant compound in bone. PFOS accumulated basically in lung, liver and kidney, while the levels of PFOS in bone and brain were very low. We hypothesized that since PFBA is a short chain compound, its predominance in lung could reflect the inhalation of contaminated dust and the industrial replacement of the eight carbons chain compounds by shorter ones. In addition, the human half-life of this compound is much shorter (3 days) (Chang et al., 2008) compared to the half-life to other longer chain compounds as those with 8 carbon-chain thereby accounting for its detection in other tissues as kidney. As aforementioned, there is an important lack of studies reporting PFASs levels in human tissues, excepting plasma. In comparison to previous results (Kärrman et al., 2009; Maestri et al., 2006; Olsen et al., 2003), the current concentrations of PFOS in liver from residents in Tarragona fall in the higher part of the range. However, this comparison can be only taken into account as a first indication.

The physical–chemical properties of each chemical are responsible for their tissue-specific accumulation profiles. However, the overall body burden can be similar although the chemicals accumulate in different tissues. In order to determine whether exposure to PFASs is related to exposures to other contaminants, the levels of PFASs in each sample were evaluated to determine whether they correlate with the concentrations of some metals and PCDD/Fs. The content of arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), tin (Sn), and thallium (Tl) had been previously determined in the same human tissue samples (unpublished results). With a few exceptions, the levels of PFASs were not associated with those of most trace elements. However, a significant Pearson correlation was noted between PFOA and As ($p < 0.001$), as well as between PFOA and Pb ($p < 0.001$). Manganese was the element presenting a significant correlation with a higher number of PFASs: PFDS, PFUDA, and PFTeDA ($p < 0.001$ in all cases). Finally, Ni correlated with PFHxDA. However, PFOS did not correlate with any of the above elements (Table S6; Supporting Information). The concentrations of PCDD/Fs had been also analyzed in adipose tissues from 15 of the same 20 individuals (Nadal et al., 2009). The mean PCDD/F concentration in adipose tissue was 14.6 pg WHO-TEQ/g of fat (range: 3.3–55.4 pg WHO-TEQ/g of fat). The total levels of PCDD/Fs, as well as those of the 17 2,3,7,8-chlorinated congeners, were compared with the concentrations of PFASs accumulated in the 5 human tissues here analyzed. Although not statistically significant, a negative correlation was

Table 1
Summary of PFAS concentrations (in ng/g wet weight) in 5 autopsy tissues from 20 individuals of Tarragona (Catalonia, Spain).

	Liver				Bone				Brain				Lung				Kidney			
	Mean	Median	Range	MLOD	% of detection	Mean	Median	Range	MLOD	% of detection	Mean	Median	Range	MLOD	% of detection	Mean	Median	Range	MLOD	% of detection
PFBA	12.9	3.0	128-Bdl.	6.00	10	Bdl.	–	–	0.03	0	13.5	1.4	137-Bdl.	2.71	25	304.2	807	4138-Bdl.	0.01	95	464	263	4026-Bdl.	0.01	95
PFPeA	1.4	Bdl.	27.1-Bdl.	0.001	5	0.8	0.8	0.8-Bdl.	1.51	0	Bdl.	–	–	0.59	0	44.5	40.8	695-Bdl.	6.006	74	Bdl.	–	–	0.006	5
PFBS	0.9	0.7	1.5-Bdl.	1.39	0	3.2	2.4	17.8-Bdl.	14.41	5	Bdl.	–	–	0.96	0	17.8	1.1	9.7-Bdl.	2.10	47	8.0	1.7	80.4-Bdl.	3.40	5
PFHxA	115	68.3	353-Bdl.	2.73	70	35.6	1.5	230-Bdl.	0.001	30	180	141	486-10.1	0.72	100	50.1	207	569-Bdl.	9.42	89	5.6	2.7	57.1-Bdl.	5.37	25
FHEA	92.6	16.7	289-Bdl.	4.40	45	42.5	2.0	494-Bdl.	3.52	25	18.6	2.0	93.1-Bdl.	4.00	25	2.4	3.9	3.9-Bdl.	5.54	0	23.7	1.4	153-Bdl.	2.85	5
PFHpA	33.3	1.5	638-Bdl.	3.00	5	77.1	2.4	309-Bdl.	2.89	45	Bdl.	–	–	2.70	0	17.4	1.5	245-Bdl.	3.00	37	7.8	2.6	94.3-Bdl.	5.26	95
PFHxS	4.6	1.8	20.6-Bdl.	3.00	10	1.8	1.2	13.8-Bdl.	2.40	5	3.2	2.3	14.4-Bdl.	4.54	5	8.1	5.7	47.6-Bdl.	3.30	32	20.8	18	37-Bdl.	4.20	5
PFOA	13.6	4.0	98.9-Bdl.	3.00	45	60.2	20.9	234-Bdl.	3.00	55	Bdl.	–	–	2.40	0	29.2	12.1	87.9-Bdl.	6.00	42	2.0	1.5	11.9-Bdl.	3.00	95
PFOS	102	41.9	405-Bdl.	3.00	90	Bdl.	–	–	3.00	0	4.9	1.9	22.5-Bdl.	3.00	20	29.1	28.4	61.8-Bdl.	3.00	89	75.6	55	269-Bdl.	6.00	45
PFNA	1.3	1.0	6.6-Bdl.	1.99	0	Bdl.	–	–	4.18	0	29.7	13.5	150-Bdl.	3.27	55	15.3	3.5	126-Bdl.	7.13	11	28.3	10.9	128-Bdl.	4.04	0
FOEA	2.8	2.8	2.8-Bdl.	5.67	0	3.6	1.6	35.7-Bdl.	3.20	5	Bdl.	–	–	8.80	0	13.2	4.9	87-Bdl.	5.60	21	Bdl.	–	–	3.55	0
PFODA	2.5	1.5	6.5-Bdl.	3.00	0	Bdl.	–	–	6.00	0	Bdl.	–	–	2.91	0	Bdl.	–	–	2.91	0	Bdl.	–	–	0.01	0
PFDA	Bdl.	–	–	0.001	0	Bdl.	–	–	0.30	0	23.4	12.4	204-Bdl.	2.94	70	17.1	1.5	108-Bdl.	2.973	32	6.2	Bdl.	90.2-Bdl.	0.001	10
PFOSA	Bdl.	–	–	2.60	0	Bdl.	–	–	2.04	0	Bdl.	–	–	2.04	0	Bdl.	–	–	10.16	0	Bdl.	–	–	3.78	0
PFDS	Bdl.	–	–	0.001	5	1.7	1.5	5.7-Bdl.	2.97	0	0.3	Bdl.	1.4-Bdl.	0.00	25	3.1	0.6	9-Bdl.	1.200	37	4.9	Bdl.	21-Bdl.	0.001	25
PFUdA	Bdl.	–	–	0.003	0	Bdl.	–	–	0.30	0	Bdl.	–	–	18.00	0	2.8	1.4	20.4-Bdl.	2.700	11	7.1	1.5	55.4-Bdl.	3.00	10
FDEA	3.7	0.7	59.3-Bdl.	3.00	5	Bdl.	–	–	0.30	0	Bdl.	–	–	2.91	0	Bdl.	–	–	0.01	0	Bdl.	–	–	9.01	0
PFDoA	2.4	1.5	20.2-Bdl.	1.45	5	16.6	5.1	169-Bdl.	0.98	70	13.2	1.5	102-Bdl.	1.32	25	20.7	Bdl.	253-Bdl.	4.76	11	14.7	4.5	91.4-Bdl.	2.30	15
PFTrDA	2.1	Bdl.	32-Bdl.	0.001	10	15.8	0.3	311-Bdl.	0.60	5	9.9	1.4	167-Bdl.	2.88	10	138.6	6.9	1582-Bdl.	2.970	42	Bdl.	–	–	6.00	0
PFTeDA	Bdl.	–	–	0.001	0	Bdl.	–	–	0.001	5	24.8	1.4	335.7-Bdl.	2.85	30	9.8	1.5	82.8-Bdl.	2.910	16	6.2	30.8	Bdl	0.002	25
PFHxDA	Bdl.	–	–	3.00	0	16.6	2.9	171.8-2.9	5.85	10	Bdl.	–	–	2.91	0	8.5	1.5	80.2-Bdl.	2.95	16	Bdl.	–	–	0.01	0

MLOD: Method Limit of Detection. Bdl.: Below limit of detection.

observed between the total sum of PCDD/Fs and the total amount of PFASs (Table S7; Supporting Information). It is well known that the toxicity of dioxins is mediated through the activation of the Aryl hydrocarbon Receptor (AhR) (White and Birnbaum, 2009). In contrast, the mode-of-action (MoA) for PFOA as well as other PFASs, is not so well understood (Post et al., 2012). Notwithstanding, it must be noted that data on PCDD/Fs were only available for adipose tissue, while PFASs levels refer to another 5 different tissues (liver, brain, kidney, bone, and lung). Therefore, these data are not entirely comparable, and consequently, this indication cannot be confirmed.

The pharmacokinetic properties of PFOA and PFOS are well studied (Loccisano et al., 2012). These parameters have been used in the development of pharmacokinetic models, aimed at describing the human distribution of PFOA and PFOS (Loccisano et al., 2011; Thompson et al., 2010), among other PFASs. Physiologically based pharmacokinetic (PBPK) models are mathematical representations of the human body, where organs are considered as compartments (Fàbrega et al., 2011). The overall goal of developing these PBPK models is to extrapolate to humans the distribution of chemicals in the body, in order to enhance the scientific basis for human health risk assessment of PFASs (Loccisano et al., 2012). According to the results of studies with experimental animals, these compounds are well absorbed orally (Loccisano et al., 2012). Therefore, ingestion should be considered a key pathway. A clear relationship between the intake of PFOA, basically through drinking water consumption, and serum concentrations in humans, has been found (Emmett et al., 2006), with a with a serum:drinking water ratio of about 100:1 (Post et al., 2012). Although a number of PBPK models have been described, most of them have been based only on animal data, while human data are still very scarce. To the best of our knowledge, we here report, for the very first time, the simultaneous accumulation of PFASs in various human tissues. This information should be beneficial for the development of theoretical PBPK models, whose validation is still incomplete. Consequently, forensic analyses offer a practical way to explore the real accumulation of those pollutants in the human body.

In the current study, PCA analysis was used to determine the variation of PFASs accumulation between tissues, as well as to extract possible relations between the individual concentrations and other factors, such as age and smoking. The PCA results are summarized in Table 2. The first PC explained a variance ranging between 12% and 29% of the total variance, for all the different tissues analyzed, while PC2 and PC3 variances ranged 19–20% and 8–15% respectively. The percentage of explained variance for those PCAs performed in the individual tissues was always higher than that in the augmented matrices. The explained variances differed in the two groups of PCAs. In the augmented matrices, they increased very slowly, not reaching 50% of the total variance until PC6. This indicates the presence of multiple independent distribution processes of PFASs in the considered tissues. On the other hand, in the individual PCAs of each tissue, the variance increased faster, reaching 50% of the total variance in the PC3 in most of the cases, indicating similar distribution processes when the same tissue is considered. Fig. 2 depicts the loadings plot for the first two PCs of the augmented and auto scaled data matrices. The first PC had positive loadings for all acidic compounds, from low to high contributions depending on the compound, except for PFHxA, PFHpA, with moderate negative loadings, and PFDOA, with a high negative loading. In this first PC, perfluoroalkyl sulphonates presented positive loadings, with higher contributions of PFHxS and PFDS. Regarding telomer acids (FHEA, FDEA and FOEA), the three compounds showed moderate loadings, negatively for FHEA and FDEA, and positively for FOEA. The second PC showed positive loadings for most acidic compounds except for PFNA, PFDA, PFUdA and PFTeDA, with especially high contributions of PFBA, PFOA, PFDA and PFTrDA. Perfluoroalkyl sulphonates presented moderate contributions to the second PC, being positive for PFBS and PFDS, and negative for the remaining two. The telomer acids presented positive loadings for FOEA and FDEA, and negative for FHEA. When plotting

Table 2
Percentages of explained variances obtained by PCAs applied to $All_{aug\text{-}auto}$, $All_{auto\text{-}aug}$ and the individual matrices of the 5 tissues.

Matrix	$All_{aug\text{-}auto}$	$All_{auto\text{-}aug}$	Liver	Brain	Bone	Lung	Kidney
PC1	11.98	12.49	28.68	22.69	25.80	18.65	20.20
PC2	9.26	11.23	19.91	17.10	16.18	16.28	14.75
	(21.25)	(23.72)	(48.59)	(39.79)	(41.98)	(34.93)	(34.96)
PC3	7.65	9.82	15.50	13.68	13.64	12.55	14.56
	(28.90)	(33.53)	(64.09)	(53.47)	(55.63)	(47.48)	(49.51)
PC4	7.49	8.28	9.78	10.96	12.52	9.66	12.17
	(36.39)	(41.81)	(73.87)	(64.43)	(68.15)	(57.14)	(61.69)
PC5	6.75	7.53	8.53	9.30	8.20	8.02	8.75
	(43.14)	(49.35)	(82.40)	(73.73)	(76.35)	(65.16)	(70.43)
PC6	6.47	6.22	5.47	7.55	6.95	7.74	8.26
	(49.61)	(55.57)	(87.87)	(81.28)	(83.30)	(72.90)	(78.70)
PC10	4.60	4.55	1.07	2.07	1.79	3.58	2.82
	(70.28)	(76.06)	(99.74)	(98.01)	(100)	(91.42)	(95.92)

In parenthesis, percentage of accumulated variance for that particular component.
$All_{aug\text{-}auto}$: augmented matrix of the 5 individual tissues and then autoscaled.
$All_{auto\text{-}aug}$: individually autoscaled matrixes of the 5 tissues and then augmented.

the scores using these two PCs, the samples can be grouped into each one of the 5 tissues analyzed (Fig. 3). This means that the profile of PFASs found in each tissue is different from the others. Thus, PC1 allowed the separation between lungs, kidney and brain, with positive contribution, while bone and liver showed a negative contribution. In turn, PC2 reflected a separation between lung and bone, with positive loadings, and the remaining three tissues, with negative loadings. When considering the remaining PCs, the behavior was similar.

Fig. S1 (Supporting Information) depicts the loadings plot for the first PC of each PCA performed in the individual matrices of each tissue. In liver, the first PC showed high positive loadings for acidic compounds with an odd number of carbons (PFPeA, PFHpA, PFNA and PFTrDA). In kidney, a similar profile was obtained, with acidic PFASs with an odd number of carbon chain (PFPeA, PFNA, PFUdA and PFTrDA) acting as prevalent compounds. In brain, acidic compounds with a pair number of carbon chain (PFBA and PFHxA) and sulphonates (PFBS, PFOS and PFDS) were the predominant compounds. Unlike other PFASs, sulphonates also showed a high contribution in bone. Lung samples also presented positive loadings for most acidic compounds with a pair number of carbon chain (PFBA, PFHxA, PFOA, PFDoA), as well as some of the sulphonates (PFOS, PFDS) and FOEA. This different profile of PC1 confirms the different distribution pattern of PFASs according to each specific tissue. The influence of smoking in the accumulation of PFAs in the lungs was also studied. As shown in Fig. 4, smoker subjects presented lower contributions of PC1 and PC2 than non-smokers. It means less accumulation of the PFASs, which contribute to these PCs. When considering the rest of PCs, a similar behavior is observed. Considering the samples included in this study, a negative correlation between smoking habits and accumulations of PFAs in lung is observed. Further investigation with a higher number of subjects should be performed to check this relationship.

The accumulation of PFASs with age was studied in the analyzed tissues. In general terms, older people (more than 60 years) showed higher concentrations of PFASs, which is a clear indication that these compounds accumulate after a long-term exposure. All middle-age (40–60 years) individuals presented fairly similar levels of PFASs in the different tissues. However, some young subjects (18–39 years) also showed relatively high levels of PFASs. These values could be due to differential accumulation factors, such as dietary intake, living habits, and/or early exposure. This was also confirmed after performing a hierarchical classification (Fig. S2). Finally, a special correlation between smoking habits and PFAS accumulation in lung was performed. Although PCs did not show a positive relation between both

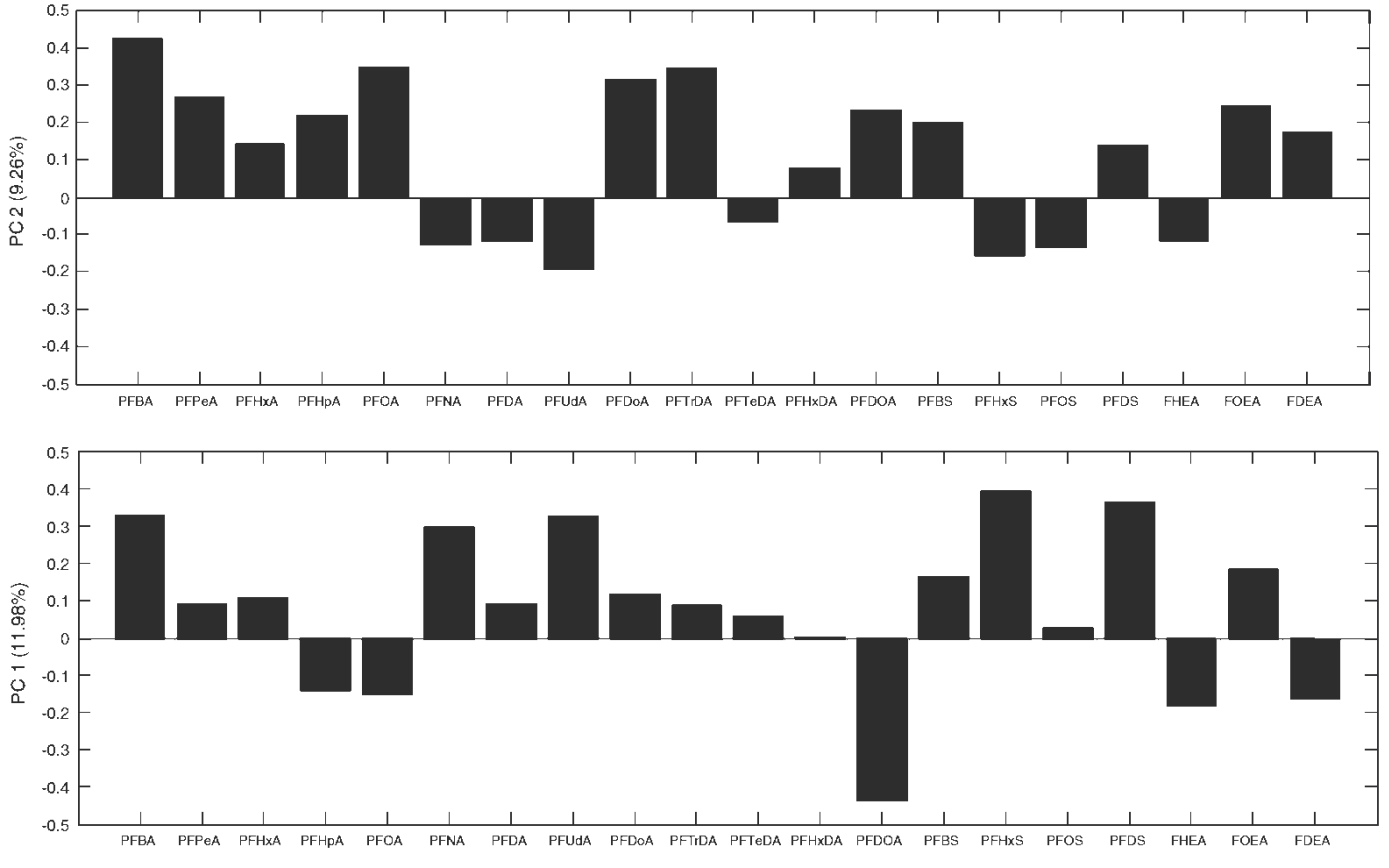

Fig. 2. Loadings of the first two principal components (PCs) for the augmented and auto scaled matrices.

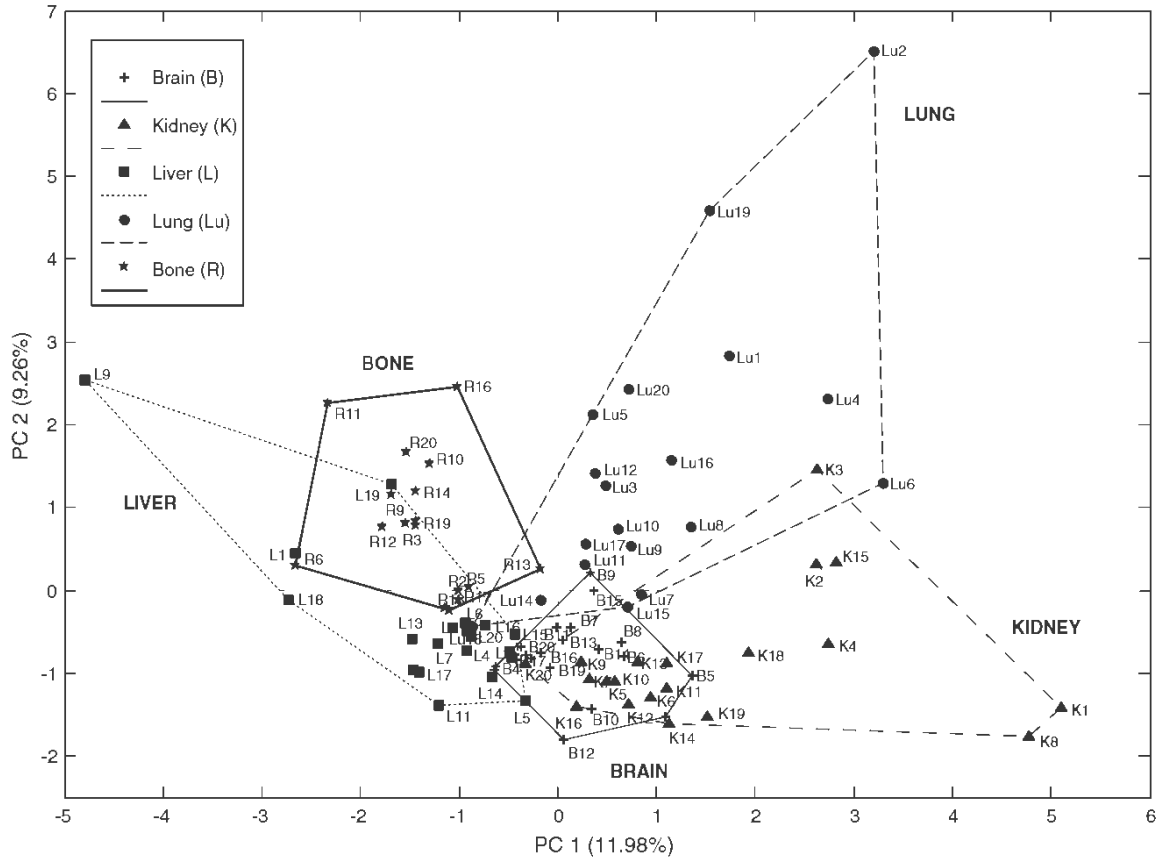

Fig. 3. Scores plots for the first two principal components (PCs) for the augmented and autoscaled matrix.

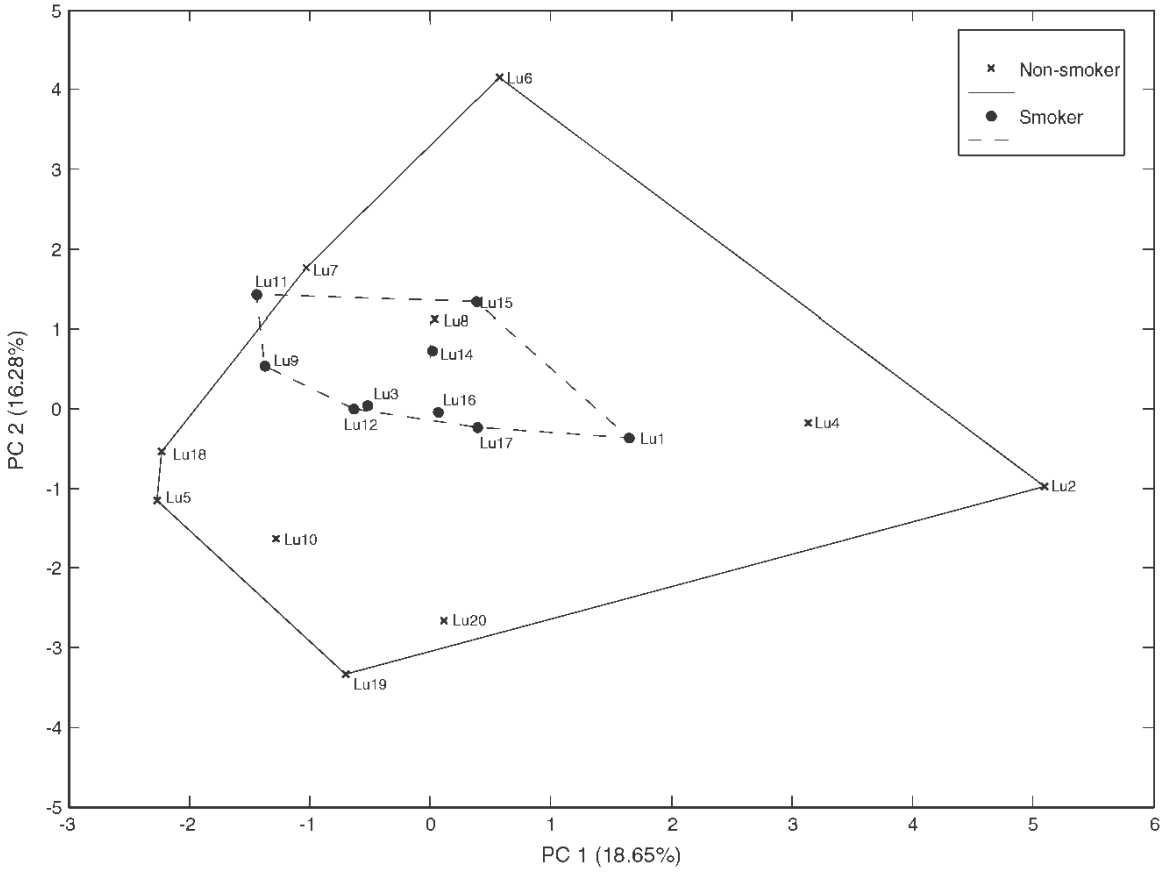

Fig. 4. Principal Component Analysis (PCA) of PFASs in lung samples.

parameters, the current number of samples was not sufficient to establish conclusions on this issue. Further investigations involving a higher number of subjects are necessary.

4. Conclusions

In this study, an effective analytical method optimized for the ultra-trace analysis of 21 PFASs in human tissues, using both small sample sizes (amount: 1 g) and a reduced sample manipulation, was addressed. The application of this approach to the analysis of 99 samples of five different tissues from 20 subjects demonstrated, for the very first time, the accumulation of certain short chain compounds, such as PFBA and PFHxA, in human tissues. Moreover, the results from the chemical analysis, together with the application of multivariate statistical techniques, showed a different accumulation pattern of the analyzed compounds in human tissues. Only few correlations were noted in the concentrations of metals and those of PFASs. However, interestingly, certain negative association between the contents of PFASs in those 5 autopsy tissues, and the levels of PCDD/Fs in adipose tissue, was observed. This finding suggests the need to fully characterize the toxicity mechanisms of PFASs, which are not currently so well understood as those of PCDD/Fs. Notwithstanding, as data refer to different biological compartments, values are not entirely comparable. In any case, the current results should be of importance for the validation of PBPK models, which are being developed for humans.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2013.06.004.

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