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 perfluoroalkyl compounds 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 2 compounds (PFBA and PFHxA) that have been identified in other monitoring studies. These compounds include:

Perfluorooctanoic acid (PFOA)
Perfluorooctane sulfonic acid (PFOS)
Perfluorohexane sulfonic acid (PFHxS)
Perfluorononanoic acid (PFNA)
Perfluorodecanoic acid (PFDeA)
Perfluoroundecanoic acid (PFUA)
Perfluoroheptanoic acid (PFHpA)
Perfluorobutane sulfonic acid (PFBuS)
Perfluorobutyric acid (PFBA)
Perfluorododecanoic acid (PFDoA)
Perfluorohexanoic acid (PFHxA)
Perfluorooctane sulfonamide (PFOSA)
2-(N-Methyl-perfluorooctane sulfonamide) acetic acid (Me-PFOSA-AcOH)
2-(N-Ethyl-perfluorooctane sulfonamide) acetic acid (Et-PFOSA-AcOH)

The term perfluoroalkyls used throughout the profile is referring to these 14 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, and 2-3 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.

Summaries of the epidemiology 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. 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. The oral data for PFOA, PFOS, and other perfluoroalkyls are presented in Tables 2-3, 2-4, and 2-5, respectively. 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 2-4, 2-5, 2-6, 2-7, and 2-8.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (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, PFDeA, PFUA, PFHpA, PFBuS, PFBA, PFDoA, PFHpA, PFOSA, Me-PFOSA-AcOH, and Et-PFOSA-AcOH. It is noted that for most health effects, there are no data for a number of the perfluoroalkyl compounds. The compound-specific discussions are further divided into Epidemiology 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 epidemiology and animal studies; the literature search framework for identifying these studies is discussed in Appendix B. As illustrated in Figures 2-1, 2-2, and 2-3, most of the health effects data come from epidemiology studies. For PFOA, PFOS, and other perfluoroalkyls, 67, 70, and 73%, respectively, of the health effect studies were in humans; it is noted that a number of epidemiology studies examined more than one perfluoroalkyl compound. Three population categories were examined in epidemiology studies: workers at facilities involved in the production or use of perfluoroalkyls (most of the studies involved workers at two U.S. facilities), 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), and populations exposed to background levels of perfluoroalkyl compounds (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 epidemiology studies. 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). Most of the epidemiology 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 epidemiology studies involves co-exposure to multiple perfluoroalkyl compounds. A number of the epidemiology studies have found strong correlations between serum levels of different perfluoroalkyl compounds. *In vitro* studies (Carr et al. 2013; Wolf et al. 2014) have shown that at lower concentrations, binary pairs of perfluoroalkyl compounds 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 epidemiology data.

Although a large number of epidemiology studies have examined the potential of perfluoroalkyl compounds to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causality. ATSDR used a weight-of-evidence approach to evaluate whether the available data supported a link between perfluoroalkyl exposure and a particular health effect. This weight-of-evidence approach takes 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 strong evidence for an association, it does not 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.

The available epidemiology studies suggest links between perfluoroalkyl exposure and several health outcomes:

- **Hepatic effects.** Increases in serum enzymes and decreases in serum bilirubin, observed in studies of PFOA, PFOS, and PFHxS, are suggestive of liver damage. In addition, the results of epidemiology studies of PFOA, PFOS, PFNA, and PFDeA suggest a link between perfluoroalkyl exposure and increases in serum lipid levels, particularly total cholesterol and LDL cholesterol.

- **Cardiovascular effects.** There is suggestive epidemiological evidence for an association between serum PFOA and PFOS and pregnancy-induced hypertension and/or pre-eclampsia.

- **Endocrine effects.** Epidemiology studies provide suggestive evidence of a link between serum PFOA and PFOS and an increased risk of thyroid disease.

- **Immune effects.** Evidence is suggestive of a link between serum PFOA, PFOS, PFHxS, and PFDeA levels and decreased antibody responses to vaccines. A possible link between serum PFOA levels and increased risk of asthma diagnosis has also been found.

- **Reproductive effects.** A suggestive link between serum PFOA and PFOS levels and an increased risk of decreased fertility has been found.

- **Developmental effects.** Evidence is suggestive of a link 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.

As presented in Figures 2-1, 2-2, and 2-3, most of the available literature on the health effects of perfluoroalkyl compounds 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, PFDeA, PFUA, PFBA, PFBuS, PFDoA, and PFHpA toxicity. The effects include increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipid levels. The 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.

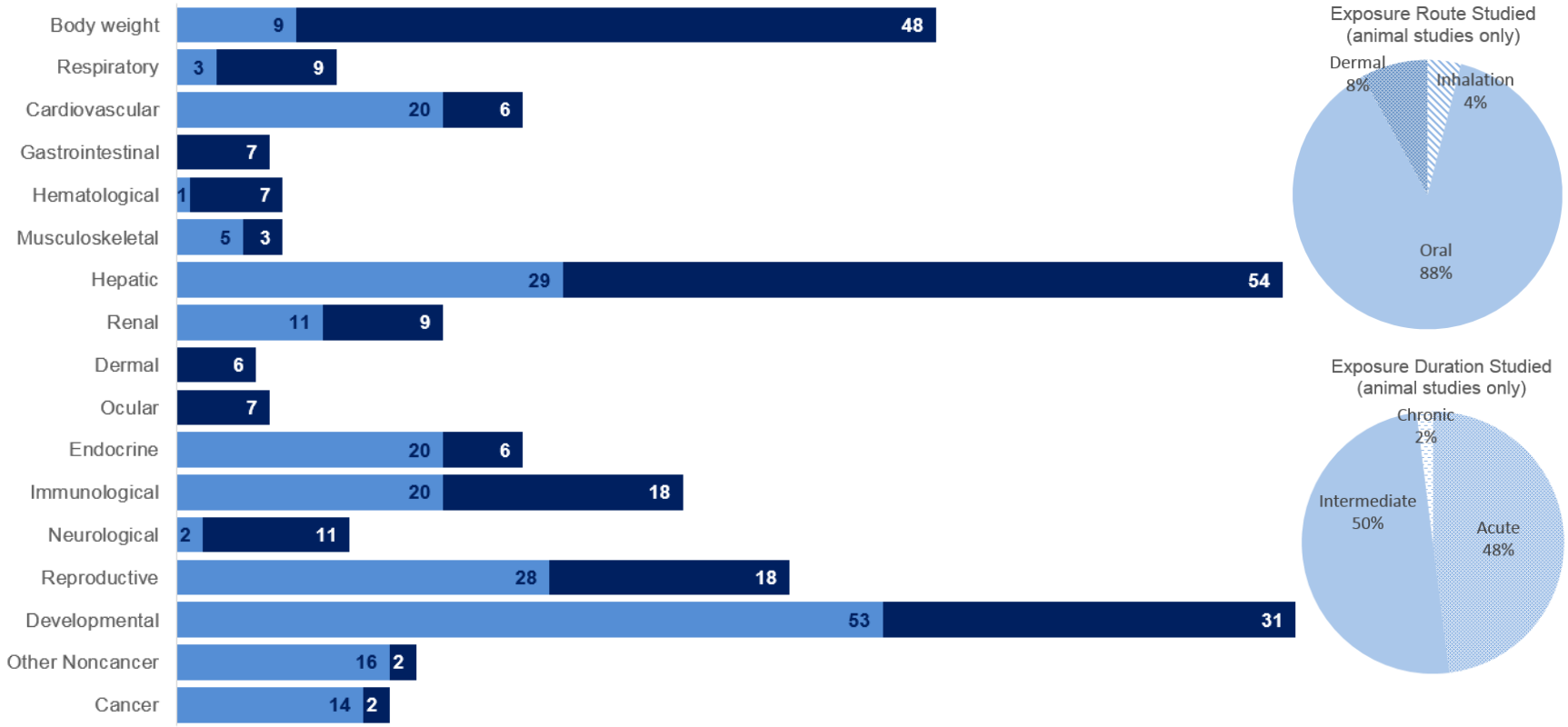
- **Immune effects.** Evidence from acute and intermediate oral studies in mice indicates that immune endpoints are sensitive targets of PFOA and PFOS toxicity. The most commonly reported effect was an impaired response to antigens. No alteration in antigen response was observed in the one study of PFNA.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

- **Reproductive effects.** Decreases in mammary gland development have been observed in mice orally exposed to PFOA. In general, studies of PFOA and PFOS have not found alterations in fertility.

- **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, PFDeA, PFUA, and PFBA toxicity. The developmental effects include decreases in pup body weight, decreases in pup survival, and alterations in locomotor activity.

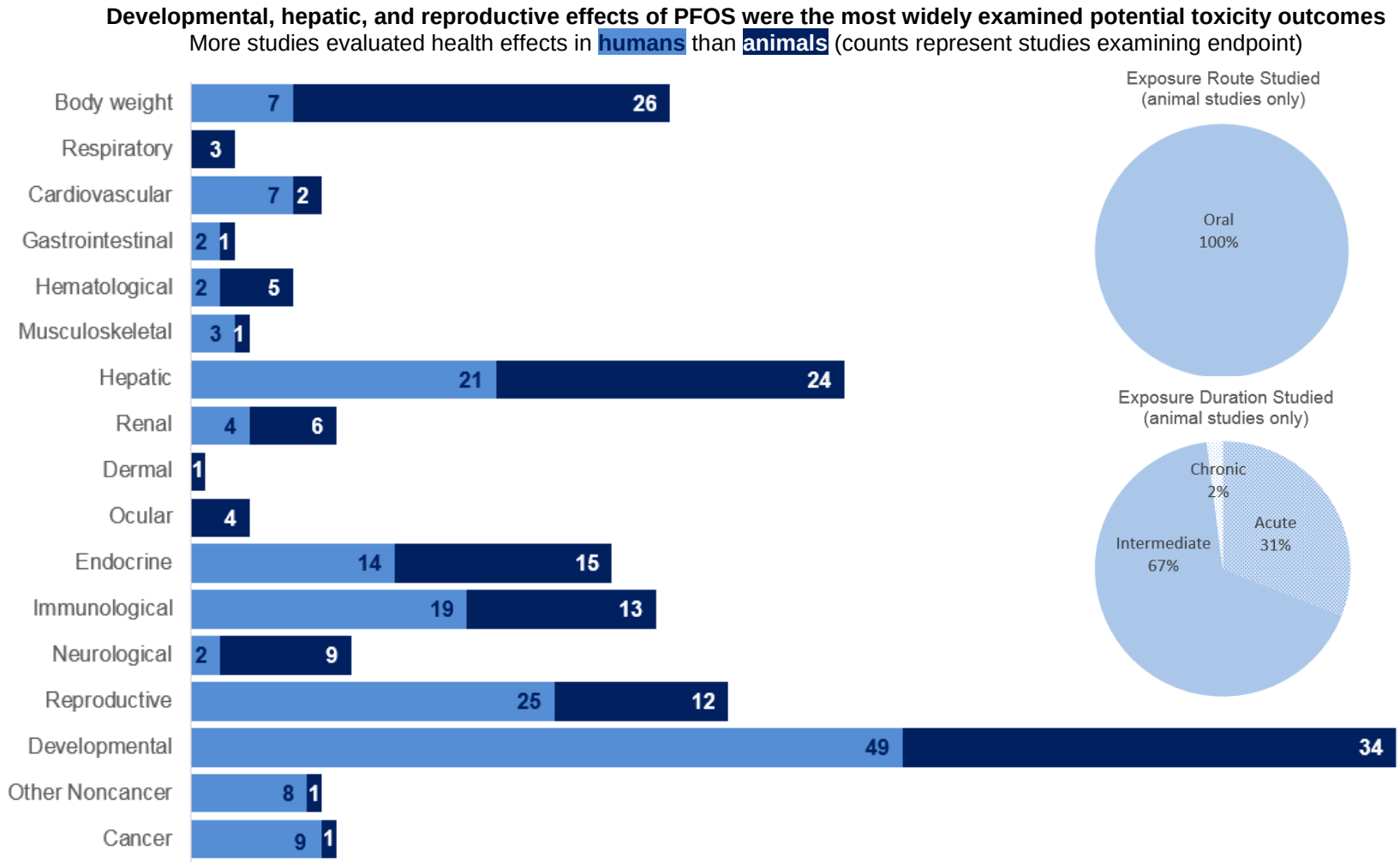
Figure 2-1. Overview of the Number of Studies Examining PFOA Health Effects*

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 271 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.

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

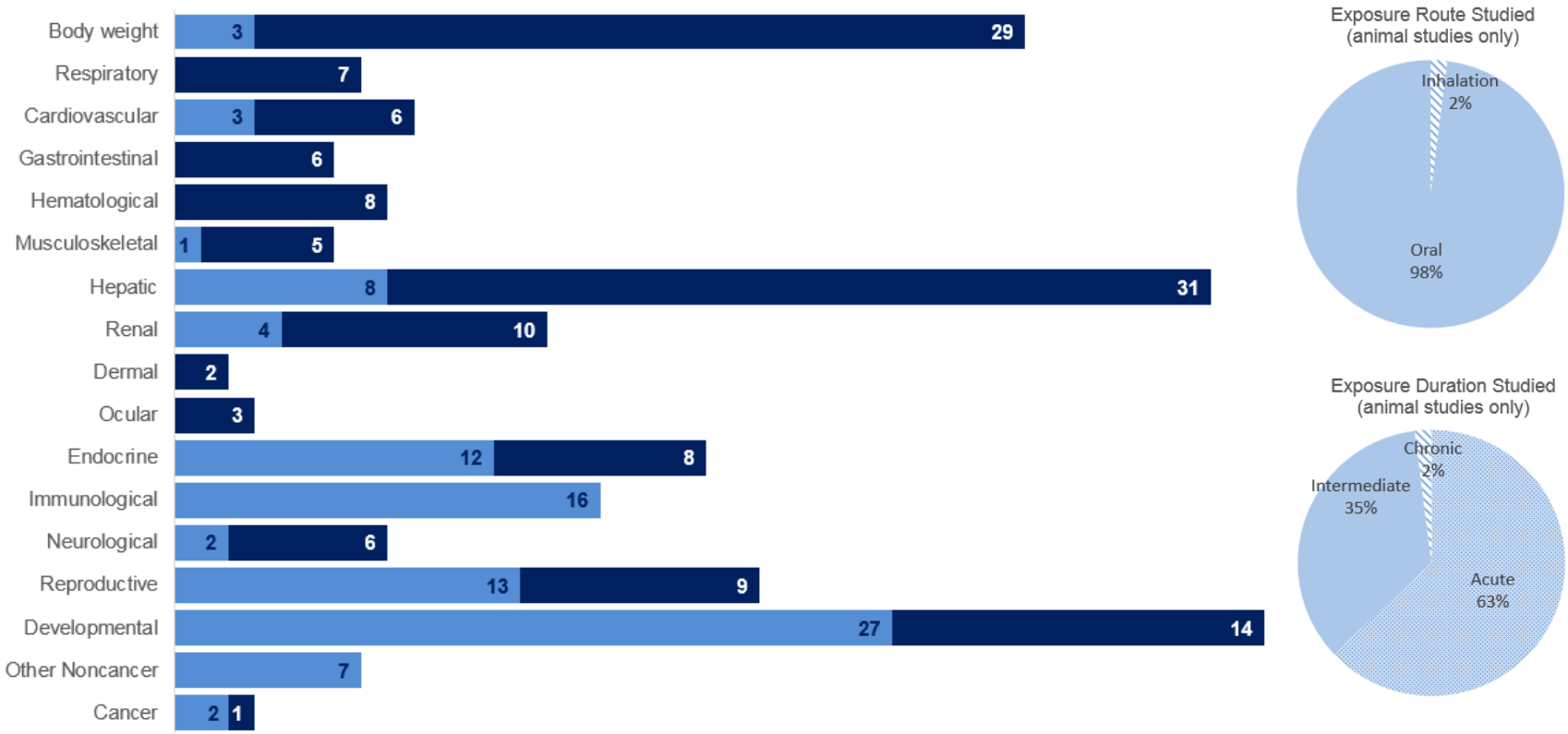


*Includes studies discussed in Chapter 2. A total of 218 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. In this figure, the number of human studies is referring to the number of publications.

Figure 2-3. 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 127 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.

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 (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	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									

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 (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effect
APFO									
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 2-4.

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 = hematology; 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 2-4. Levels of Significant Exposure to PFOA – Inhalation
Acute (≤14 days)

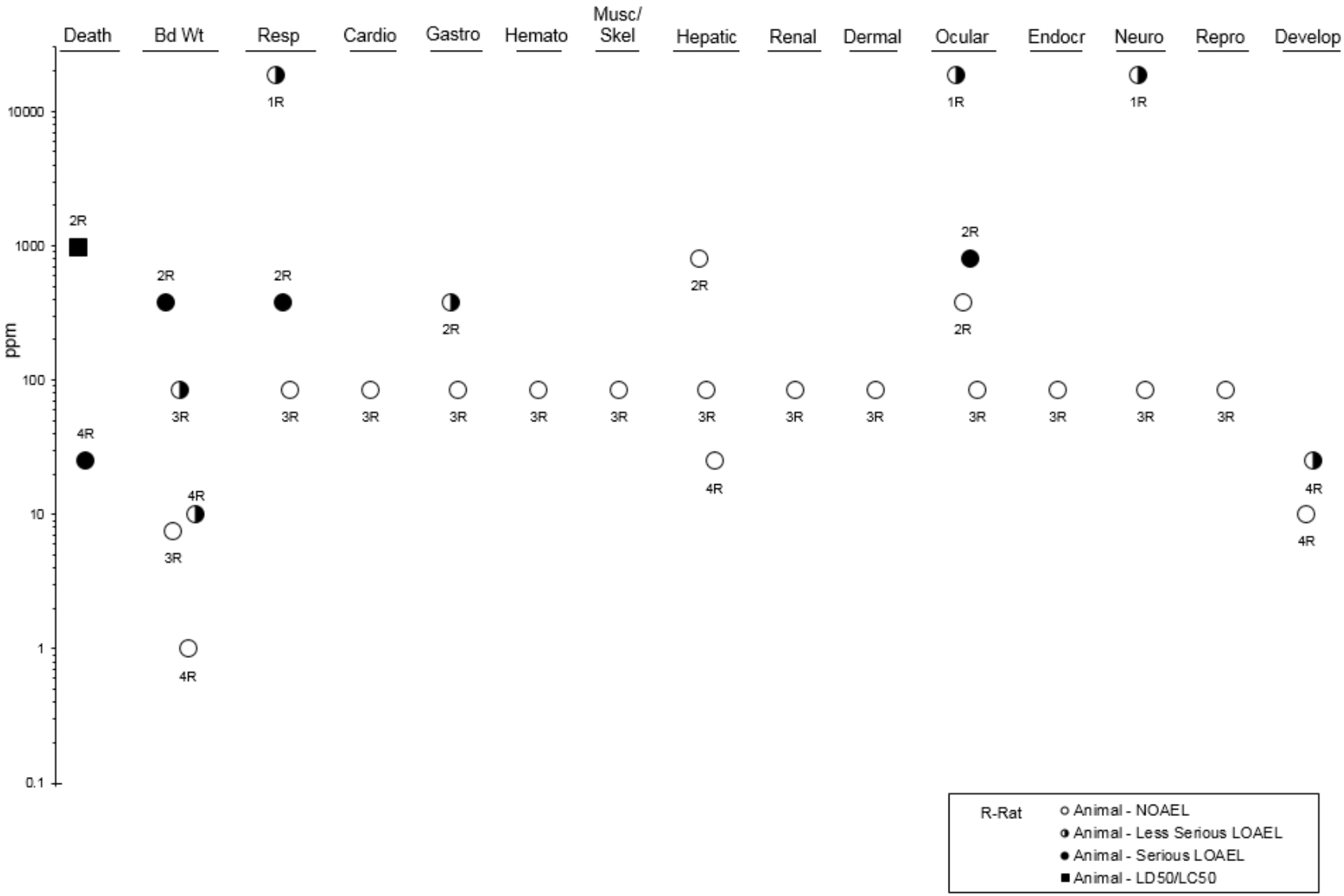


Table 2-2. Levels of Significant Exposure to Other Perfluoroalkyls – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	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 2-5.

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 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Inhalation
Acute (≤14 days)

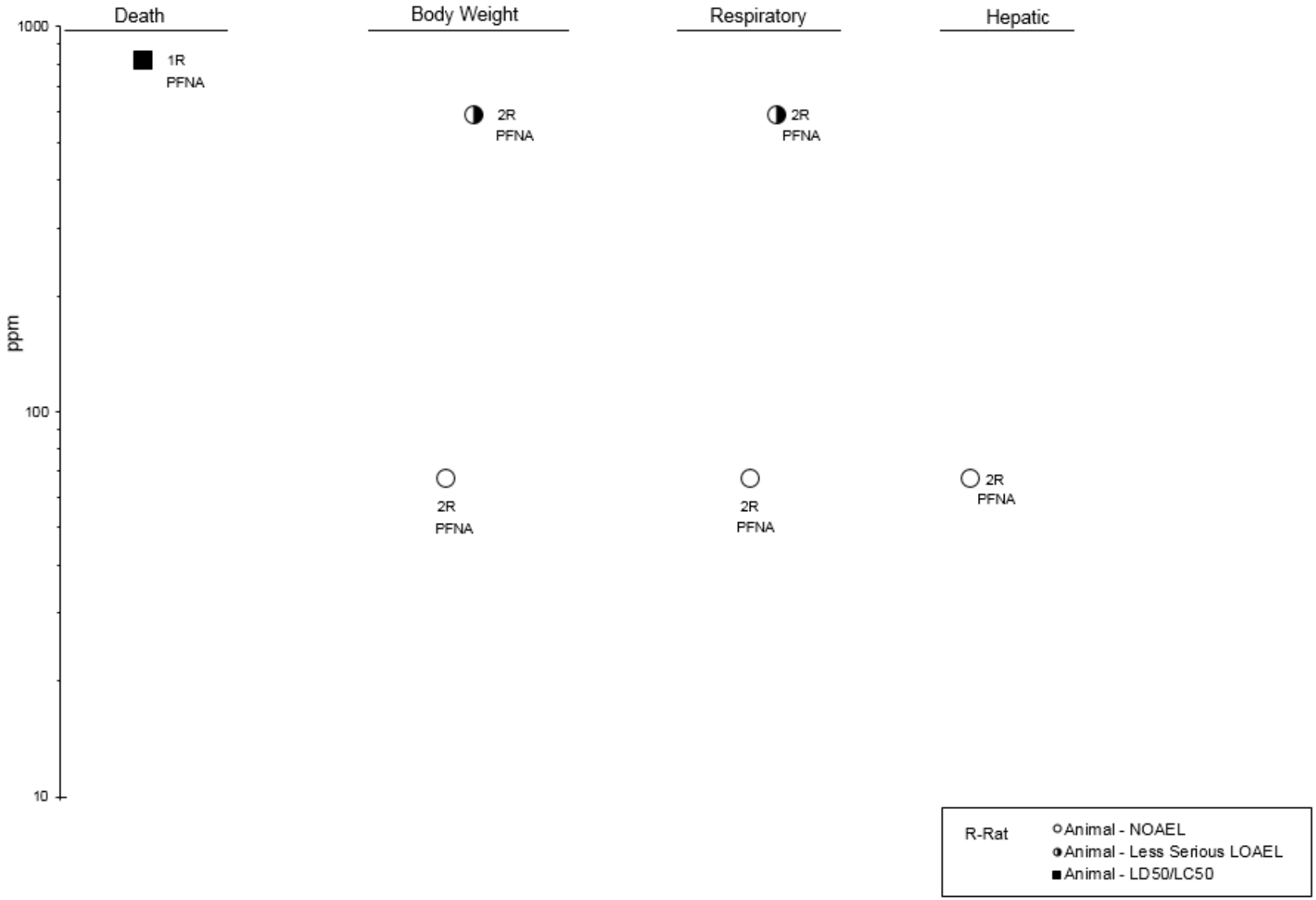


Table 2-3. Levels of Significant Exposure to PFOA – Oral

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 4 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 (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									
17	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									
18	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									
19	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									

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
20	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									
21	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									
22	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									
23	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									
24	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									

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
25	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									
26	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									

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
27	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									
28	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									
29	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									
30	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									

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
31	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									
32	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									
33	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									
34	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									
35	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									

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
INTERMEDIATE EXPOSURE									
36	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									
37	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

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	100			No histological alterations in testes or ovaries
Griffith and Long 1980									
APFO									
38	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									
39	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									
40	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

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
					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									
41	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									
42	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									
43	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									

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 (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									
45	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			
					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									
46	Rat (Sprague-Dawley) 10 dams; 12–13 pups	GDs 12–18 (GO)	0, 5, and 20	BC, BW, DX, OW	Bd Wt	5	20		30% reduction in body weight of dams
					Develop	5	20		13% reduction in body weight of male pups
Li 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
47	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									
48	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									
49	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.									
50	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									

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
51	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									
52	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									
53	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									
54	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									

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
55	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									
56	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									
57	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									
58	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									

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) 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									
60	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									
61	Mouse (C57BL/6) 6 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									
62	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									

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
63	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									
64	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									
65	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									
66	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									
67	Mouse (C57BL/6/Bk1) 6 F	GDs 1–21 ad lib (F)	0, 0.3		Develop		0.3[b]		Increased locomotor activity in adult offspring
Onishchenko 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
68	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									
69	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									
70	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									
71	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									

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 (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									
73	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									
74	Mouse (C57BL/6N) 7–8 M	3 weeks (F)	0, 5	HP	Hepatic		5		Hepatocellular hypertrophy and degeneration
Tan et al. 2013									
PFOA									
75	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									
76	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									

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) 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									
78	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									
79	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									
80	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									
81	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									

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
82	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									
83	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
CHRONIC EXPOSURE									
84	Rat (Sprague-Dawley) 65 M,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	1.5 M		15 M	Lung hemorrhage
					Cardio	15			
					Gastro	15			
					Hemato	15			
					Hepatic	1.5	15		Increased serum ALT and AST and hepatocellular hypertrophy at ≥1.5 mg/kg/day; hepatocellular necrosis at 15 mg/kg/day after 1 year of exposure
					Renal	15			
					Ocular	15			
					Endocr	15			
					Immuno	15			
					Neuro	15			
					Repro	1.5 M	15 M		Vascular mineralization in the testes
							1.5 F		Tubular hyperplasia in the ovaries
					Other noncancer		1.5 M		Inflammation of the salivary gland
					Cancer				No significant increases in the incidence of neoplastic lesions
3M 1983									
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
85	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				No significant increases in the incidence of neoplastic lesions
Biegel et al. 2001									
APFO									

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

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 = hematology; 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; OF = organ function; OP = ophthalmology; OW = organ weight; PFOA = perfluorooctanoic acid; PND = postnatal day; PPARα = peroxisome proliferator-activated receptor-α; Repro = reproductive; Resp = respiratory; sRBC = sheep red blood cell; TSH = thyroid-stimulating hormone; TT4 = total thyroxine; TWA = time-weighted average; UR = urinalysis; W = water; WI = water intake

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

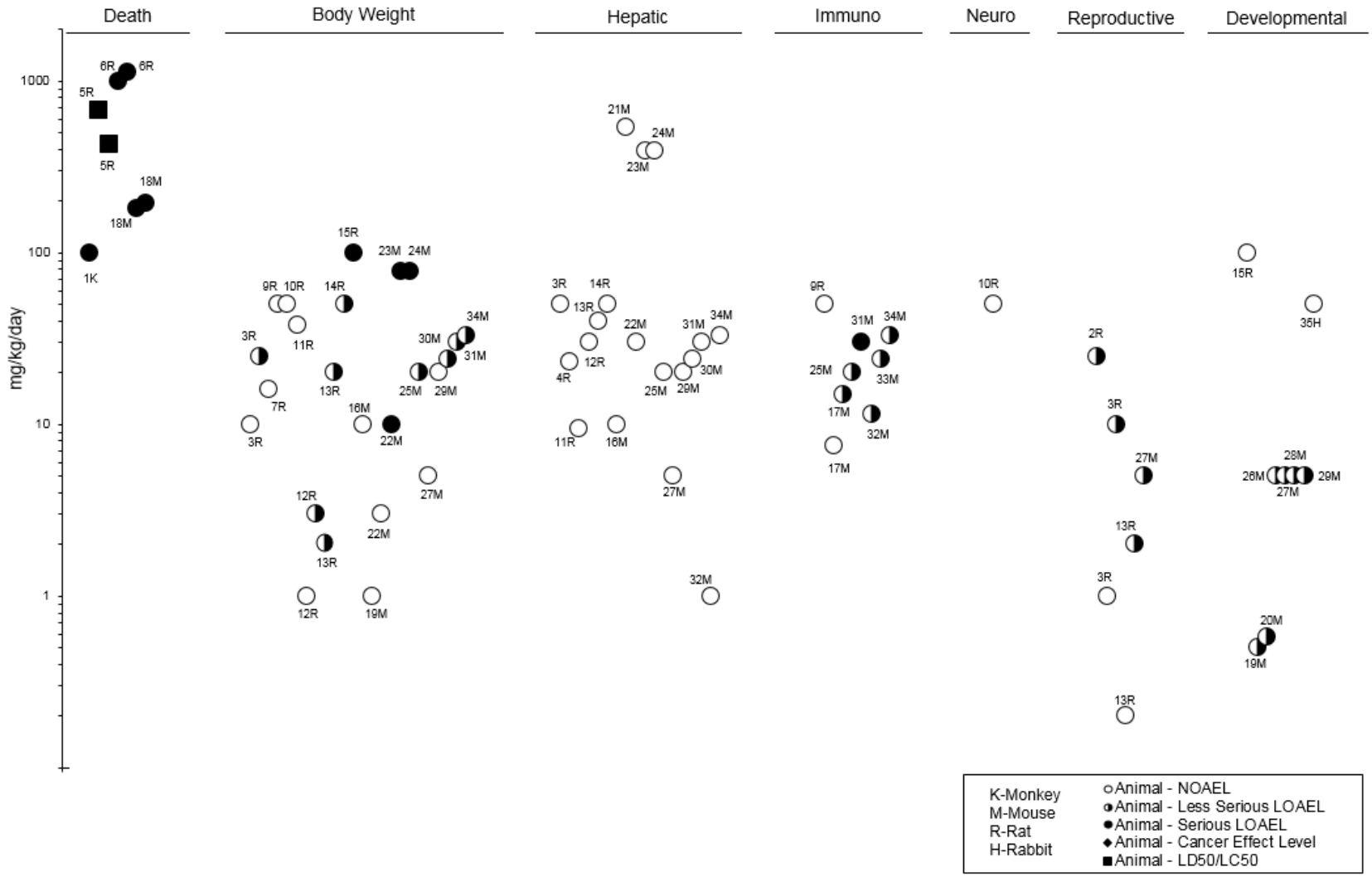


Figure 2-6. Levels of Significant Exposure to PFOA – Oral
Intermediate (15–364 days)

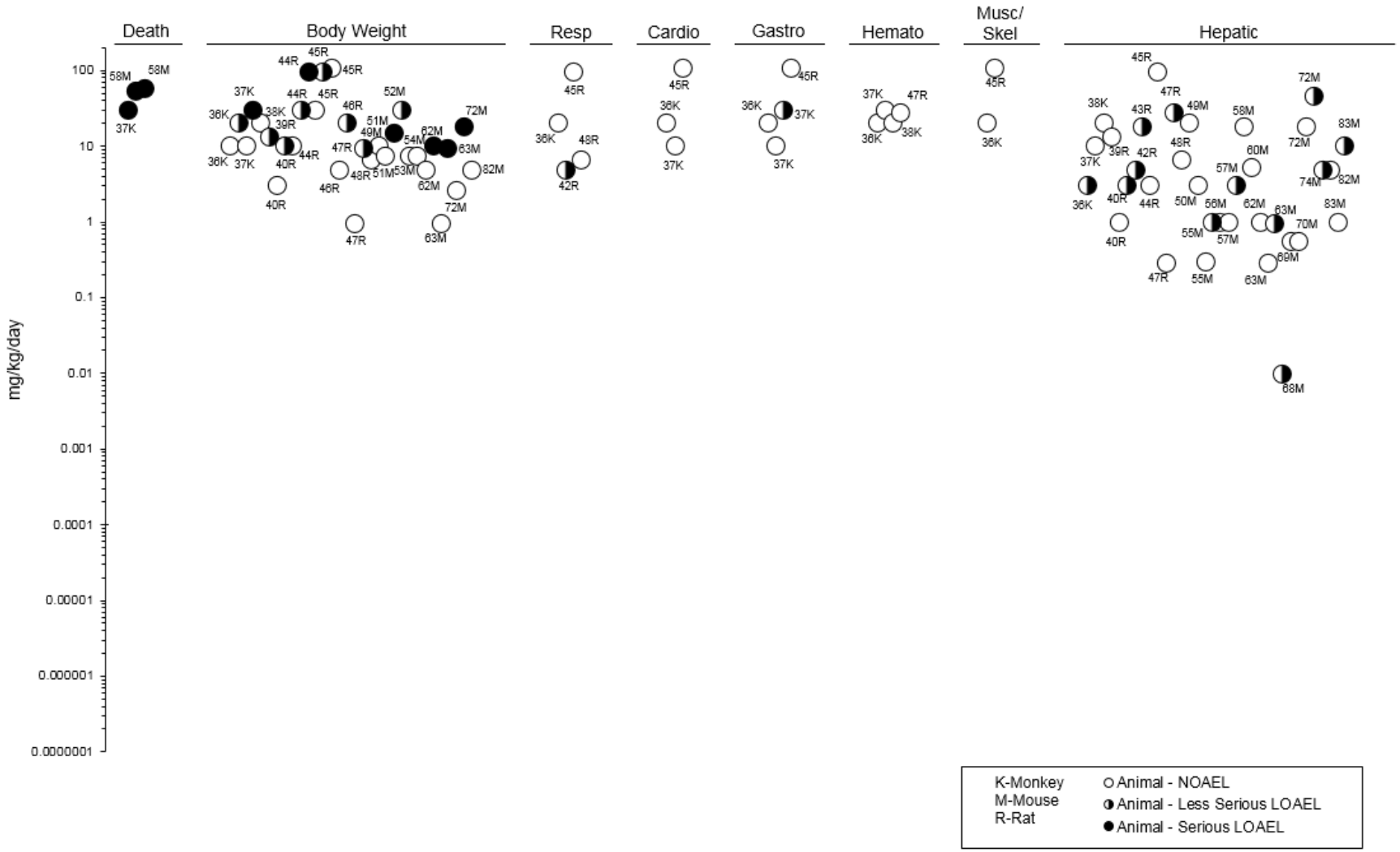


Figure 2-6. Levels of Significant Exposure to PFOA – Oral
Intermediate (15–364 days)

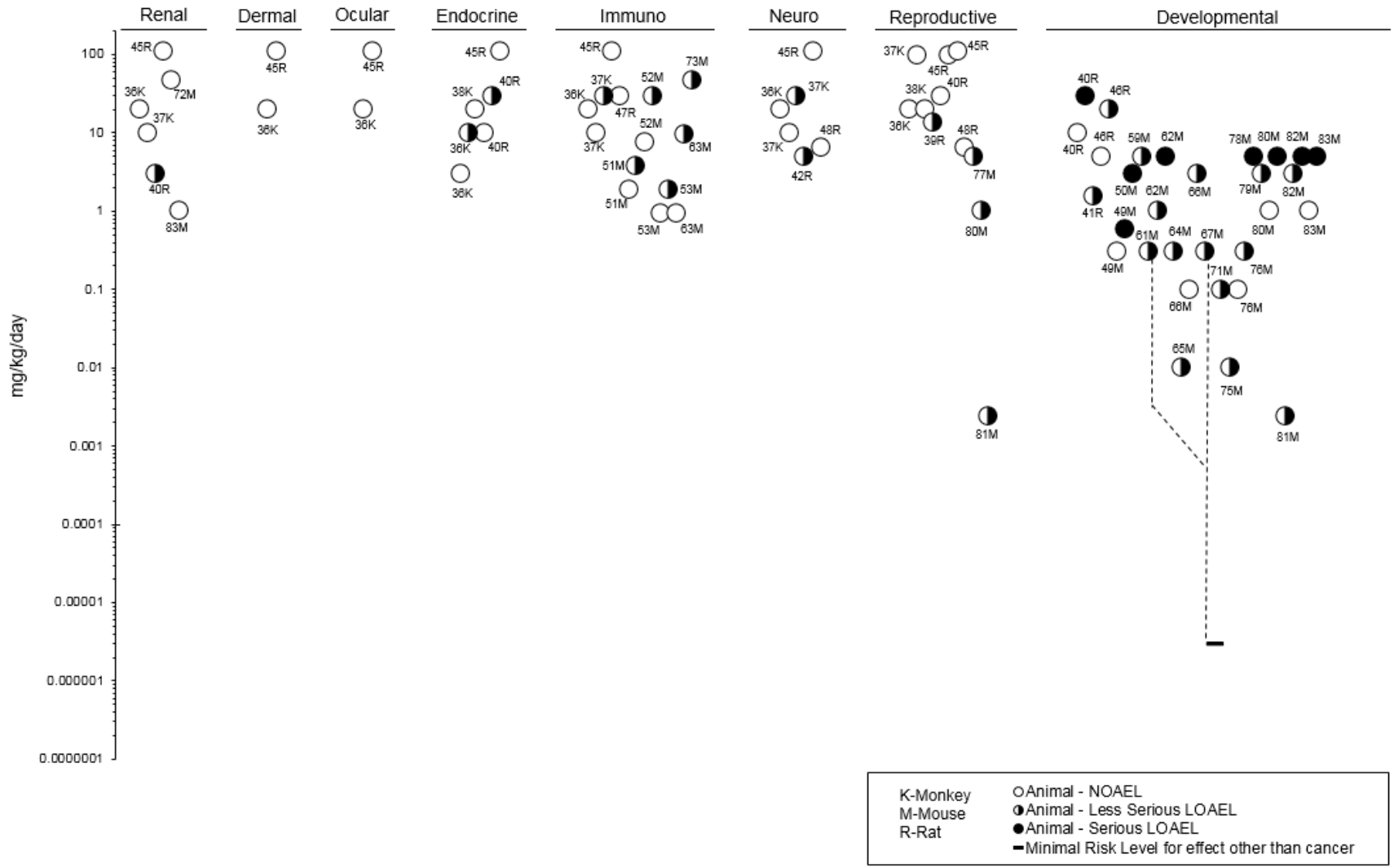


Figure 2-6. Levels of Significant Exposure to PFOA – Oral
Chronic (≥365 days)

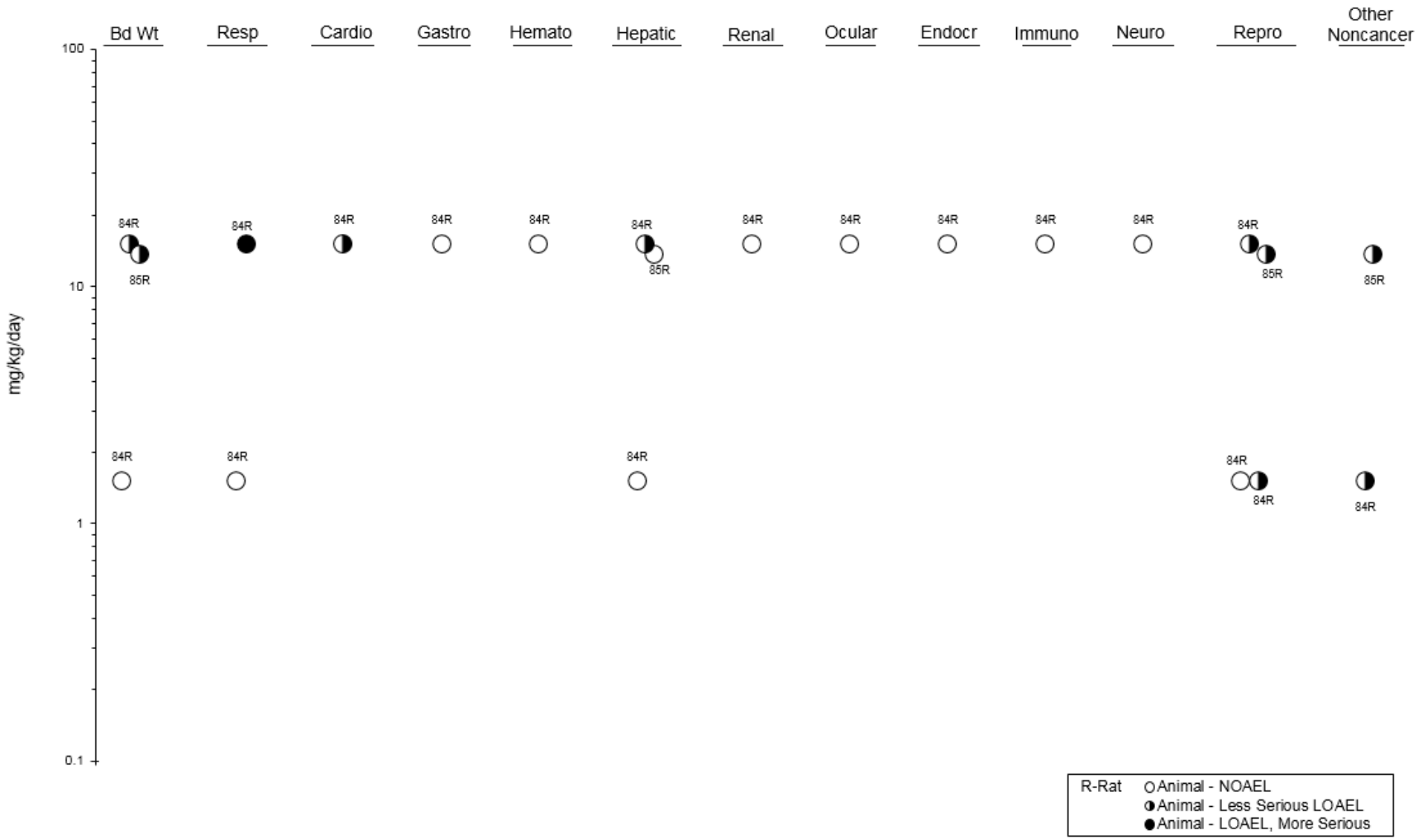


Table 2-4. Levels of Significant Exposure to PFOS – Oral

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	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**									
2	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**									
3	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**									
4	Rat (Sprague-Dawley) 10 M	7 day (F)	0, 1.79, 8.96	HP	Hepatic	8.96			Hepatocellular hypertrophy, incr liver weight, decreased serum cholesterol at ≥1.79 mg/kg/day
					Endocr	8.96			
Elcombe et al. 2012b **PFOS potassium salt**									
5	Rat (Sprague-Dawley) NS F	2 day GDs 19–20 1 x/d (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**									

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	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									
7	Rat (Sprague-Dawley) NS F	2 days GDs 19–20 1 x/d (G)	0, 25, 50		Develop			25	Increased neonatal mortality
Grasty et al. 2005									
PFOS potassium salt									
8	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									
9	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									
10	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									

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 (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									
12	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									
13	Mouse (CD-1) 8–10 F	GDs 12–18 (GW)	0, 6	DX	Develop		6		Decreased distanced traveled in open field test at 3 months of age
Fuentes et al. 2007a									
PFOS potassium salt									
14	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									
15	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
16	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									
17	Mouse (BALB/c) 5 F	7 days (GW)	20	BW, OW, BC	Bd Wt		20		41% decrease 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									
18	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									
19	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									

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
20	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									
21	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									
22	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									
23	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									
24	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									
25	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
26	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									
27	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									
28	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									
29	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
30	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									
31	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									
32	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	1.33 M	3.21 M		12% decrease in terminal body weight
					Hepatic	6.34 M			Increased relative liver weight at ≥0.14 mg/kg/day
					Immuno	6.34 M			
Lefebvre et al. 2008									
PFOS									
33	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									

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
34	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									
35	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									
36	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
					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									

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) 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									
38	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									
39	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									

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
40	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									
41	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
42	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									
43	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									
44	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									
45	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
46	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									
47	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									
48	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									
49	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									
50	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
PFOS potassium salt									
Fuentes et al. 2007c									

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 (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									
52	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									
53	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									
54	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									
55	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									

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
56	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									
57	Mouse (C57BL/6J-Apc+/+) 20–21 F	GDs 1–7 (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									
58	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									
59	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									

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
60	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									
61	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									
62	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
					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									
63	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									

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
64	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									
65	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									

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
66	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									
67	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			
					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

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
					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 2-7.
[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.

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 = hematology; 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; sRBC = sheep red blood cell; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; TT4 = total thyroxine; TWA = time-weighted average; W = water

Figure 2-7. Levels of Significant Exposure to PFOS – Oral
Acute (≤14 days)

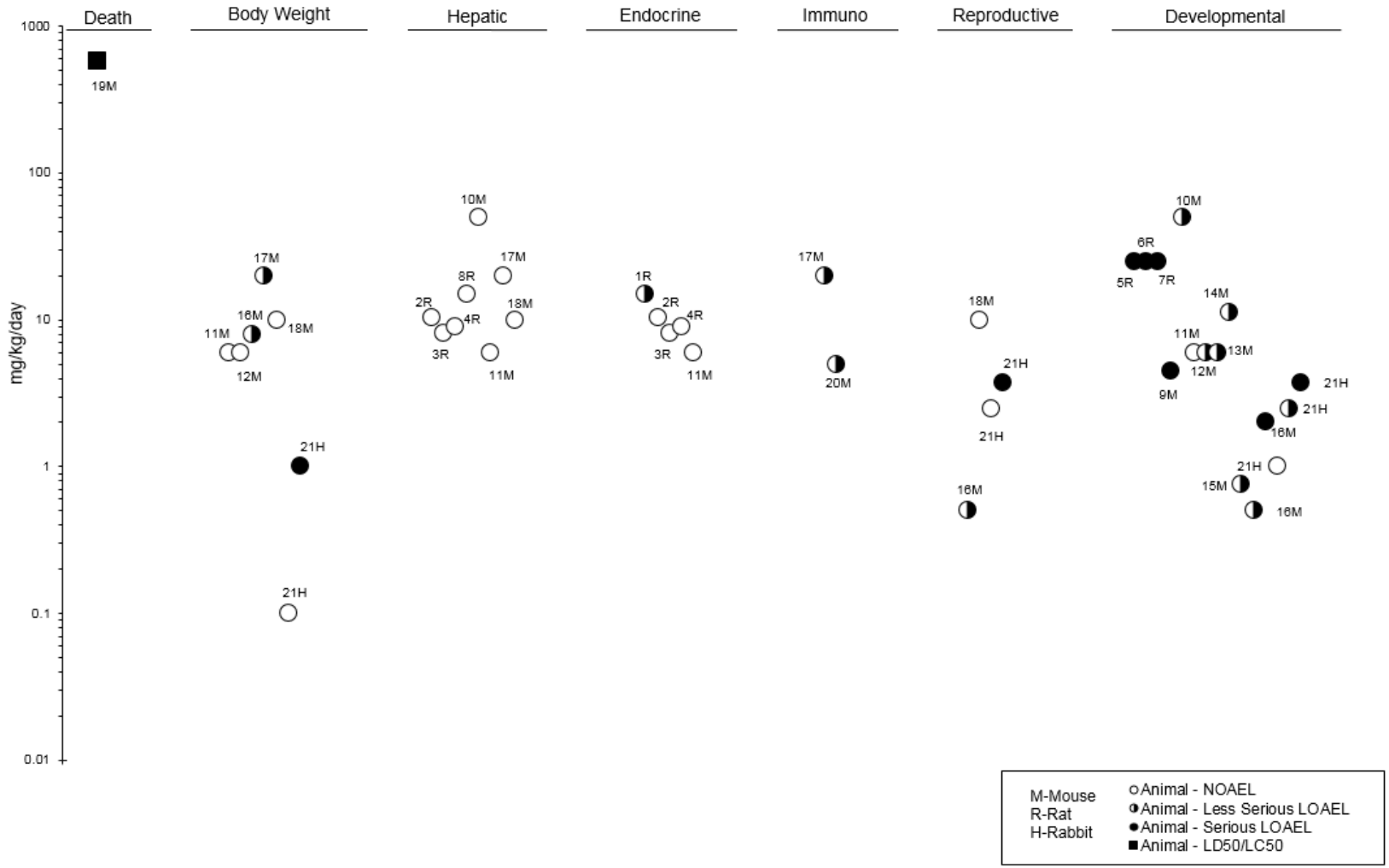


Figure 2-7. Levels of Significant Exposure to PFOS – Oral
Intermediate (15–364 days)

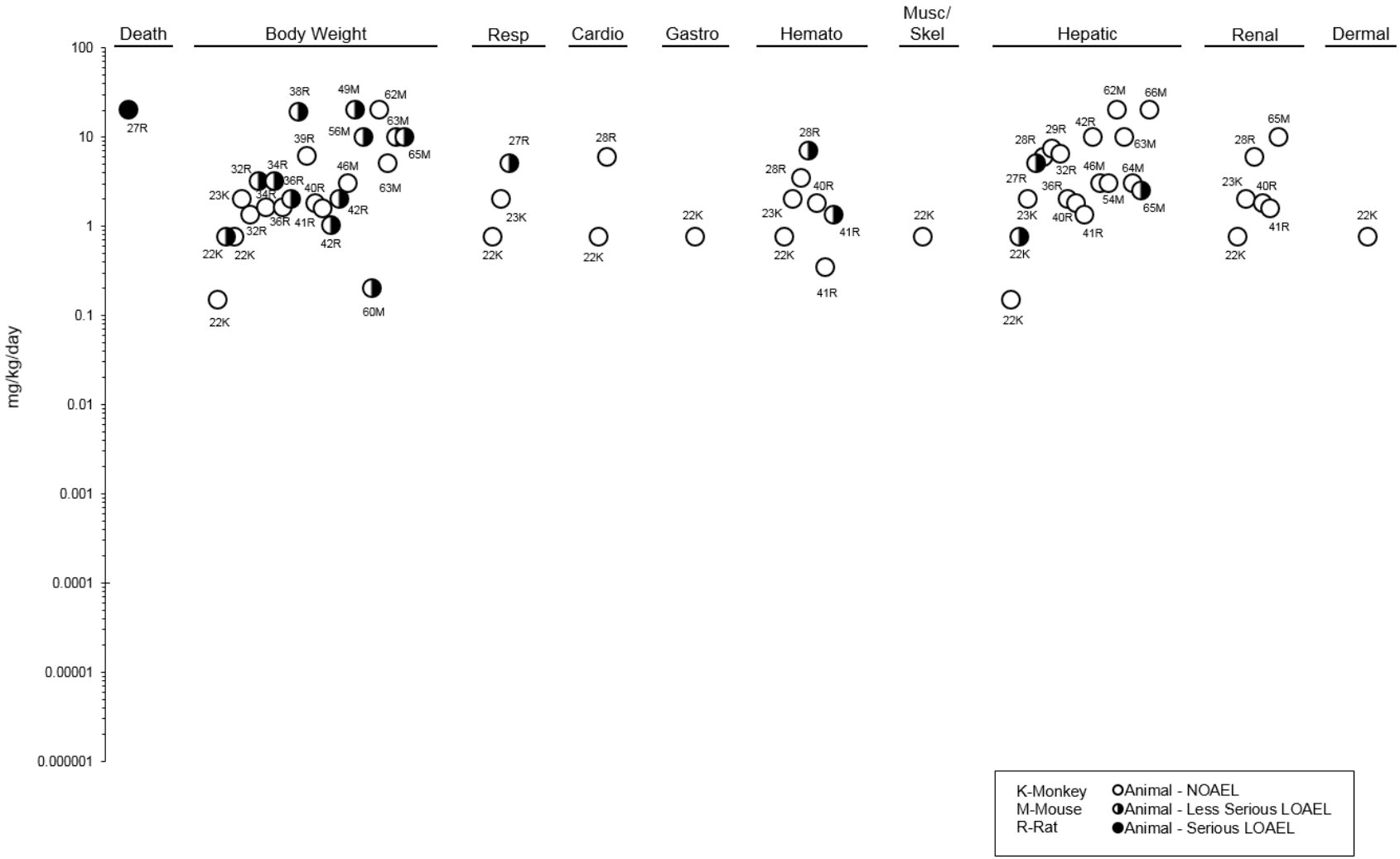


Figure 2-7. Levels of Significant Exposure to PFOS – Oral
Intermediate (15–364 days)

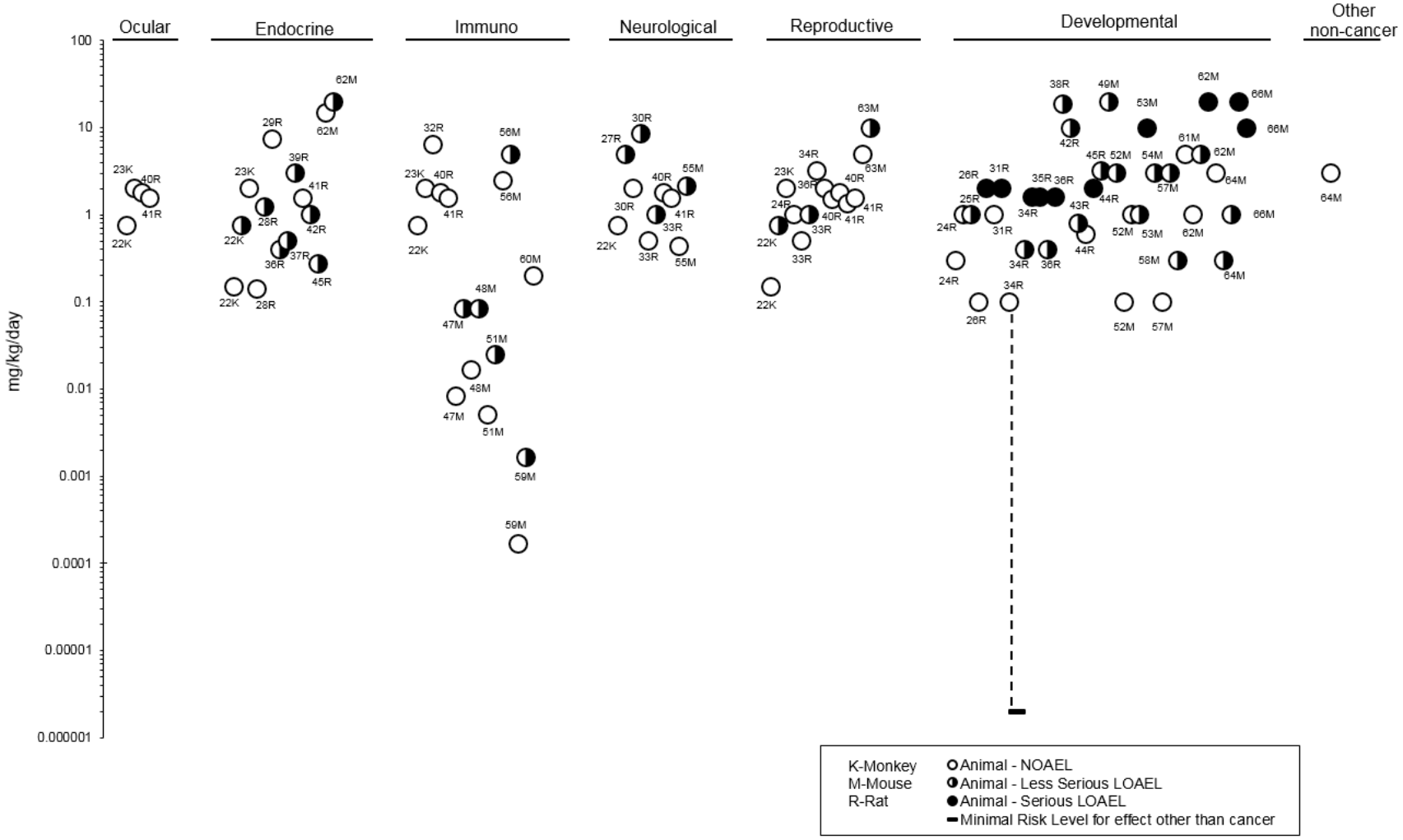


Figure 2-7. Levels of Significant Exposure to PFOS – Oral
Chronic (≥365 days)

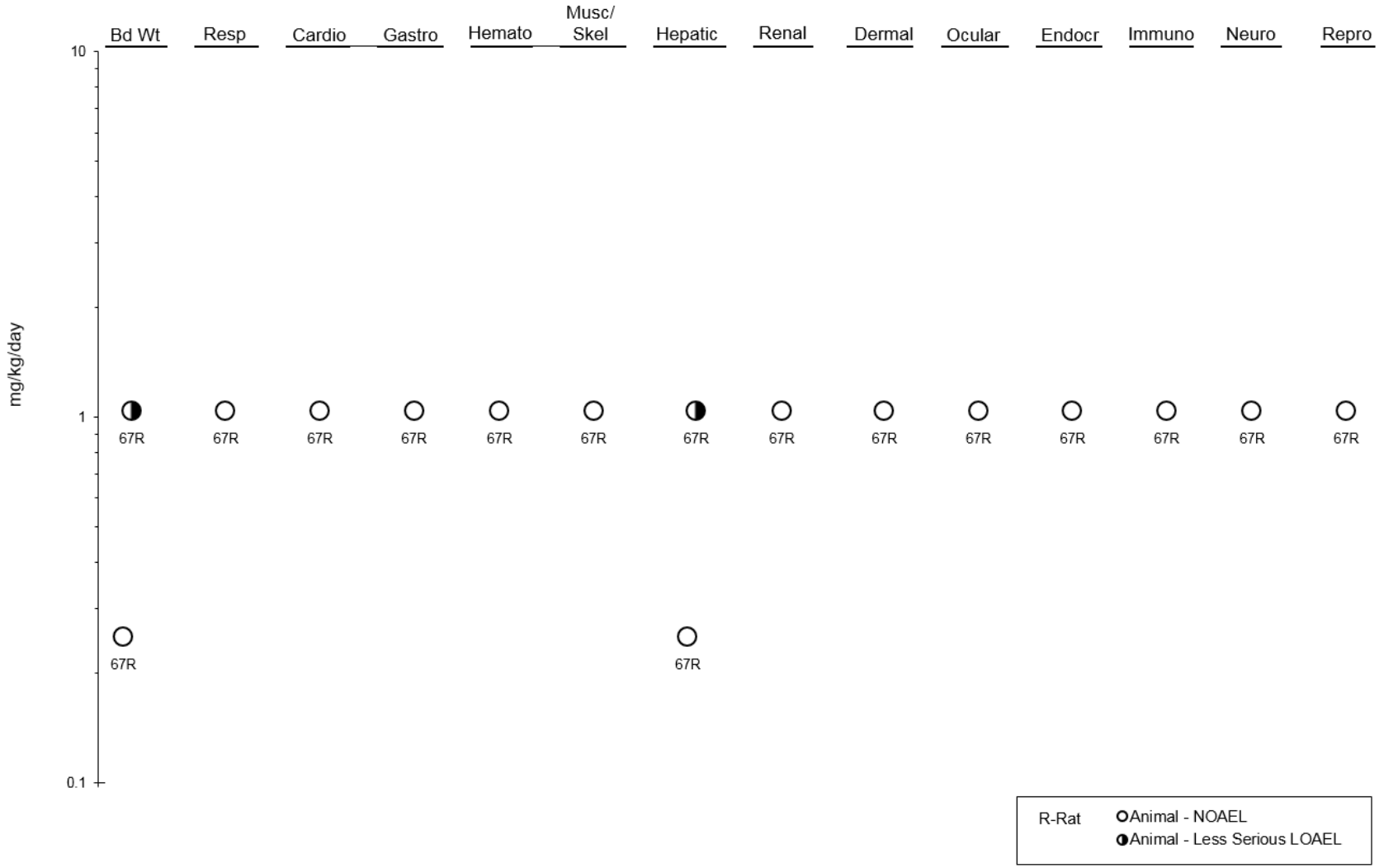


Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

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									
PFDeA									
14	Rat (Wistar) 5–12 M	Once (GO)	0, 50 PFDeA	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 PFDeA	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 PFDeA	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 PFDeA	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 PFDeA	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 PFDeA	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 PFDeA	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									
PFDoA									
24	Rat (Wistar) 5–12 M	Once (GO)	0, 5, 20, 50 PFDoA	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 PFDoA	FX	Neuro	50			No alterations in open field activity
Kawabata et al. 2017									
26	Rat (Wistar) 8 M	Once (GO)	0, 50 PFDoA	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 PFDoA	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 PFDoA	OW, BI, HP	Hepatic	10			Increased liver weight, increase in increased hepatic triglyceride and cholesterol levels at ≥5 mg/kg/day; increased serum triglyceride levels at 10 mg/kg/day
Zhang et al. 2008									
PFOSA									
29	Rat (Sprague-Dawley) 15 M	Once (GO)	0, 5 PFOSA	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									

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									
32	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; Hoberman and York 2003									
33	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									

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									
34	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									
35	Mouse (CD-1) 8–10F	GDs 1–17 (GW)	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									
36	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[c]	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									

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
37	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									
PFUA									
38	Rat (Crl:CD[SD]) 12 M,F (main); 5 M,F (other)	41–46 days (GO)	0, 0.1, 0.3, 1.0 PFUA	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%)
					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									

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
PFBuS									
39	Rat (Sprague-Dawley)	28 days 1 time/day (GO)	0, 100, 300, 900 PFBuS	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									
40	Rat (Sprague-Dawley) 10 NS	90 days (G)	0, 60, 200, 600 PFBuS	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			
					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									

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	Rat (Sprague-Dawley)	P0: starting 70 days prior to mating; F1: starting at weaning (G)	0, 30, 100, 300, 1,000 PFBuS	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									
42	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 30 PFBuS	BW, FI, BC, OW	Bd wt	30			
					Hepatic	30			Decreased plasma triglyceride levels
Bijland et al. 2011									
PFBA									
43	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
44	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									
45	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									
PFDoA									
46	Rat (Sprague-Dawley) 8 F	28 days PNDs 24–72 1 time/day (GW)	0, 0.5, 1.5, 3 PFDoA		Develop	1	3		40% reduced serum estradiol and 20% increased serum cholesterol in pubertal females
Shi 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
CHRONIC EXPOSURE									
PFHxA									
47	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 red blood cell 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 2-8.
[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.

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; 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 = hematology; HP = histopathology; Immuno = immunotoxicological; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; PFBA = perfluorobutyric acid; PFBuS = perfluorobutane sulfonic acid; PFDeA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOSA = perfluorooctane sulfonamide; PFUA = perfluoroundecanoic acid; PPARα = peroxisome proliferator-activated receptor-α; Repro = reproductive; Resp = respiratory; TG = teratogenicity; UR = urinalysis

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

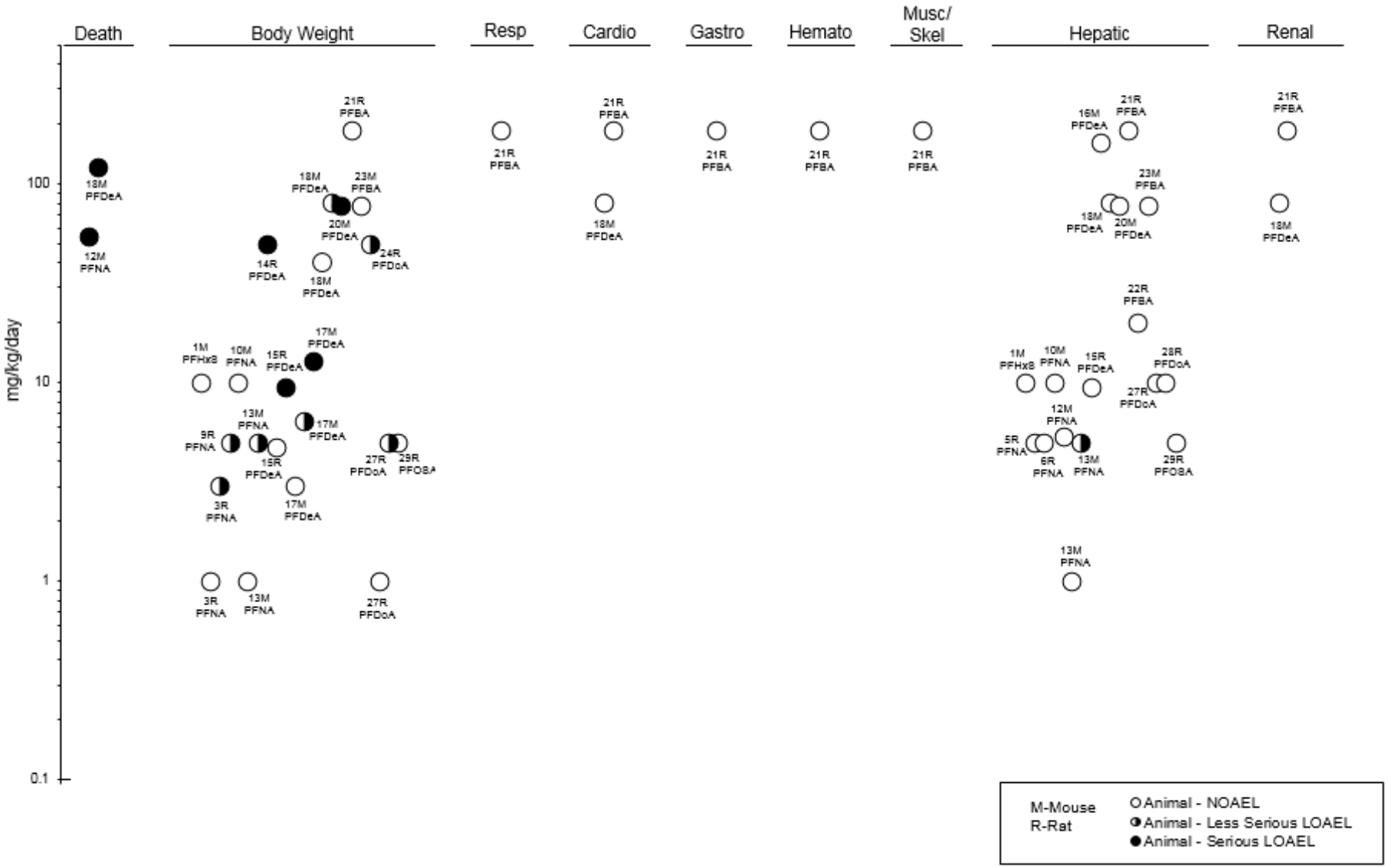


Figure 2-8. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Acute (≤14 days)

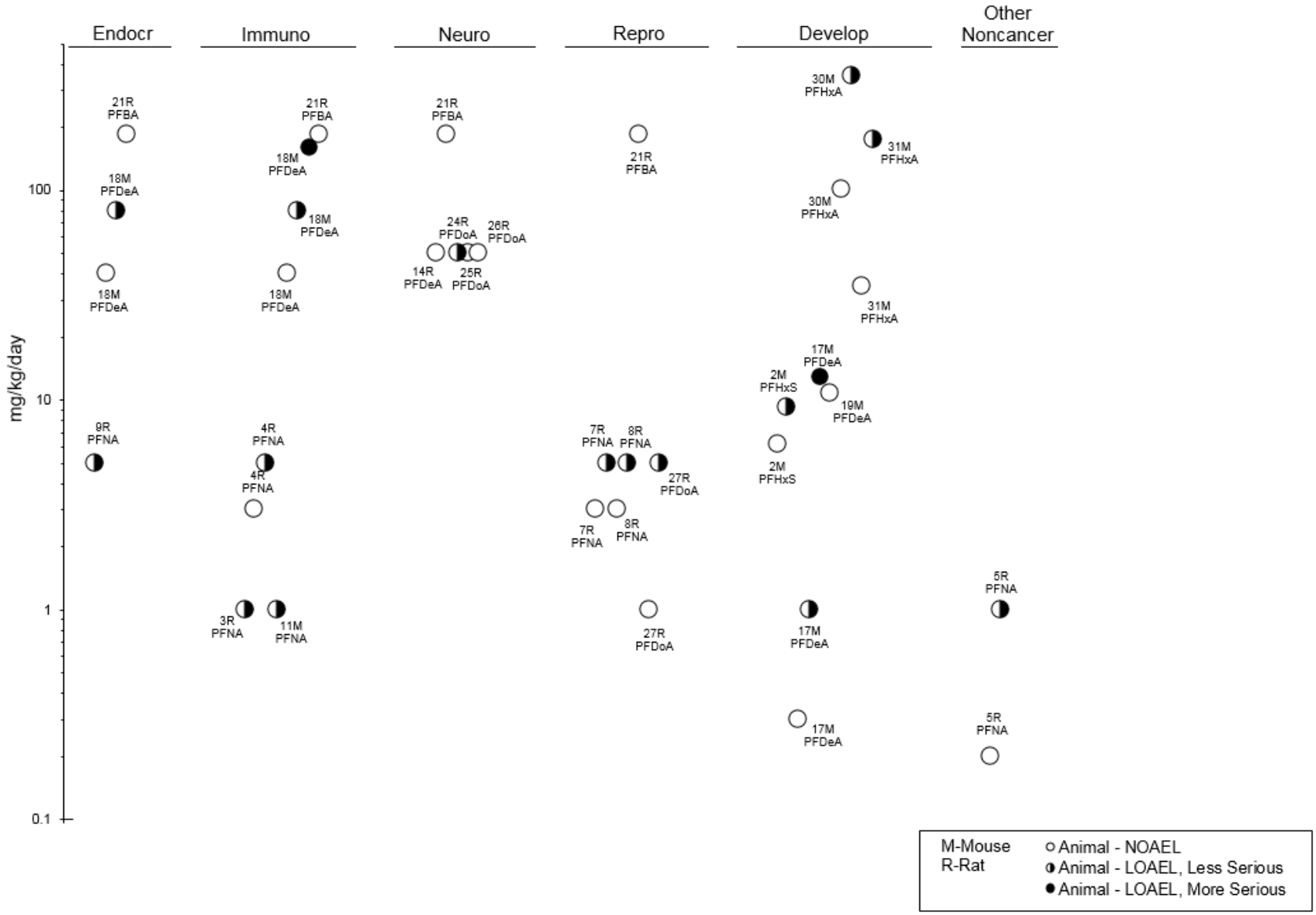


Figure 2-8. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Intermediate (15–364 days)

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

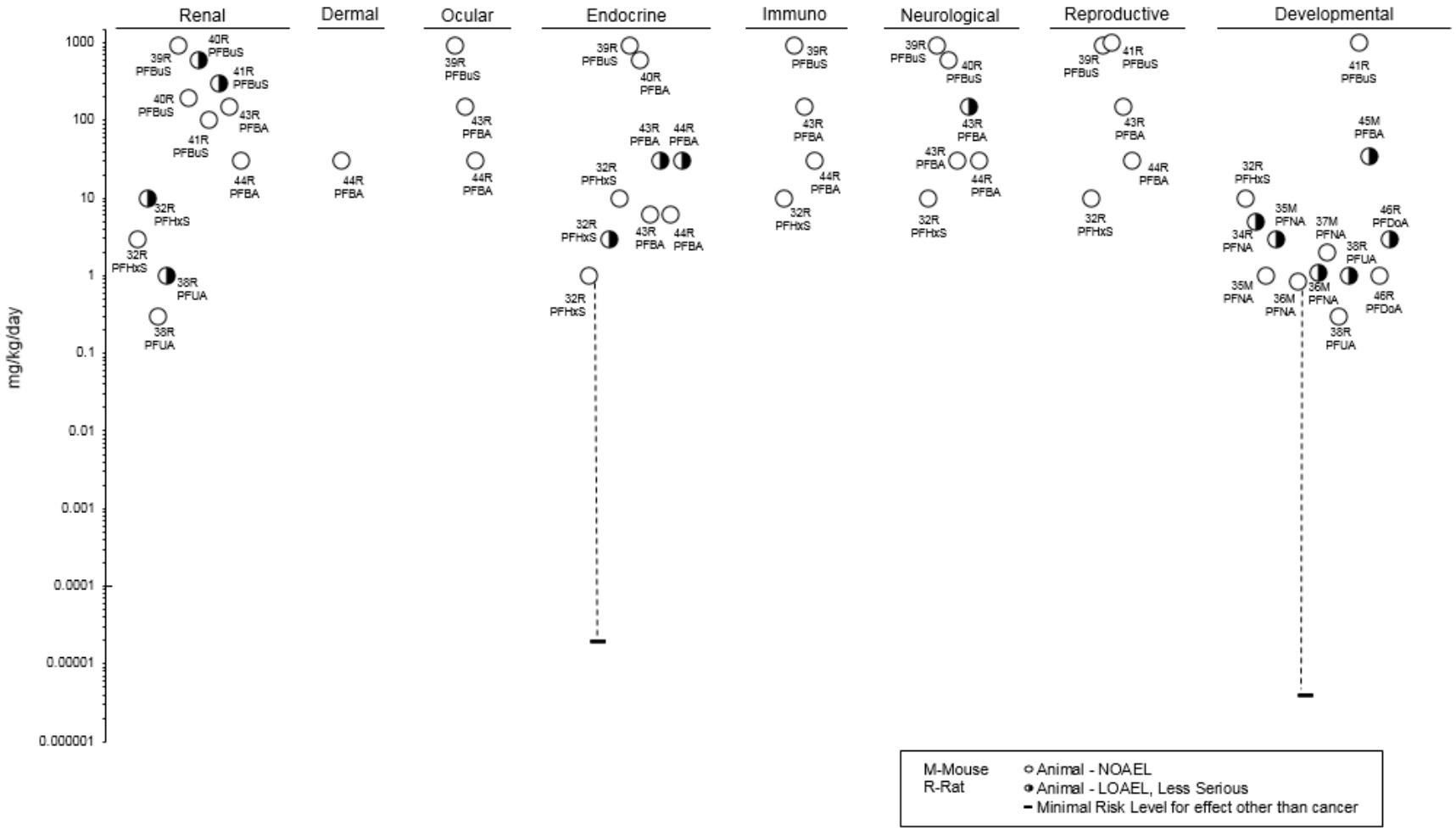


Figure 2-8. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Chronic (≥365 days)

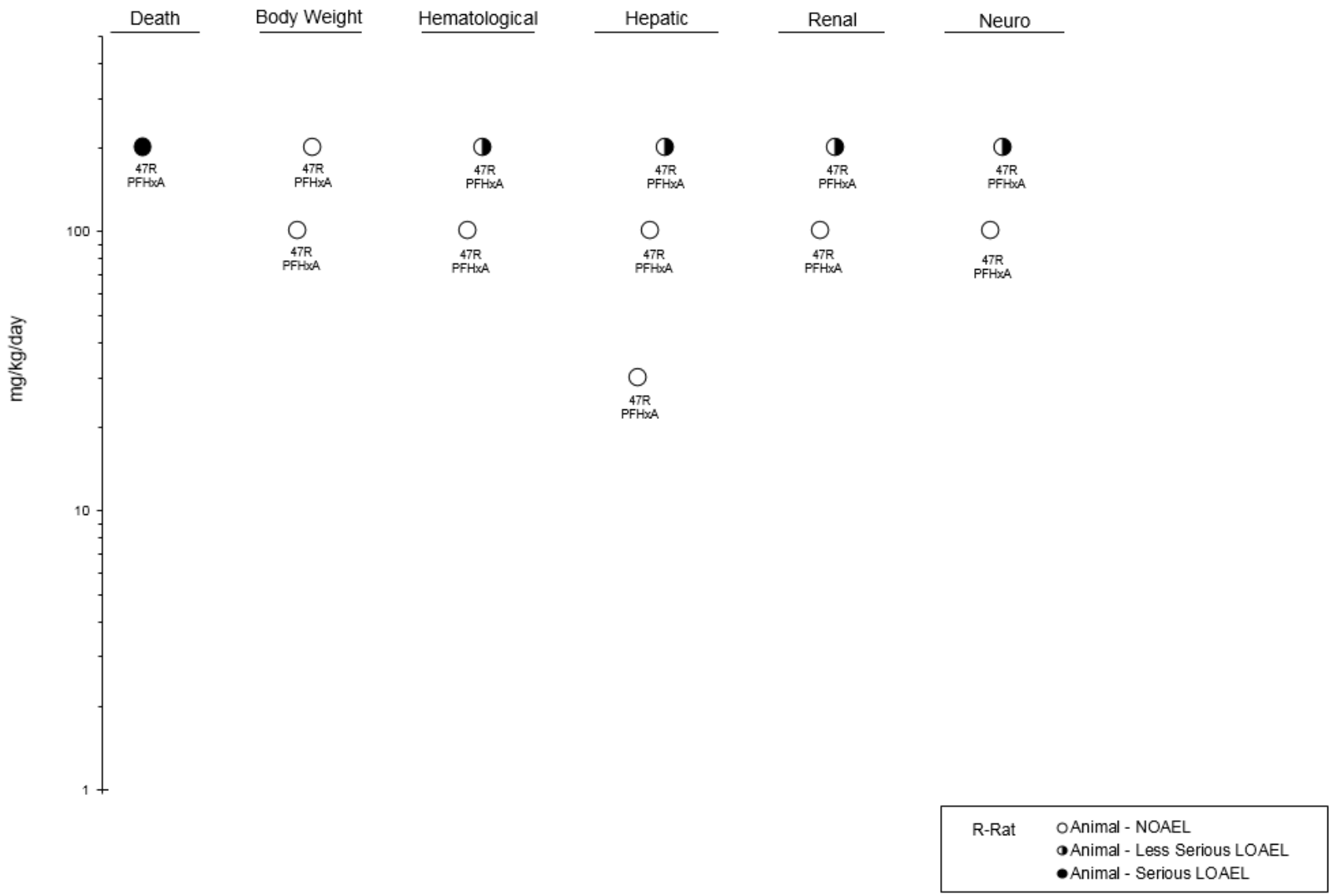


Table 2-6. Levels of Significant Exposure to PFOA – Dermal

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 = hematology; 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. Laboratory animal studies have measured LC_{50} and LD_{50} values and reported deaths following inhalation, oral, or dermal exposure to perfluoroalkyls. These data are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and Figures 2-4, 2-5, 2-6, 2-7, and 2-8.

PFOA

Epidemiology 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.

Laboratory Animal Studies. Limited data are available regarding death in animals following inhalation exposure to perfluoroalkyl compounds. 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 ≤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 4th 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

Epidemiology 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 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).

PFDeA

Laboratory Animal Studies. An LD_{50} of 120 mg/kg was estimated for PFDeA in female C57BL/6N mice administered single doses between 20 and 320 mg/kg/day PFDeA 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 PFDeA. 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 PFDeA in male Fischer-344 rats was 57 mg/kg.

PFHxA

Laboratory Animal Studies. 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. Animals in the 200 mg/kg/day dose group had rales and increased incidence of struggling behavior.

2.3 BODY WEIGHT

Overview. Epidemiology studies have examined the possible associations between *in utero* and/or early life exposure to perfluoroalkyls and body weight, body mass index (BMI), 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 epidemiology studies are summarized in Table 2-7, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 1. 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 2-4, 2-5, 2-6, 2-7, and 2-8.

PFOA

Epidemiology 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.

Table 2-7. Summary of Childhood Growth 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 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
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
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)
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**

Table 2-7. Summary of Childhood Growth in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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
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			
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)

Table 2-7. Summary of Childhood Growth in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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
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
Maisonet et al. 2012 General population (n=447 girls)	>23.0 ng/mL (maternal PFOS 3rd tertile)	Body weight at 20 months (adjusted for birth weight)	**β 438.4 g (71.09–805.65 g, p=0.0195)*, 3rd tertile**
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			
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
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)

Table 2-7. Summary of Childhood Growth in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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
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)
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)
PFDeA			
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 PFDeA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
PFUA			
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 PFUA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
PFDoA			
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 PFDoA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**

Table 2-7. Summary of Childhood Growth in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOSA			
Halldorsson et al. 2012 General population (n=665 20 year olds)	1.1 ng/mL (maternal median PFOSA)	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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BMI = body mass index; HR = hazard ratio; NR = not reported; NS = not significant; OR = odds ratio; PFDeA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFUA = perfluoroundecanoic acid; RR = relative risk

A study by de Cock et al. (2014) of infants 1–11 months of age did not find associations between cord blood PFOA levels and weight, height, 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. 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 (Andersen et al. 2013; Braun et al. 2016a; Høyer et al. 2015b), 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).

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).

Overall, the available epidemiology data do not suggest a connection between serum PFOA levels and body weight or risk of being overweight/obese.

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 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 perfluoroalkyl compounds. 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, in acute-duration studies, rats administered 25 mg/kg/day APFO for 14 days had a mean terminal body weight 14% lower than controls (Cook et al. 1992). 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) 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 control 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

Epidemiology Studies. General population studies have evaluated body weight, height, and BMI in infants, children, and adults to assess whether there were links 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. 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) 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). Overall, the epidemiology studies do not suggest a connection between serum PFOS and body weight or the risk of being overweight/obese.

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 weight loss during treatment, whereas treatment on GDs 10–13, 14–17, or 17–20 resulted in significant reductions in 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). 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).

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

Epidemiology Studies. Two studies have evaluated the influence of *in utero* PFHxS exposure on childhood growth and found no associations between maternal PFHxS levels and body weight at 20 months of age (Maisonet et al. 2012), changes in BMI scores between 2 and 8 years of age (Braun et al. 2016a), or the risk of childhood overweight/obesity (Braun et al. 2016a).

Laboratory Animal Studies. Treatment of rats with up to 10 mg/kg/day PFHxS by gavage for 40–60 days did not significantly affect body weight (Butenhoff et al. 2009a; Hoberman and York 2003); the mean terminal body weights were within 10% of the body weight of the control group. Food consumption was not affected by treatment with PFHxS.

PFNA

Epidemiology Studies. Several studies have examined the influence of maternal PFNA levels on childhood growth. These studies did not find associations between maternal PFNA levels and growth during childhood (Wang et al. 2016), BMI (Braun et al. 2016a; Halldorsson et al. 2012), or overweight/obesity risk (Braun et al. 2016a).

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).

PFDeA

Epidemiology Studies. One study examined the effect of PFDeA levels on childhood growth; Wang et al. (2016) reported decreases in weight and height in girls associated with increasing maternal serum PFDeA levels.

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 PFDeA in the diet resulted in a 32% weight loss in rats (Kawashima et al. 1995); the NOAEL was 4.7 mg/kg/day.

Body weight of female C57BL/6N mice administered a single gavage dose of 80 mg/kg PFDeA was reduced 12% relative to controls 30 days after dosing (Harris et al. 1989); no significant effect was seen at 40 mg/kg PFDeA. In a developmental study, pregnant mice dosed with 6.4 mg/kg/day PFDeA on GDs 6–15 gained 92% less weight (adjusted weight) on GDs 6–18 than controls; mice dosed with 12.8 mg/kg/day lost weight (Harris and Birnbaum 1989). Food consumption data were not provided in any of these studies. Weight loss was also observed in C57BL/6N mice exposed to 78 mg/kg/day PFDeA in the diet for 10 days (Permadi et al. 1992, 1993).

PFUA

Epidemiology Studies. Wang et al. (2016) found an inverse association between maternal serum PFUA levels and weight and height in girls.

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).

PFBuS

Laboratory Animal Studies. No significant alterations in body weight gain were observed in Sprague-Dawley rats administered ≤900 mg/kg/day PFBuS via gavage for 28 days (3M 2001) or in Sprague-Dawley rats administered ≤1,000 mg/kg/day PFBuS via gavage for at least 70 days (Lieder et al. 2009b).

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).

PFDoA

Epidemiology Studies. Wang et al. (2016) found an inverse association between maternal serum PFDoA levels and growth (weight and height) in girls.

Laboratory Animal Studies. Dosing of Sprague-Dawley rats with 5 mg/kg/day PFDoA 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 PFDoA (Kawabata et al. 2017).

PFHxA

Laboratory Animal Studies. Gavage administration of 100 or 200 mg/kg/day for 2 years did not result in alterations in body weight gain in male or female rats, respectively (Klaunig et al. 2015).

PFOSA

Epidemiology Studies. Halldorsson et al. (2012) did not find associations between maternal serum PFOSA 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 PFOSA (Seacat and Luebker 2000).

2.4 RESPIRATORY

Overview. A small number of epidemiology studies have examined the potential of perfluoroalkyl compounds to damage the respiratory tract; detailed descriptions of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 2. 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).

PFOA

Epidemiology 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). 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 ($p<0.05$). 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 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 for 4 weeks had no effect of 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 by gavage in a reproductive study (40–60 days of dosing) showed no treatment-related effects (Butenhoff et al. 2009a; Hoberman and York 2003).

PFNA

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

PFBuS

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

2.5 CARDIOVASCULAR

Overview. Epidemiology and laboratory animal studies have evaluated the toxicity of perfluoroalkyls to the cardiovascular system. The epidemiology 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 epidemiology data are available for other perfluoroalkyls; general population studies for PFOS, PFHxS, PFNA, PFDeA, PFUA, and PFHpA have not found increases in the risk of cardiovascular disease. Most of the available epidemiology studies did not find a link between serum PFOA and hypertension, and the one study for PFOS did not find an association. 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 PFDeA and pre-eclampsia; one study on PFUA found an inverse association.

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). The laboratory animal studies did not find increases in the incidence of histological alterations in the heart following exposure to PFOA, PFOS, PFHxS, PFDeA, PFBuS, or PFBA.

PFOA

Epidemiology 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). Two 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

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)	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 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
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
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)
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**

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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**
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)***
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
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)***
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

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
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			
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)***
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
PFDeA			
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.2 ng/mL (median PFDeA 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 PFDeA)	Pre-eclampsia	HR 0.88 (0.75–1.04), per ln unit
PFUA			
Lin et al. 2013a, 2013b General population (n=644)	6.59 ng/mL (median PFUA)	Carotid intima media thickness	NS (p=0.953 for trend)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.2 ng/mL (median PFUA 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 PFUA)	Pre-eclampsia	**HR 0.78 (0.66–0.92)*, per ln unit**
PFHpA			
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
PFDoA			
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.02 ng/mL (median PFDoA in cases and controls)	Coronary artery disease	OR 0.63 (0.35–1.11), 4th quartile

[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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CHD = coronary heart disease; HR = hazard ratio; LHWA = Little Hocking Water Authority; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFDeA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUA = perfluoroundecanoic acid; SMR = standardized mortality ratio; SPR = standard prevalence ratio

(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, another NHANES study did not find an association between serum PFOA and physician-diagnosed coronary artery disease, angina, and/or heart attack (Melzer et al. 2010). A general population study did not find an association between serum PFOA levels and carotid intima media thickness (Lin et al. 2013a).

Epidemiology 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. 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). 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. The fourth study (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. Another study of participants in the C8 Health Project that used measured serum perfluoroalkyl levels found significant increases in the odds ratios (ORs) for pregnancy-induced hypertension in women with higher PFOA (≥6.9 ng/mL) levels (Darrow et al. 2013). A third study of highly exposed residents reported a weak association between

serum PFOA and 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. 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). 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

Epidemiology 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. 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.

Epidemiology Studies—Hypertension. No increases in the risk of hypertension associated with serum PFOS levels were observed in adolescent NHANES participants (Geiger et al. 2014a). Two studies found increases in the risk of pregnancy-induced hypertension (Darrow et al. 2013) or 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. 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

Epidemiology Studies. Two 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 pre-eclampsia (Starling et al. 2014b) were found.

Laboratory Animal Studies. Dosing of rats with≤10 mg/kg/day PFHxS by gavage for 40–60 days did not cause morphological alterations in the heart (Butenhoff et al. 2009a; Hoberman and York 2003).

PFNA

Epidemiology 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. Other general population studies have not found increases in the risk of coronary heart disease (Mattsson et al. 2015) or pre-eclampsia (Starling et al. 2014b) associated with increasing serum PFNA levels.

PFDeA

Epidemiology Studies. The risks of coronary artery disease (Mattsson et al. 2015) and pre-eclampsia (Starling et al. 2014b) were not associated with serum PFDeA levels in general population studies.

Laboratory Animal Studies. Death in female C57BL/6N mice following administration of a single lethal dose of 160 or 320 mg/kg PFDeA 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).

PFUA

Epidemiology Studies. Starling et al. (2014b) found an inverse association between serum PFUA levels and the risk of pre-eclampsia in pregnant women. No association between serum PFUA levels and carotid intima artery thickness was observed in a general population study (Lin et al. 2013a). A third general population study (Mattsson et al. 2015) did not find an increase in the risk of coronary artery disease associated with serum PFUA levels.

PFHpA

Epidemiology 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.

PFBuS

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

PFBA

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).

PFDoA

Epidemiology Studies. No increase in the risk of coronary heart disease associated with serum PFDoA levels was found in a general population study (Mattsson et al. 2015).

2.6 GASTROINTESTINAL

Overview. Available epidemiology 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. 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 2-4, 2-6, and 2-7. Studies on PFOA and PFBuS 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, or PFBA.

PFOA

Laboratory Animal Studies. 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). 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

Epidemiology 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 PFHxS via gavage for 42–56 days (Butenhoff et al. 2009a; Hoberman and York 2003).

PFBuS

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 PFBuS via gavage for 90 days (Lieder et al. 2009a); these lesions were likely due to irritation from the repeated gavage administration with PFBuS. In another study, no morphological alterations were observed in the gastrointestinal tract of rats administered ≤900 mg/kg/day PFBuS 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).

2.7 HEMATOLOGICAL

Overview. A small number of epidemiology 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. 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 perfluoroalkyl compounds (Tables 2-1, 2-3, 2-4, 2-5, and 2-6). Some laboratory animal studies have reported alterations in hematological indices following exposure to higher doses of PFOA, PFOS, PFHxS, PFUA, PFBuS, PFBA, or PFHxA.

PFOA

Epidemiology 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).

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).

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

Epidemiology 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; Hoberman and York 2003). 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; Hoberman and York 2003).

PFUA

Laboratory Animal Studies. Treatment of rats with 1.0 mg/kg/day PFUA 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.

PFBuS

Laboratory Animal Studies. A 90-day exposure to PFBuS 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 PFBuS 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 or up to 150 mg/kg/day for 28 days did not result in significant alterations in hematological parameters (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a). 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.

PFHxA

Laboratory Animal Studies. In a 104-week gavage study, treatment of female Sprague-Dawley rats (60 or 70/dose) with 200 mg/kg/day PFHxA resulted in significant decreases in mean red blood cell counts (8.1%) and hemoglobin (5.2%), as well as significant increases in reticulocyte counts at weeks 25 and 51 (23.6 and 53.6%, respectively) (Klaunig et al. 2015). There were no alterations in hematological parameters in males. The NOAEL was 100 mg/kg/day.

2.8 MUSCULOSKELETAL

Overview. Several epidemiology 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 a link 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. No morphological alterations were noted in bone or skeletal muscle in laboratory animals following exposure to PFOA, PFOS, PFBuS, or PFBA; these data are summarized in Tables 2-1, 2-3, 2-4, and 2-5 and Figures 2-4, 2-6, 2-7, and 2-8.

PFOA

Epidemiology 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 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 (Khalil et al. 2016; 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). Two studies of adult NHANES participants found no associations

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)	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
		Osteoarthritis risk (women)	**OR 1.84 (1.17–2.90; p=0.008)*, per ln-PFOA increase**
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
		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

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		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**
		Osteoarthritis risk (women)	OR 1.14 (0.68–1.94; p=0.619), per ln-PFOS increase
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
		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

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		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
		Osteoarthritis risk (women)	**OR 1.64 (1.14–2.38; p=0.008)*, per ln-PFHxS increase**
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
		Osteoarthritis risk (women)	**OR 1.45 (1.02–2.05; p=0.001)*, per ln-PFNA increase.**

[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 compound; 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

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. 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 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. The investigators noted that PFOA concentrations were 4–5 times higher in the PFOA-exposed animals than in controls.

PFOS

Epidemiology Studies. Several epidemiology 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 osteoarthritis 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.

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

Epidemiology Studies. A study of NHANES participants found an increase in the risk of osteoarthritis 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).

PFNA

Epidemiology Studies. Khalil et al. (2016) found an increase in the risk of osteoarthritis 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.

PFBuS

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

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).

2.9 HEPATIC

Overview. Epidemiology 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 epidemiology 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 epidemiology 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 epidemiology 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. Although there is considerable variability across studies, the evidence is adequate for PFOA, PFOS, and PFHxS, particularly for ALT levels; consistent results were not found for PFNA. The results of available epidemiology studies suggest associations between increases in serum lipids, particularly total cholesterol and LDL cholesterol, and serum PFOA, PFOS, PFNA, and PFDeA. For PFHxS, PFUA, PFHpA, PFBuS, PFBA, and PFDoA, 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.

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 2-4, 2-5, 2-6, 2-7, and 2-8. The results of these studies provide strong evidence that the liver is a sensitive target of PFOA, PFOS, PFHxS, PFNA, PFUA, PFBuS, and PFBA 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).

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,

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)	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)	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 compound; 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 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)	Cumulative 16.5 ng/mL (median PFOA in 2005/2006)	ALT	**Association (p<0.0001 for trend)*, 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)*, 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	**Higher levels (p<0.05)*, males only with no adjustments**
		Risk of abnormal ALT	OR 2.1 (0.6–7.3)
		GGT	**Difference (p<0.05)*, females only with no adjustments**
		Risk of abnormal GGT	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	11.3 ng/mL (median PFOS)	ALT	NS (p>0.01)
General population (NHANES) (n=4,333)		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	27.39 and 22.20 ng/mL (geometric mean PFOS in males and females)	ALT	NS (p=0.066)
General population (NHANES) (n=2,216)		GGT	NS (p=0.808)
		Total bilirubin	NS (p=0.223)
Yamaguchi et al. 2013	5.8 ng/mL (mean PFOS)	ALT	**Association (p=0.03)***
General population (n=608)		AST	**Association (p=0.01)***
		GGT	**Association (p=0.03)***
PFHxS			
Gleason et al. 2015	1.8 ng/mL (median PFHxS)	ALT	**Association (p<0.01)***
General population (NHANES) (n=4,333)		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	2.29 and 1.72 ng/mL (geometric mean PFHxS in males and females)	ALT	NS (p=0.691)
General population (NHANES) (n=2,216)		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	NR	ALT	NS, longitudinal analysis
Occupational (n=592)		AST	NS, longitudinal analysis
		GGT	NS, longitudinal analysis
		Bilirubin	NS, longitudinal analysis
Gleason et al. 2015	1.4 ng/mL (median PFNA)	ALT	**Association (p<0.001)***
General population (NHANES) (n=4,333)		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	0.89 and 0.72 ng/mL (geometric mean PFNA in males and females)	ALT	NS (p=0.131)
General population (NHANES) (n=2,216)		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 compound; 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 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)	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 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)
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
		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

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)***
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**
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)***

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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
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**

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	**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)***
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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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 triglyceride	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**
		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)
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]
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**
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)***
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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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
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]
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
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)***
PFDeA			
Fu et al. 2014a General population (n=133)	0.19 ng/mL (median PFDeA)	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)

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)	0.09 ng/mL (50th PFDeA percentile)	Total cholesterol	NS (β 1.84, -2.12–5.79), per ln-unit increase in PFDeA
		LDL cholesterol	NS (β 0.19, -3.30–3.69), per ln-unit increase in PFDeA
		HDL cholesterol	**Association (β 2.54, 1.22–3.87)*, per ln-unit increase in PFDeA**
		Triglycerides	NS (β -0.03, -0.07–0.01), per ln-unit increase in PFDeA
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	1.0 and 1.0 ng/mL (mean PFDeA 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)
PFUA			
Fu et al. 2014a General population (n=133)	0.26 ng/mL (median PFUA)	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)
Starling et al. 2014a General population (n=854 pregnant women)	0.22 ng/mL (50th PFUA percentile)	Total cholesterol	NS (β 0.89, -3.28–5.06), per ln-unit increase in PFUA
		LDL cholesterol	NS (β -2.36, -5.97–1.25), per ln-unit increase in PFUA
		HDL cholesterol	**Association (β 4.05, 2.75–5.35)*, per ln-unit increase in PFUA**
		Triglycerides	NS (β -0.04, -0.08–0.00), per ln-unit increase in PFUA

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
PFBuS			
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	0.5 and 0.4 ng/mL (mean PFBuS 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)
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)
PFDoA			
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	4.5 and 4.4 ng/mL (mean PFDoA 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 compound; 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 = perfluorobutyric acid; PFBuS = perfluorobutane sulfonic acid; PFDeA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUA = perfluoroundecanoic acid; VLDL = very low-density lipoprotein

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 adapted 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

Epidemiology 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).

Epidemiology 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) 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 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 epidemiology 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.

Epidemiology 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 2-9, 2-10, 2-11, and 2-12.

Figure 2-9. Serum Total Cholesterol Levels Relative to Serum PFOA Levels
(Presented as percent change in cholesterol levels)

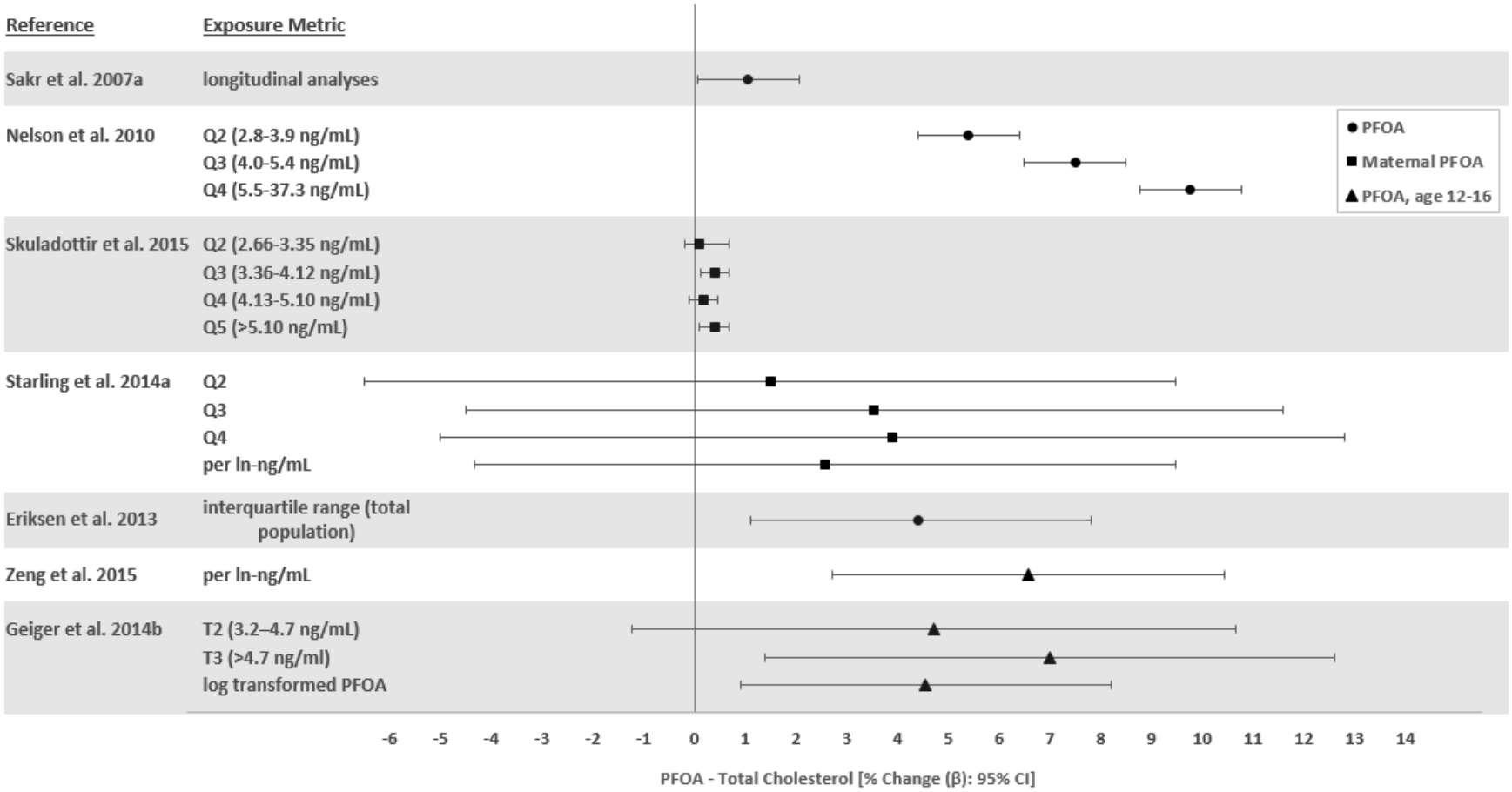


Figure 2-10. Risk of Abnormal Cholesterol Levels Relative to PFOA Levels (Presented as Adjusted Ratios)

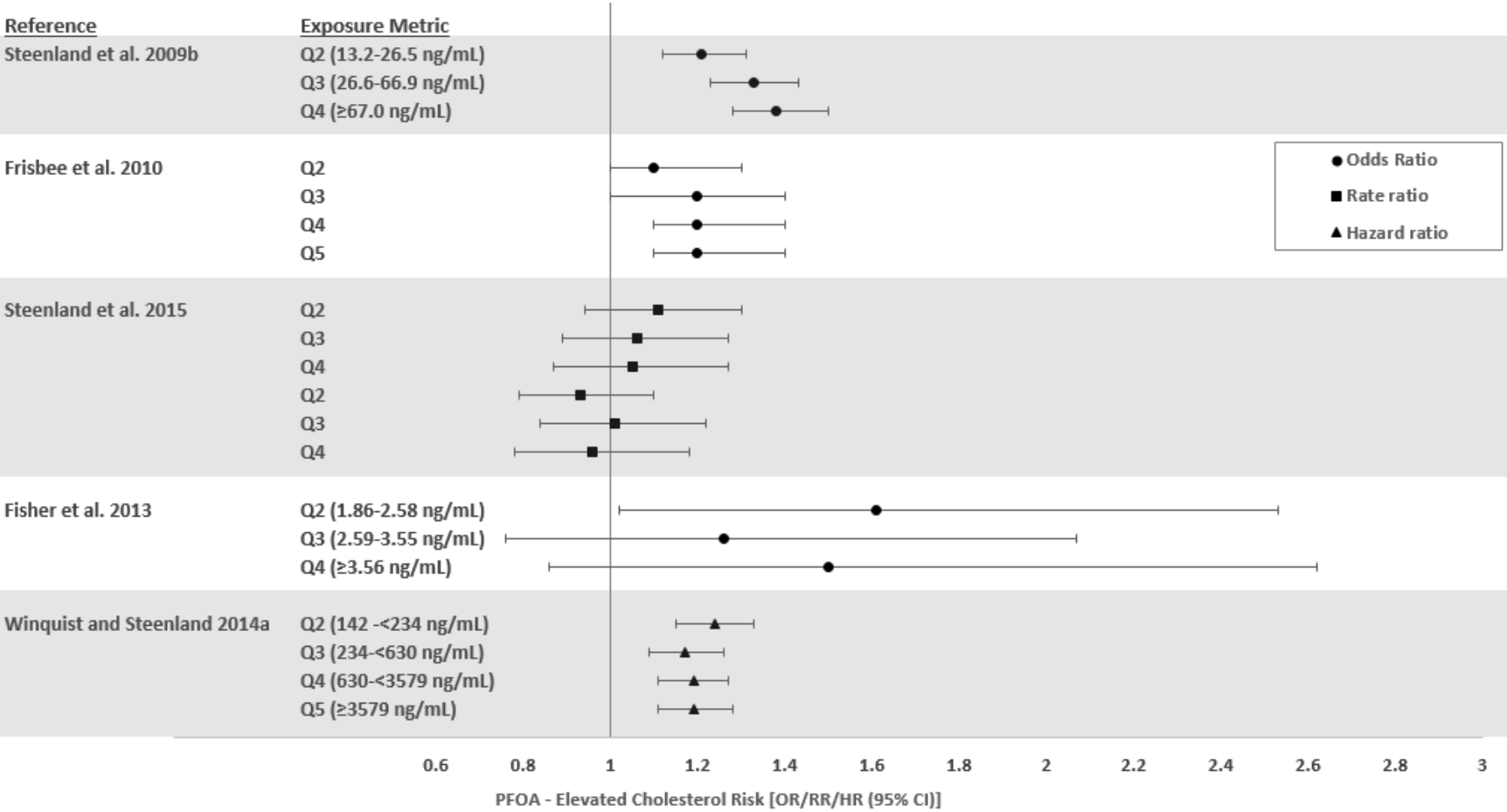


Figure 2-11. Serum LDL Cholesterol Levels Relative to Serum PFOA Levels
(Presented as percent change in LDL cholesterol levels)

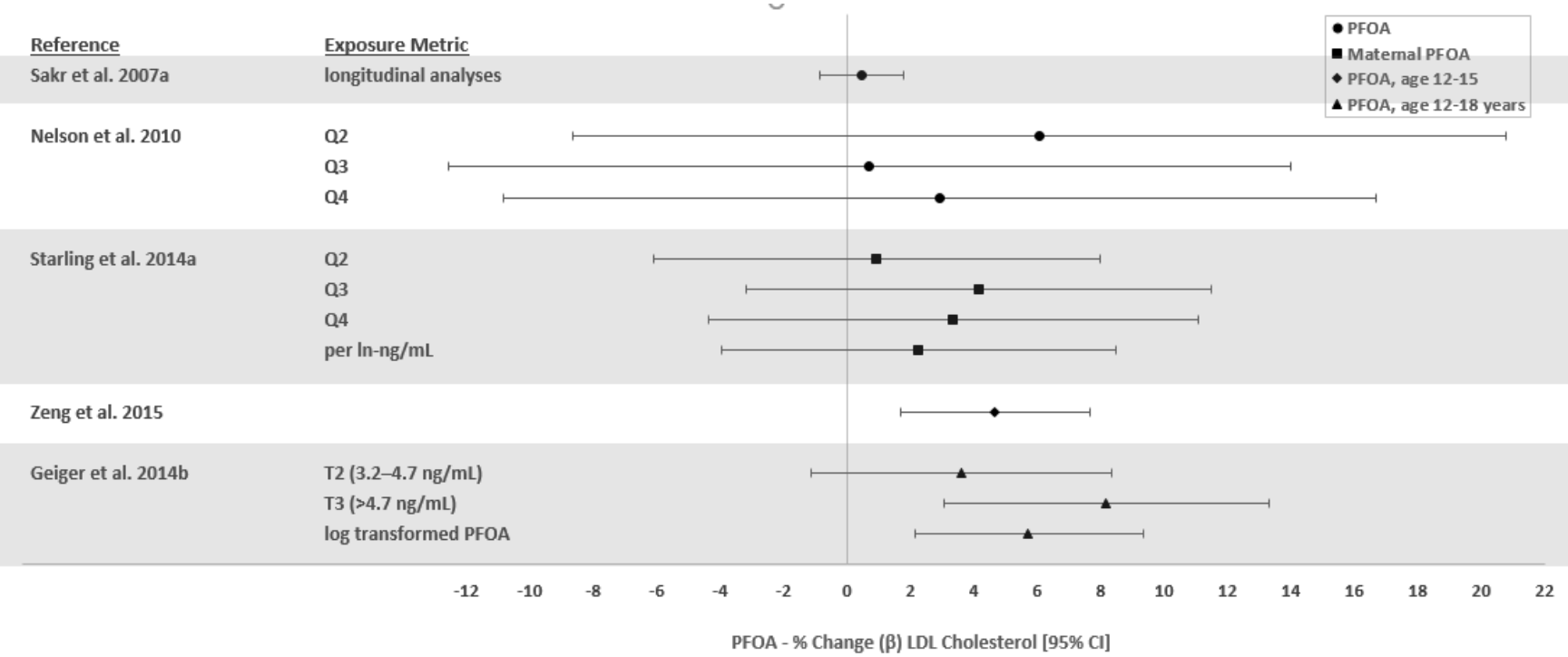
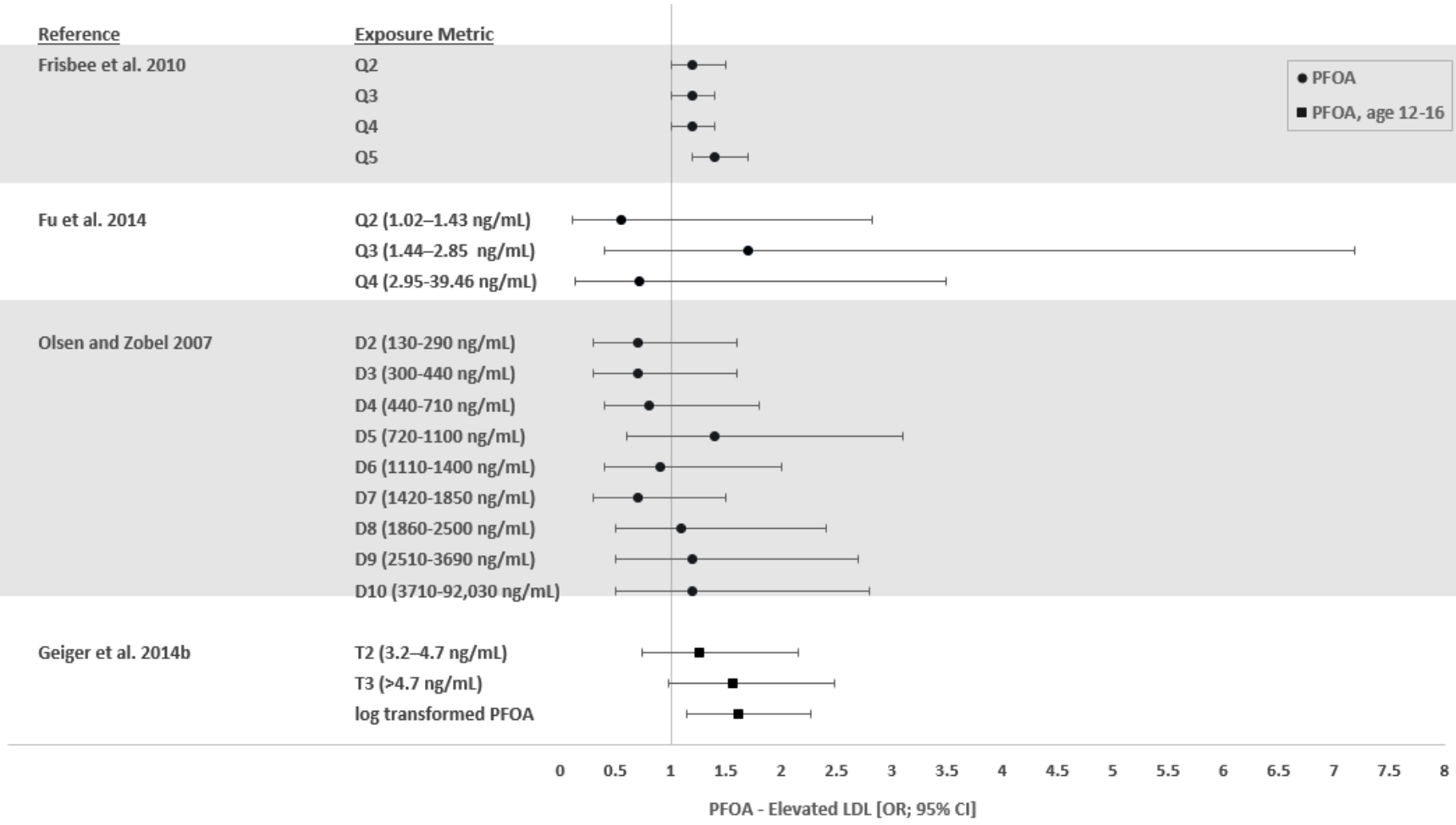


Figure 2-12. Risk of Abnormal LDL Cholesterol Levels Relative to PFOA Levels (Presented as Adjusted Ratios)



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) 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, 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 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 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 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, Japan, 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.

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. 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 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 epidemiology 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 (results only available as an abstract), administration of APFO to patients with advanced refractory solid tumors at doses of 50–1,200 mg weekly for 6 weeks resulted in decreases in serum LDL cholesterol levels (it is unclear if this effect was observed at all dose levels) (MacPherson et al. 2011). 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 2-12). 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 that this was not 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 perfluoroalkyl compounds in animals following short- or long-term oral exposures. The hepatic response to exposure to many perfluoroalkyl compounds, 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. 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). 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 (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 hepatomegalocytosis, hepatocellular necrosis, and portal mononuclear cell infiltration after a 1-year exposure to 15 mg/kg/day in the diet (3M 1983). A 2-year exposure to 15 mg/kg/day resulted in hepatomegalocytosis, cystoid degeneration, and portal mononuclear cell infiltration (3M 1983). 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 toxicity. 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 not involving peroxisome proliferation. 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. Epidemiology 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 epidemiology 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 epidemiology studies, an experimental study in humans exposed to PFOA (MacPherson et al. 2011) 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

Epidemiology 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).

Epidemiology 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.

Epidemiology 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 2-13, 2-14, 2-15, and 2-16.

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 level was 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 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

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

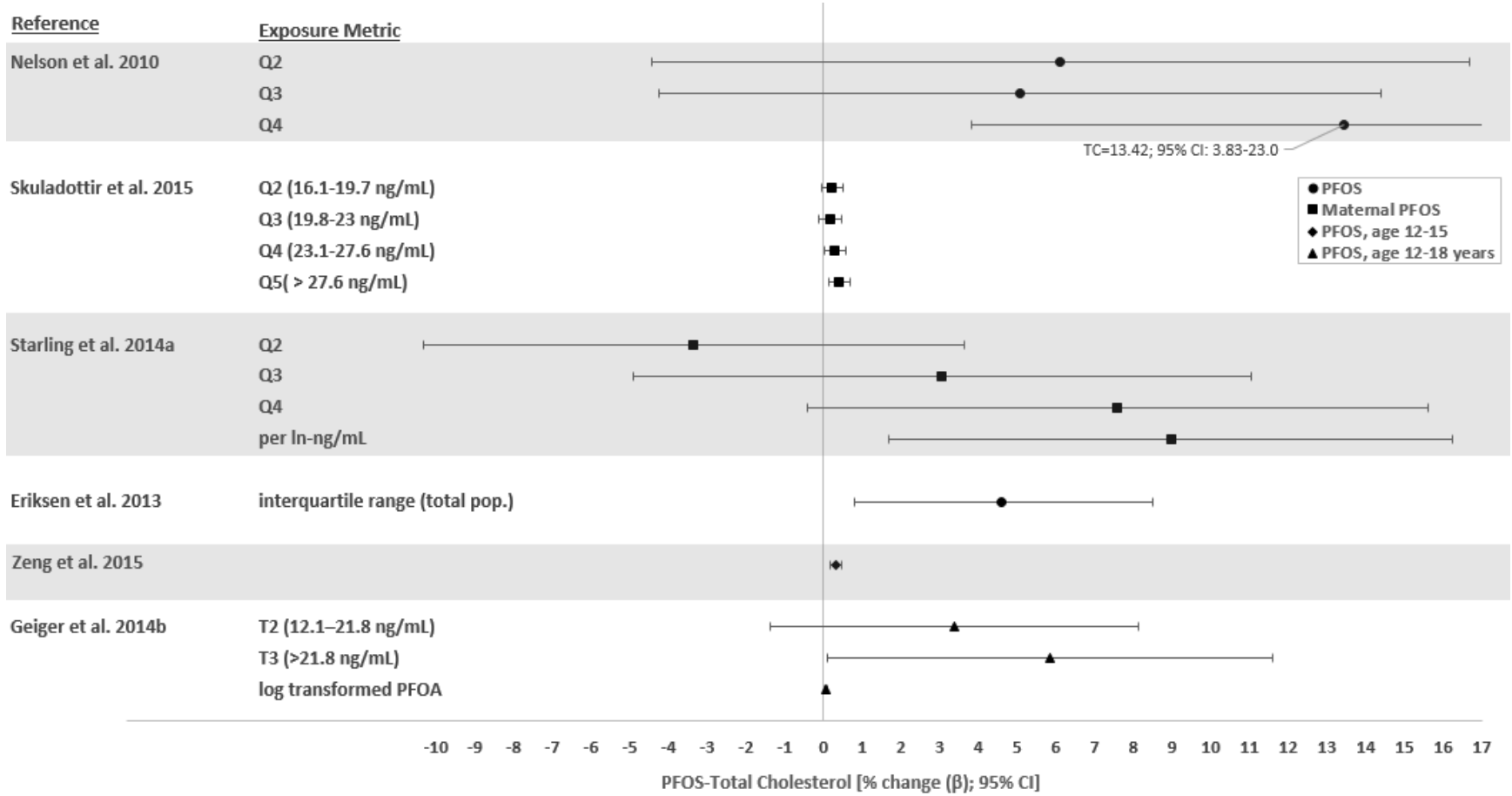


Figure 2-14. Risk of Abnormal Cholesterol Levels Relative to PFOS Levels (Presented as Adjusted Ratios)

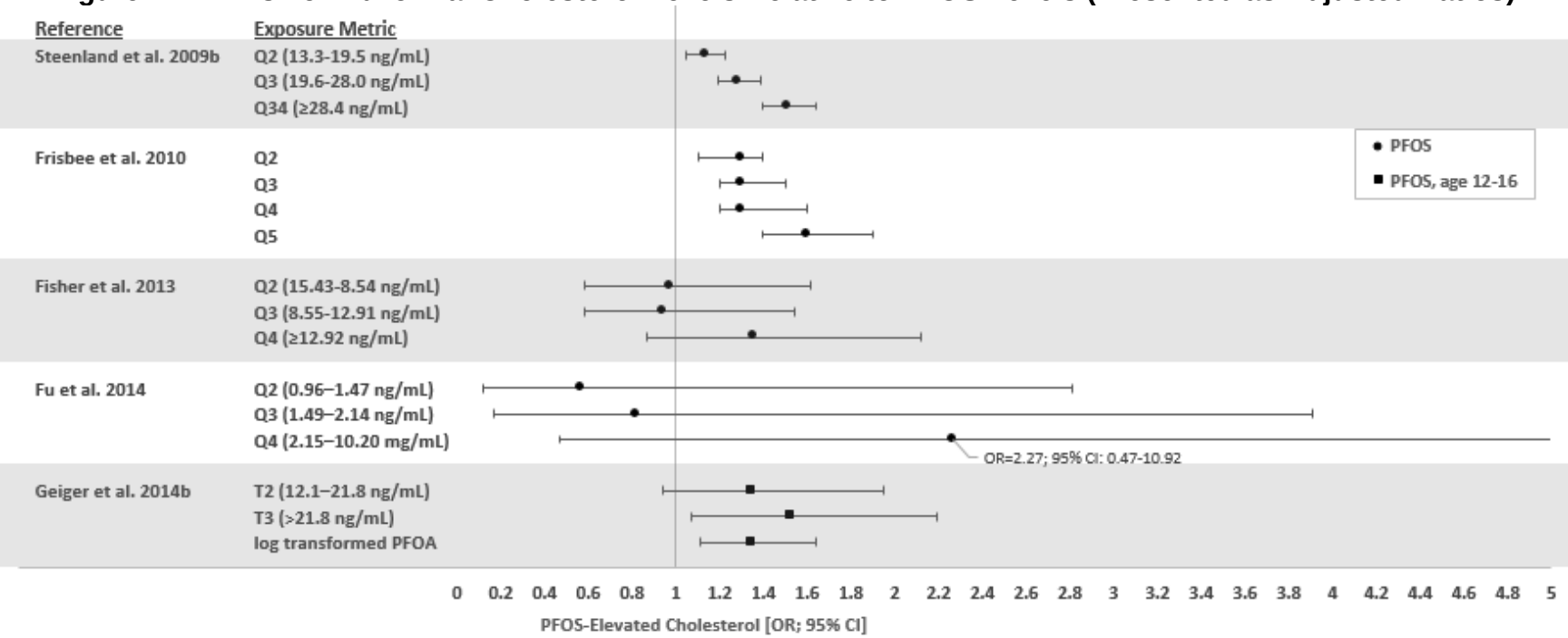


Figure 2-15. Serum LDL Cholesterol Levels Relative to Serum PFOS Levels
(Presented as percent change in LDL cholesterol levels)

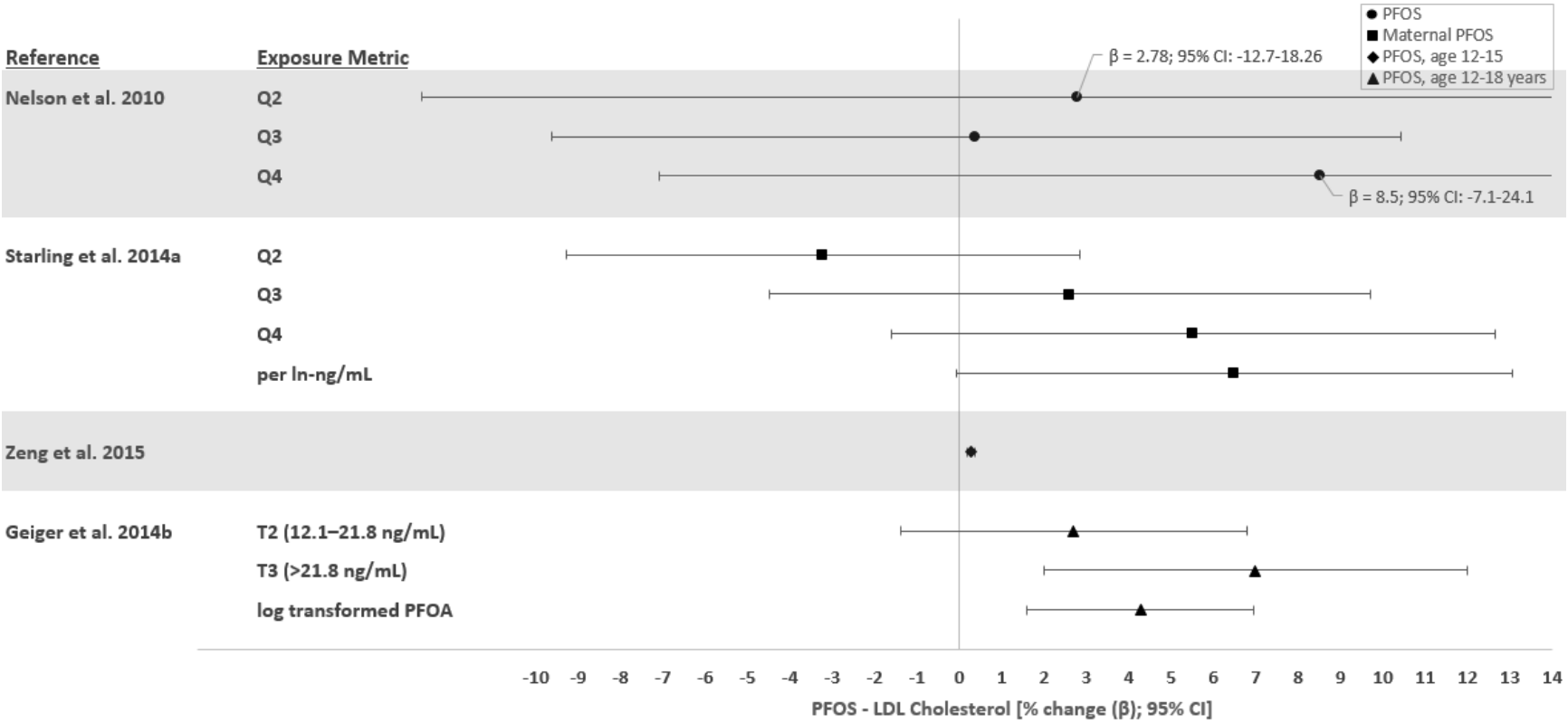
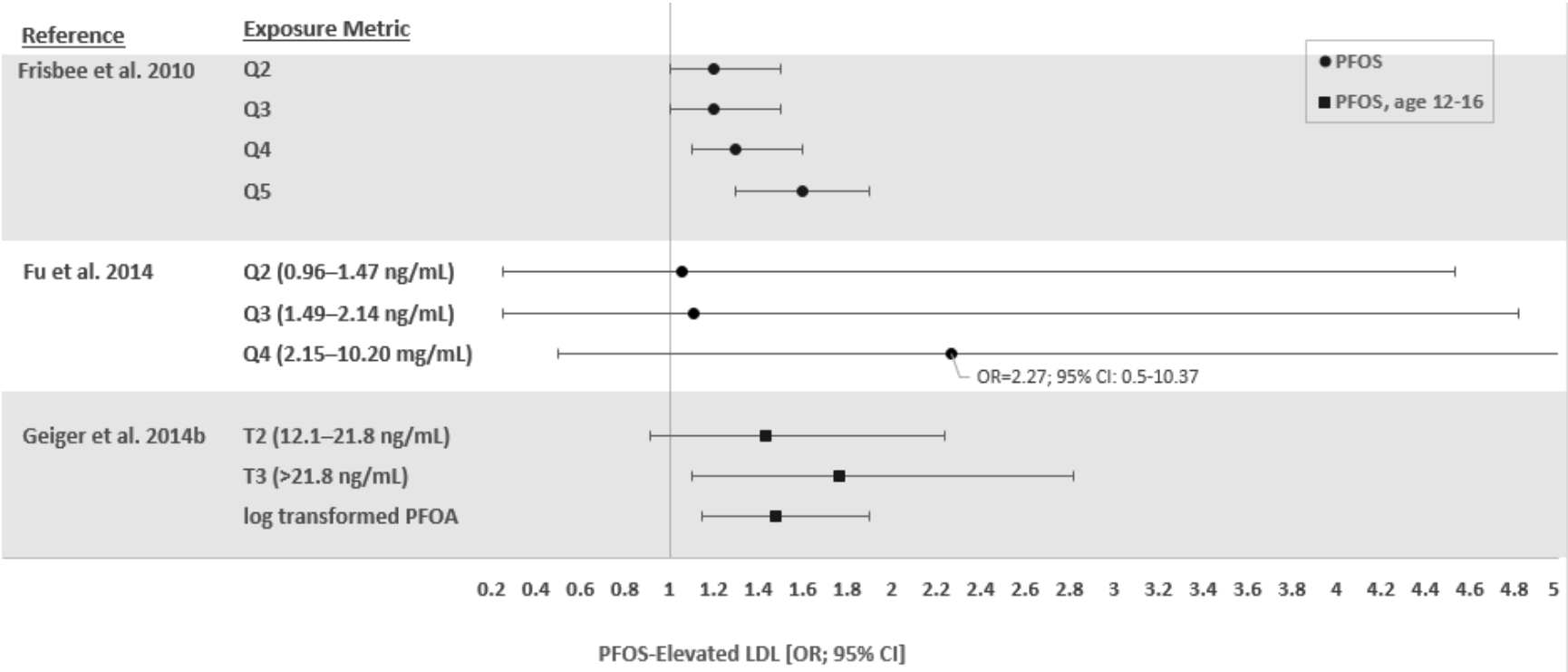


Figure 2-16. Risk of Abnormal LDL Cholesterol Levels Relative to PFOS Levels (Presented as Adjusted Ratios)



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. The investigators noted that the odds of high cholesterol from the 1st to the 5th quartile 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 (Châtaeu-Degat et al. 2010); inverse associations between serum PFOS and HDL cholesterol (Starling et al. 2014a) and triglycerides (Châtaeu-Degat et al. 2010) were also found. However, other studies in adults have not found associations between serum PFOS and total cholesterol (Châtaeu-Degat et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Fu et al. 2014a), non-HDL cholesterol (Fisher et al. 2013), LDL cholesterol (Châtaeu-Degat et al. 2010; Fisher et al. 2013; Fu et al. 2014a; Starling et al. 2014a), HDL cholesterol (Fisher et al. 2013; Fu et al. 2014a), or triglycerides (Fu et al. 2014a; Starling et al. 2014a). 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 (Châtaeu-Degat et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Nelson et al. 2010). 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. 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.

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.

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. Epidemiology 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 epidemiology 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 applicability of the hepatic hypertrophy and serum lipid alterations observed in rodent studies to humans has been questioned due to species differences in PPARα receptor activation, the presumed mechanism of action for these effects in rodents.

PFHxS

Epidemiology 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.

Epidemiology Studies—Serum Lipids. Four 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. 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).

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 ≥3 mg/kg/day induced a significant increase in absolute and relative liver weight in males (Butenhoff et al. 2009a; Hoberman and York 2003). 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. 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

Epidemiology 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. The Gleason et al. (2015) study also did not find increased risks for elevated ALT, AST, GGT, or bilirubin.

Epidemiology 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 (Zeng et al. 2015); one study in pregnant women did not find an association (Starling et al. 2014a). Several studies have also found associations with LDL cholesterol (Fu et al. 2014a; 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; 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; Zeng et al. 2015) or triglycerides (Fu et al. 2014a; Starling et al. 2014a). Exceptions were the Starling et al. (2014a) study of pregnant women, which found a positive association for HDL cholesterol, 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. Nine studies have evaluated the hepatic toxicity of PFNA. The observed effects are consistent with effects observed for other perfluoroalkyl compounds. Alterations in serum lipid levels consisted of decreases in serum HDL cholesterol levels in rats administered via gavage ≥1 mg/kg/day for 14 days (Fang et al. 2012a) and decreases in serum triglyceride and cholesterol levels in mice receiving gavage doses of ≥1 mg/kg/day (Wang et al. 2015a). Increases in liver weight were observed in rats nose-only exposed to ≥67 mg/m^3 (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). Hepatocellular vacuolation was observed in mice administered via gavage 5 mg/kg/day 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 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 for 10 days.

PFDeA

Epidemiology Studies—Serum Lipids. Three general population studies have evaluated the potential relationships between serum PFDeA and serum lipids and reported inconsistent results. Fu et al. (2014a) found an association between serum PFDeA and total cholesterol in adults; studies in pregnant women (Starling et al. 2014a) and children (Zeng et al. 2015) did not find associations. Both Fu et al. (2014a) and Starling et al. (2014a) found positive associations with HDL cholesterol; this was not found in the Zeng et al. (2015) study. None of the studies found associations between serum PFDeA and LDL cholesterol or triglycerides (Fu et al. 2014a; 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 PFDeA include alterations in liver weight and morphology. Increases in liver weight have been observed in mice following a single gavage dose of PFDeA; 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 PFDeA 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 PFDeA, 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).

PFUA

Epidemiology Studies—Serum Lipids. Neither of the two studies (Fu et al. 2014a; Starling et al. 2014a) examining possible relationships between serum PFUA and serum lipids found associations between serum PFUA and for total cholesterol, LDL cholesterol, or triglyceride levels. Starling et al. (2014a) found an association of serum PFUA 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 PFUA. In an intermediate-duration study of rats administered PFUA 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

Epidemiology Studies—Serum Lipids. Epidemiology 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.

PFBuS

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

Laboratory Animal Studies. Treatment of male rats with 900 mg/kg/day PFBuS 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. The NOAEL for liver weight effects was 300 mg/kg/day. 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 PFBuS 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

Epidemiology Studies—Serum Lipids. Only one epidemiology 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 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 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 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.

PFDoA

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

Laboratory Animal Studies. Dosing of male Sprague-Dawley rats with 10 mg/kg/day PFDoA 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 PFDoA, 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. Single doses ≥1 mg/kg/day significantly induced the expression of PPARα and PPARγ and their target genes to enhance fatty acid β-oxidation. 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]).

PFHxA

Laboratory Animal Studies. 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.

PFOSA

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 PFOSA.

2.10 RENAL

Overview. Epidemiology 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 epidemiology studies evaluating kidney disease and renal function are summarized in Table 2-13; Table 2-14 contains the studies

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)	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)	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 compound; 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 adolescents)	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 adolescents)	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	1.4 ng/mL (median PFNA)	Serum uric acid	**Association (p<0.001)***
General population (NHANES) (n=4,333)		Hyperuricemia risk	NS (p=0.42 for trend)
Kataria et al. 2015	≥1.5 ng/mL (4th PFNA quartile)	Serum uric acid	NS (p>0.05)
General population (NHANES) (n=1,960 adolescents)			
Qin et al. 2016	0.8 ng/mL (median PFNA)	Serum uric acid	NS (p>0.05)
General population (n=225 adolescents)		Hyperuricemia risk	OR 1.28 (0.83–1.96)
PFDeA			
Qin et al. 2016	0.9 ng/mL (median PFDeA)	Serum uric acid	NS (p>0.05)
General population (n=225 adolescents)		Hyperuricemia risk	OR 1.26 (0.82–1.92)
PFBuS			
Qin et al. 2016	0.5 ng/mL (median PFBuS)	Serum uric acid	NS (p>0.05)
General population (n=225 adolescents)		Hyperuricemia risk	OR 1.23 (0.86–1.75)
PFDoA			
Qin et al. 2016	2.7 ng/mL (median PFDoA)	Serum uric acid	NS (p>0.05)
General population (n=225 adolescents)		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 compound; 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; PFBuS = perfluorobutane sulfonic acid; PFDoA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

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 epidemiology 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 epidemiology studies or the inconsistency of the results, possible associations between other perfluoroalkyls (PFHxS, PFNA, PFDeA, PFBuS, or PFDoA) and renal functions cannot be assessed.

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 2-4, 2-6, 2-7, and 2-8. In general, the laboratory animal studies have not found evidence of impaired renal function or morphological damage following exposure to PFOA, PFOS, PFHxS, PFDeA, PFUA, PFBuS, PFBA, or PFHxA.

PFOA

Epidemiology Studies—Kidney Disease. Several epidemiology 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 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 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 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).

Epidemiology Studies—Biomarkers of Renal Function. Several biomarkers of renal function have been evaluated in epidemiology 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.

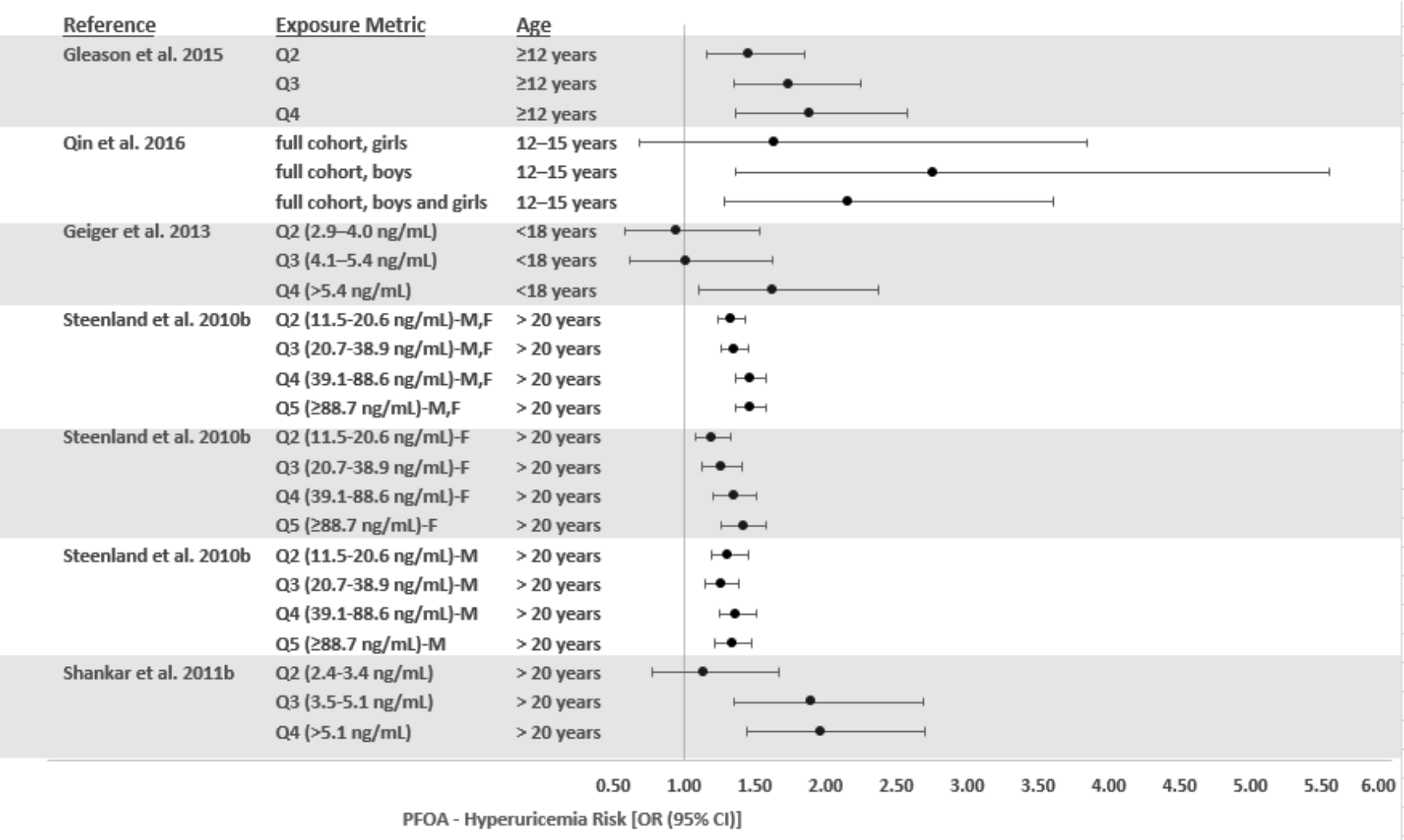
Epidemiology Studies—Alterations in Uric Acid Levels. Associations between serum PFOA levels and serum uric acid levels have been found in several occupational, community, and general population studies. Costa et al. (2009) and Sakr et al. (2007b) reported associations between serum PFOA levels and serum 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 2-17.

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). 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. Epidemiology 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

Figure 2-17. Risk of Hyperuricemia Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



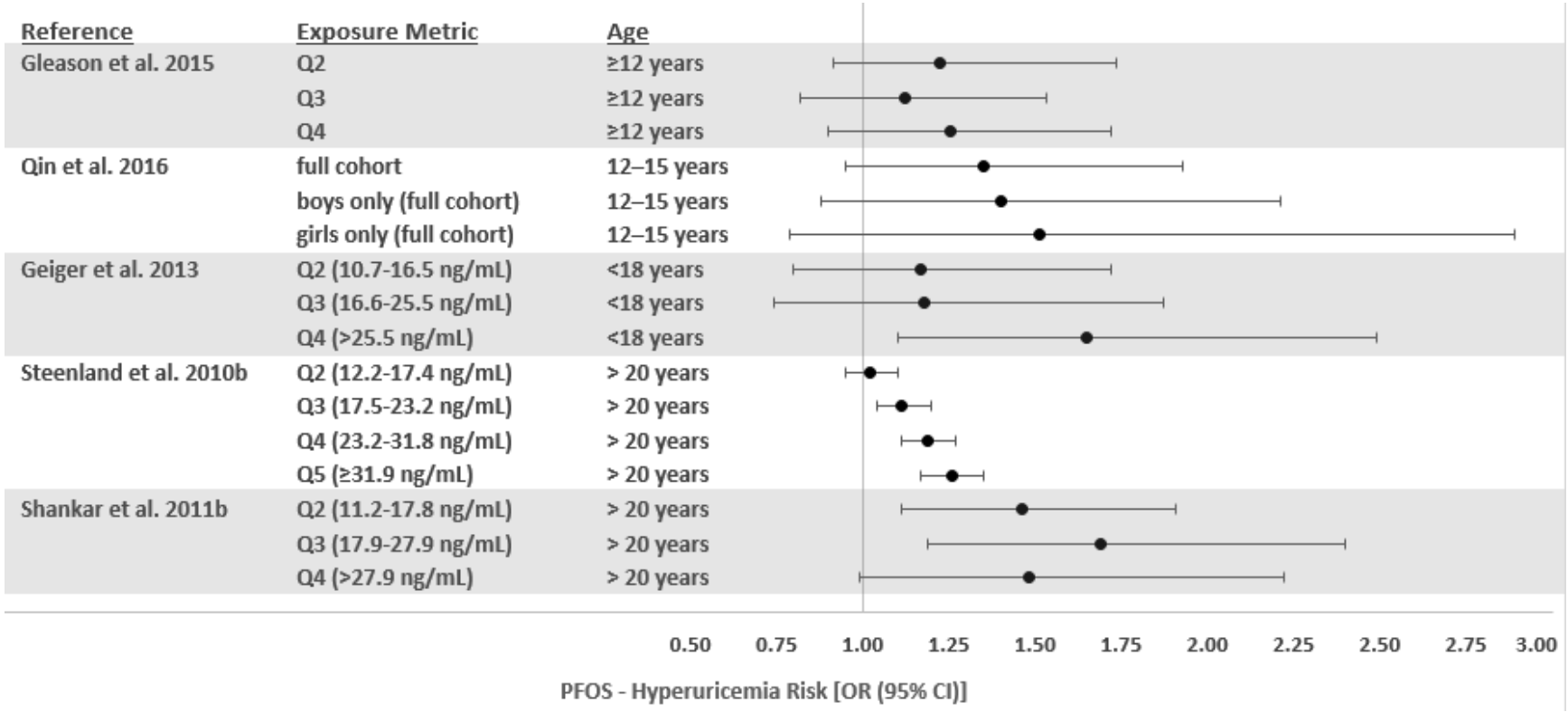
glomerular filtration rate; however, there is suggestive evidence that this association may be due to reverse causation rather than a direct effect. Associations between serum PFOA levels and serum uric acid levels have been consistently observed in occupational, community, and general populations. Laboratory animal studies have not found evidence of alterations in renal function or histological alterations.

PFOS

Epidemiology 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.

Epidemiology 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). Two studies of adolescent NHANES participants also found associations between serum PFOS and serum uric acid levels (Gleason et al. 2015; Kataria et al. 2015); a third 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; this was not found in the Gleason et al. (2015) study. 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 2-18.

Figure 2-18. Risk of Hyperuricemia Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



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. 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

Epidemiology Studies—Biomarkers of Renal Function. A small number of epidemiology studies have evaluated biomarkers of renal function. In a study of C8 Health Project participants, 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). A comparison between the reported median PFHxS level in the Watkins et al. (2013) study (5.2 ng/mL) and the lower end of the 4th quartile serum PFHxS level in the Kataria et al. (2015) study (≥4 ng/mL) suggests that the serum PFHxS levels were higher in the Watkins et al. (2013) study.

Epidemiology Studies—Alterations in Uric Acid Levels. In adolescent NHANES participants, no associations between serum PFHxS levels and serum uric acid levels (Gleason et al. 2015; Kataria et al. 2015) 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; Hoberman and York 2003); the NOAEL was 3 mg/kg/day. No significant effect on BUN was reported in female rats.

PFNA

Epidemiology Studies—Biomarkers of Renal Function. Two epidemiology 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.

Epidemiology Studies—Alterations in Uric Acid Levels. Gleason et al. (2015) found an association between serum PFNA and serum uric acid levels in adolescents; this association was not found in other 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.

PFDeA

Epidemiology Studies—Alterations in Uric Acid Levels. Epidemiology studies examining renal outcomes are limited to a study of Taiwanese adolescents that found no association between serum PFDeA 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 PFDeA 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.

PFUA

Laboratory Animal Studies. Treatment of male and female rats with 1.0 mg/kg/day PFUA 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.

PFBuS

Epidemiology Studies—Alterations in Uric Acid Levels. Serum PFBuS 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 PFBuS 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 300 mg/kg/day PFBuS. In a 90-day rat study, PFBuS 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).

PFDoA

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

PFHxA

Epidemiology 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. 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, PFBuS, or PFBA (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) 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).

PFBuS

No gross and microscopic alterations were observed in the eyes of rats administered ≤900 mg/kg/day PFBuS 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).

2.13 ENDOCRINE

Overview. Epidemiology 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 epidemiology 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. The results of community and general population studies suggest an association between serum PFOA and an increased risk of thyroid disease. There is also limited support of an association between serum PFOS and thyroid disease. Although some associations between serum PFOA or PFOS and thyroid stimulating hormone (TSH), triiodothyronine (T3), or thyroxine (T4) levels have been found, the results are not consistent across studies. A small number of studies have evaluated PFHxS, PFNA, and PFDeA and most studies have not found consistent associations between serum perfluoroalkyl levels and thyroid hormone levels.

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, or PFDeA. Histopathological alterations have been observed in the thyroid of some laboratory animal studies for PFHxS and PFBA; the investigators noted that these effects were likely secondary to the hepatocellular hypertrophy. In general, the pituitary, parathyroid, thyroid, and adrenal glands do not appear to be sensitive targets following exposure to PFOA, PFOS, or PFBuS.

PFOA

Epidemiology Studies. A number of epidemiology 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. Another study reported an association between serum PFOA and TSH, but this was only observed at one time point (Olsen et al. 1998b). A fourth occupational study reported that TSH, T4, and T3 levels were within the reference range (Sakr et al. 2007b).

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
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)	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 year, men ≤50 years, women >50 years, men >50 years)	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 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**
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 hypothryoxinemia 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)
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)

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)	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**
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
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)
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)
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)	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 year, men ≤50 years, women >50 years, men >50 years)	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**
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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)***
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)
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**
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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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)
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

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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			
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)
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)
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)
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)

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)	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)
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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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
		TSH	β -1.1 (CI 0.7–1.5), per interquartile shift
		Total T4	B 0.8 (-0.4–2.0), per interquartile shift
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)
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)	2.09 ng/mL (median PFNA)	TSH	NS (p=0.1354)
		T4	NS (p=0.7436)
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)

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)	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)
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)
PFDeA			
Berg et al. 2015 General population (n=391 pregnant women)	0.31–2.34 ng/mL (4th PFDeA quartile)	T3	**Association (p=0.03) (4th quartile)**

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 PFDeA)	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 PFDeA)	TSH	NS (p=0.2721)
		T4	NS (p=0.2176)
Wang et al. 2013a General population (903 pregnant women)	0.09 ng/mL (geometric mean PFDeA)	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 PFDeA)	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 PFDeA)	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)
PFUA			
Berg et al. 2015 General population (n=391)	0.4–0.96 ng/mL (4th PFUA quartile)	Free T3	**Association (p=0.00)*, 4th quartile**

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)	0.20 ng/mL (geometric mean PFUA)	TSH	NS (p=0.527)
		Free T4	NS (p=0.204)
Ji et al. 2012 General population (n=633)	1.75 ng/mL (median PFUA)	TSH	NS (p=0.5368)
		T4	NS (p=0.0642)
Wang et al. 2013a General population (n=903 pregnant women)	0.20 ng/mL (geometric mean PFUA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	3.26 ng/mL (median PFUA)	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 PFUA)	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)
PFDoA			
Ji et al. 2012 General population (n=633)	0.92 ng/mL (median PFDoA)	TSH	NS (p=0.6925)
		T4	NS (p=0.7153)
Wang et al. 2014 General population (285 pregnant women)	0.36 ng/mL (median PFDoA)	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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Yang et al. 2016a	0.046 ng/mL (mean PFDoA)	TSH	**Inverse association (p<0.01)***
General population (n=157 pregnant women)		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)***
Me-PFOSA-AcOH			
Jain 2013	NR	TSH	NS (p>0.05)
General population (NHANES) (n=1,525)		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 compound; 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; PFDeA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUA = perfluoroundecanoic acid; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

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 cumulative serum PFOA and hyperthyroidism or hypothyroidism were found in retrospective analysis (Winquist and Steenland 2014b). However, in prospective analysis, an association between 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. 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 four studies found associations between serum PFOA and T3 levels (Jain 2013; Lewis et al. 2015; Webster et al. 2016; Wen et al. 2013), four other studies did not find these associations (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 (Bloom et al. 2010; Jain 2013; Ji et al. 2012; Lewis et al. 2015; 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) levels were found in the general population studies, with the exception of the study by Lewis et al. (2015), which found an association for TSH and T4 levels.

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). 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 (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

Epidemiology Studies. A number of epidemiology 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. 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 two general population studies (Berg et al. 2015; Wang et al. 2014). In contrast, two other studies found inverse associations for TSH (Dallaire et al. 2009;

Yang et al. 2016a). An occupational exposure study (Olsen et al. 1998a) and eight general population studies (Bloom et al. 2010; Jain 2013; Ji et al. 2012; 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 (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 (Jain 2013; Ji et al. 2012; Lewis et al. 2015; Raymer et al. 2012; 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).

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. In a 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

Epidemiology Studies. Ten 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 epidemiology studies did not find associations for TSH, T3, or T4 (Bloom et al. 2010; Jain 2013; Ji et al. 2012; Lewis et al. 2015; Wang et al. 2013a, 2014; Webster et al. 2016; Yang et al. 2016a). Chan et al. (2011) did not find an increase in the risk of hypothyroxinemia associated

with serum PFHxS levels, but Wen et al. (2013) found increases in the risk of subclinical hypothyroidism and subclinical hyperthyroidism among women, but not men.

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; Hoberman and York 2003). 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.

PFNA

Epidemiology 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 (Bloom et al. 2010; Jain 2013; Ji et al. 2012; Lopez-Espinosa et al. 2012; 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). 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) or in NHANES participants (Wen et al. 2013).

PFDeA

Epidemiology Studies. Most general population studies did not find associations between serum PFDeA levels and TSH, T3, or T4 levels (Bloom et al. 2010; Ji et al. 2012; Wang et al. 2013a, 2014; Yang et al. 2016a). The exceptions were studies by Berg et al. (2015) and Wang et al. (2014), which found associations with T3 levels in pregnant women, and a study by Yang et al. (2016a), which found an inverse association with TSH levels in pregnant women.

Laboratory Animal Studies. Administration of a single dose of 80 mg/kg PFDeA 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).

PFUA

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

PFBuS

Laboratory Animal Studies. Treatment of rats with up to 900 mg/kg/day PFBuS 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.

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.

PFDoA

Epidemiology Studies. Three general population studies have evaluated the effect of PFDoA on thyroid hormone levels. Wang et al. (2014) reported inverse associations between serum PFDoA 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 PFDoA and TSH or T4.

Me-PFOSA-AcOH

Epidemiology Studies. In the only available study examining thyroid outcomes, Jain (2013) found no associations between serum Me-PFOSA-AcOH levels and TSH, T3, or T4 levels in NHANES participants.

2.14 IMMUNOLOGICAL

Overview. Epidemiology 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 epidemiology 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. In general, the epidemiology studies identify the immune system as a target of perfluoroalkyl toxicity. The strongest evidence of the immunotoxicity of perfluoroalkyls in humans comes from epidemiology studies finding associations evaluating the antibody response to vaccines. Associations have been found for PFOA, PFOS, PFHxS, and PFDeA. There is also some limited evidence for decreased antibody response for PFNA, PFUA, and PFDoA, 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. Epidemiology studies have also found increases in the asthma diagnosis that were associated with serum PFOA levels. There is also marginal evidence for PFOS, PFHxS, PFNA, PFDeA, PFBuS, and PFDoA; 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 autoimmunity; epidemiology 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 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

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
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**
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**
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**

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)	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)
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)

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 pregnant women)	1.3 ng/mL (maternal median PFOA)	Cord IgE levels	
		Males	NS (p>0.05)
		Females	**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)
Okada et al. 2014 General population (n=2,603 infants)	2.67 ng/mL (maternal mean PFOA)	Risk of allergic diseases	
		Males	OR 0.63 (0.63–1.37), 4th quartile
		Females	**OR 0.64 (0.42–0.97)*, 4th quartile**
		Eczema	
		Males	OR 0.75 (0.48–1.18), 4th quartile
		Females	OR 0.65 (0.39–1.09), 4th quartile
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)	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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Steenland et al. 2015 Occupational (n=3,713)	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**
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)

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. 2016b General population (n=78 adults receiving influenza vaccine)	2.28 ng/mL (geometric mean)	Seroconversion Hemagglutinin Immunohistochem.	 NS (p=0.07 for trend) 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)
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 IL-5 IFN-γ IL-2	 **Association (p=0.001 for trend)*** **Association (p=0.004 for trend)*** NS (p>0.05 for trend) 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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
		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
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
		Diphtheria antibody levels at age 5	NS, maternal PFOS NS, PFOS 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]
		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)
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)

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)	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)
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
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

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)	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**
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)

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)	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)
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)

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	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
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)
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	OR 0.81 (0.56–1.16), 4th quartile
		Females	OR 1.13 (0.75–1.69), 4th quartile
		Eczema	
		Males	OR 0.78 (0.51–1.19), 4th quartile
		Females	OR 0.82 (0.49–1.36), 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)	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**
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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		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)
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
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
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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		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)
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 Females	 OR 0.95 (0.66–1.38), 4th quartile **OR 0.55 (0.36–0.82)*, 4th quartile**
		Eczema Males Females	 OR 0.96 (0.61–1.52), 4th quartile OR 0.63 (0.38–1.02), 4th quartile
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)
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)

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		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]
PFDeA			
Dalsager et al. 2016 General population (n=359 children aged 1–4 years)	0.27 ng/mL (median maternal serum PFDeA 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 PFDeA 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**
Grandjean et al. 2012 General population (n=456 and n=464 children 5 and 7 years of age)	0.28 ng/mL (geometric mean PFDeA at age 5 years) 0.28 ng/mL (geometric mean maternal PFDeA)	Tetanus antibody levels at age 5	NS, maternal PFDeA **β -19.9% (-33.1 to -3.9), per 2-fold increase PFDeA levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFDeA **β -22.3 (-35.8 to -5.8), per 2-fold increase PFDeA levels at age 5**
		Diphtheria antibody levels at age 5	NS, maternal PFDeA NS, PFDeA at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFDeA NS, PFDeA at age 5
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)*, PFDeA at age 7** NS (p=0.258), PFDeA at age 13
		Diphtheria antibody levels at age 13	**Association (p=0.008)*, PFDeA at age 7** NS (p=0.726), PFDeA 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]
Kielsen et al. 2016 General population (n=12 adults)	0.30 ng/mL (median PFDeA)	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 PFDeA)	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
Smit et al. 2015 General population (n=1,024 children)	0.16 and 0.42 ng/mL (maternal mean PFDeA 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)***
PFUA			
Kielsen et al. 2016 General population (n=12 adults)	0.21 ng/mL (median PFUA)	Diphtheria antibody levels	**Inverse association (p=0.036)*, unadjusted**
		Tetanus antibody levels	**Inverse association (p=0.039)*, 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)	1.50 ng/mL (maternal mean PFUA)	Risk of allergic diseases	
		Males	OR 1.13 (0.79–1.63), 4th quartile
		Females	**OR 0.58 (0.39–0.86)*, 4th quartile**
		Eczema	
		Males	OR 1.16 (0.75–10.81), 4th quartile
		Females	**OR 0.50 (0.30–0.81)*, 4th quartile**
Smit et al. 2015 General population (n=1,024 children)	0.16 and 0.68 ng/mL (maternal mean PFUA 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

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

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFBuS			
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 PFBuS 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
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 PFBuS 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)
PFDoA			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children)	5.8 and 4.5 ng/mL (mean serum PFDoA 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
Kielsen et al. 2016 General population (n=12 adults)	0.039 ng/mL (median PFDoA)	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 PFDoA)	Risk of allergic diseases	
		Males	OR 0.93 (0.65–1.34), 4th quartile
		Females	OR 0.58 (0.39–0.85)*, 4th quartile
		Eczema	
		Males	OR 1.00 (0.64–1.55), 4th quartile
		Females	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 PFDoA 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

[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 compound; 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; PFBuS = perfluorobutane sulfonic acid; PFDeA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUA = perfluoroundecanoic acid; RR= relative risk; TNF-α =tumor necrosis factor-α

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 2-6, 2-7, and 2-8. 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 PFDeA. 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

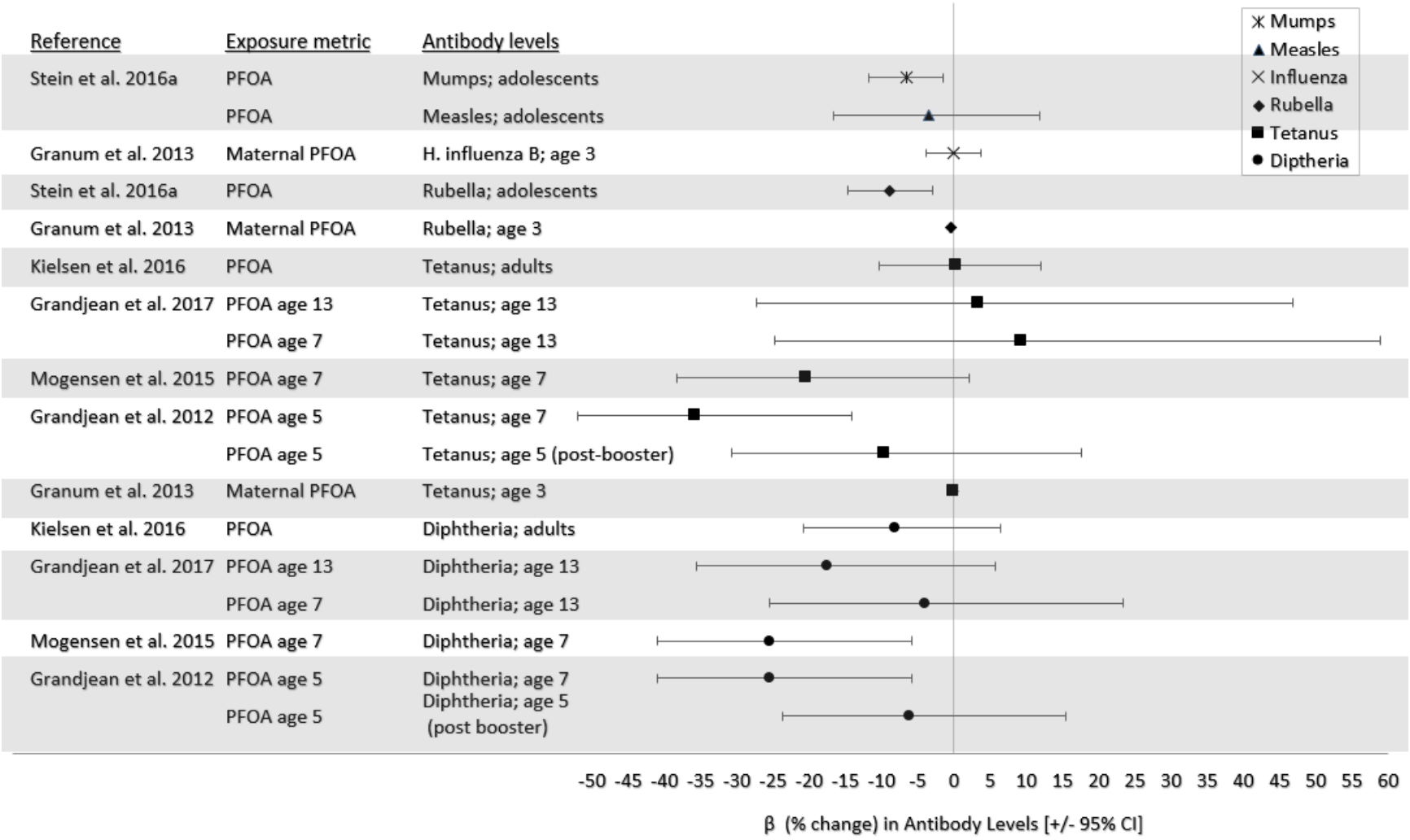
Epidemiology Studies—Immunosuppression Outcomes. Studies evaluating the immunosuppressive effects of PFOA have examined disease resistance and antibody responses. One study found associations between maternal serum PFOA and the number of episodes of the common cold and other respiratory tract infections 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. 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), or between maternal PFOA and the risk of number of days with cough, nasal discharge, diarrhea, or vomiting (Dalsager et al. 2016).

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 2-19. 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 Islands (Heilmann et al. 2010). 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).

Epidemiology 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) and adults (Anderson-Mahoney et al. 2008; Zhu et al. 2016).

Figure 2-19. Antibody Responses Relative to Serum PFOA Levels in Epidemiology Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFOA)

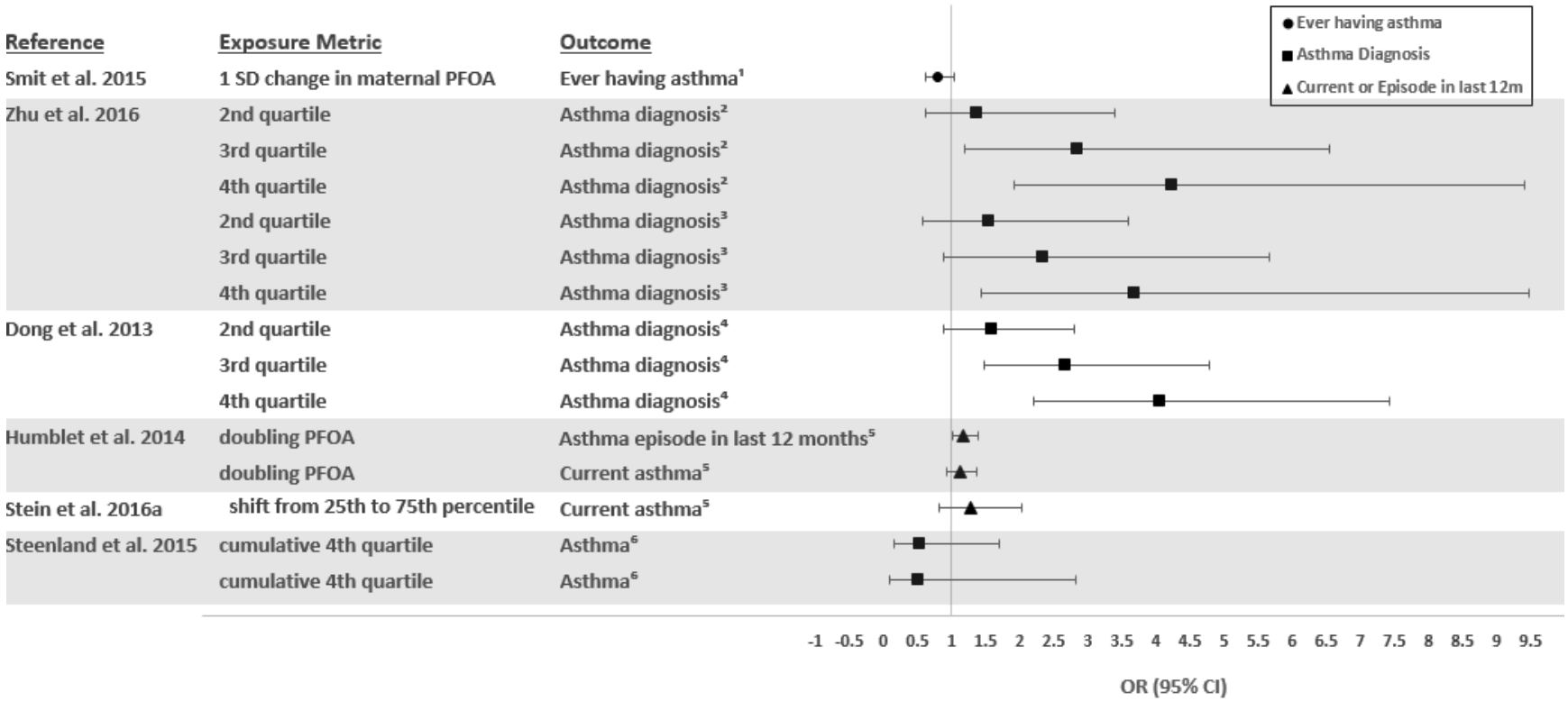


However, other studies have found no association between estimated cumulative serum PFOA levels and incidence of asthma being treated with medication in workers (Steenland et al. 2015) or asthma in the general population (Stein et al. 2016a). In children, no associations between maternal serum PFOA levels and asthma-related health outcomes were observed in 3-year-old children (Granum et al. 2013) or 5–9-year-old children (Smit et al. 2015), 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), or children 5–9 years of age (Smit et al. 2015) 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 2-20. 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).

No association between current serum PFOA levels and risks of allergy or allergic sensitization were found in adults (Stein et al. 2016a). 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

Figure 2-20. Risk of Asthma Diagnosis Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



[1]age 5–9 years
[2]males, age 9–16 years
[3]females age 9–16 years
[4]age 10–15 years
[5]age 12–19 years
[6]adult

al. 2015). NTP (2016b) concluded that there is low confidence that exposure to PFOA during childhood is associated with increased hypersensitivity responses.

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.

Epidemiology 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 epidemiology 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 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).

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).

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.

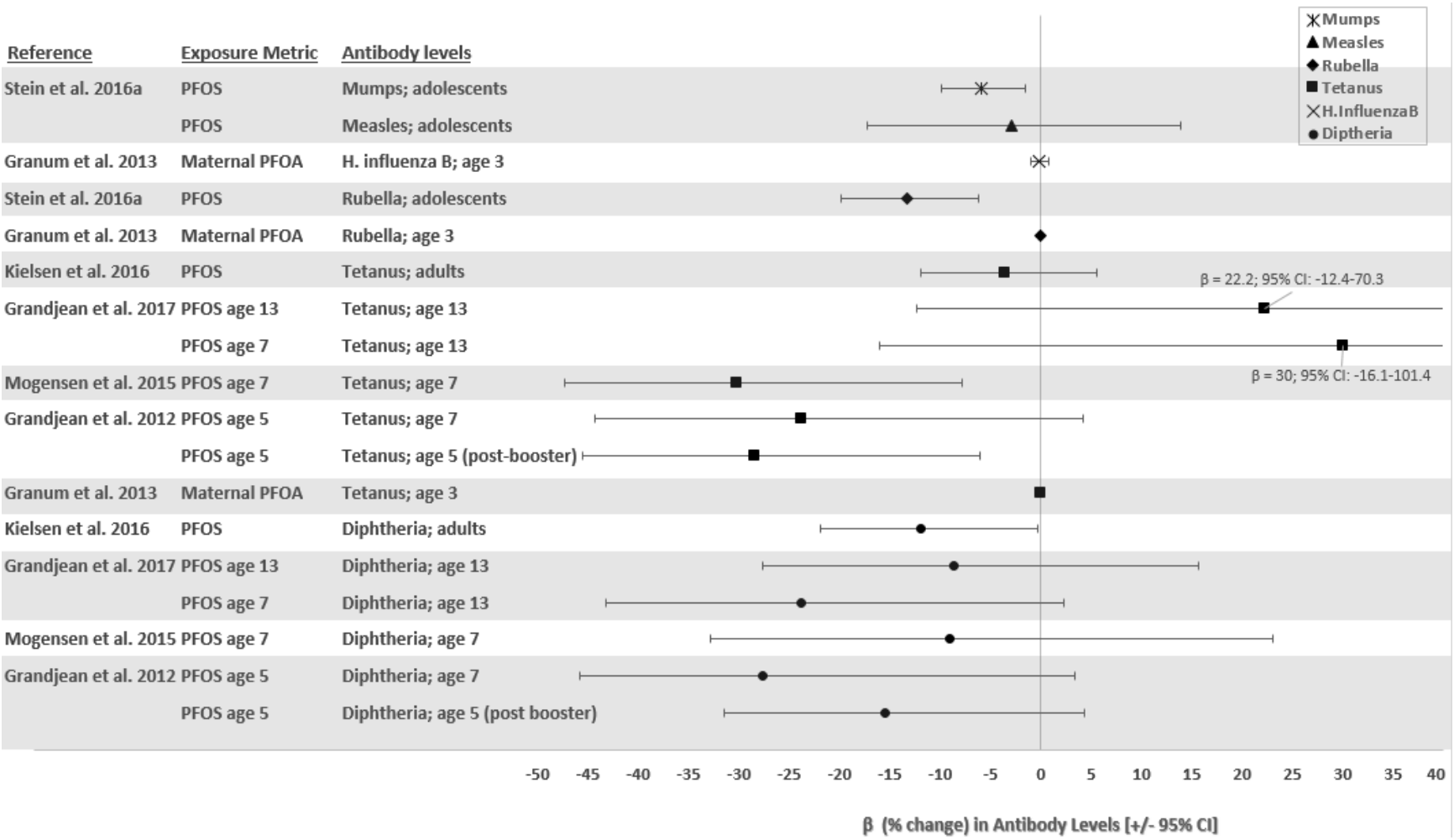
Summary. Epidemiology studies have evaluated several aspects of immunotoxicity including immunosuppression, hypersensitivity, and autoimmunity. A number of general populations studies have found significant inverse associations between serum PFOA levels and antibody responses to vaccines. However, no 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

Epidemiology Studies—Immunosuppression Outcomes. Several epidemiology 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). However, associations between the number of days with symptoms of infection and maternal PFOS levels were observed in children (Dalsager et al. 2016).

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 2-21. 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 (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 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 (Grandjean et al. 2012) and antibody levels at age 13 were not associated with child PFOS levels at age 7 or 13 (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).

Figure 2-21. Antibody Responses Relative to Serum PFOS Levels in Epidemiology Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFOS)



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).

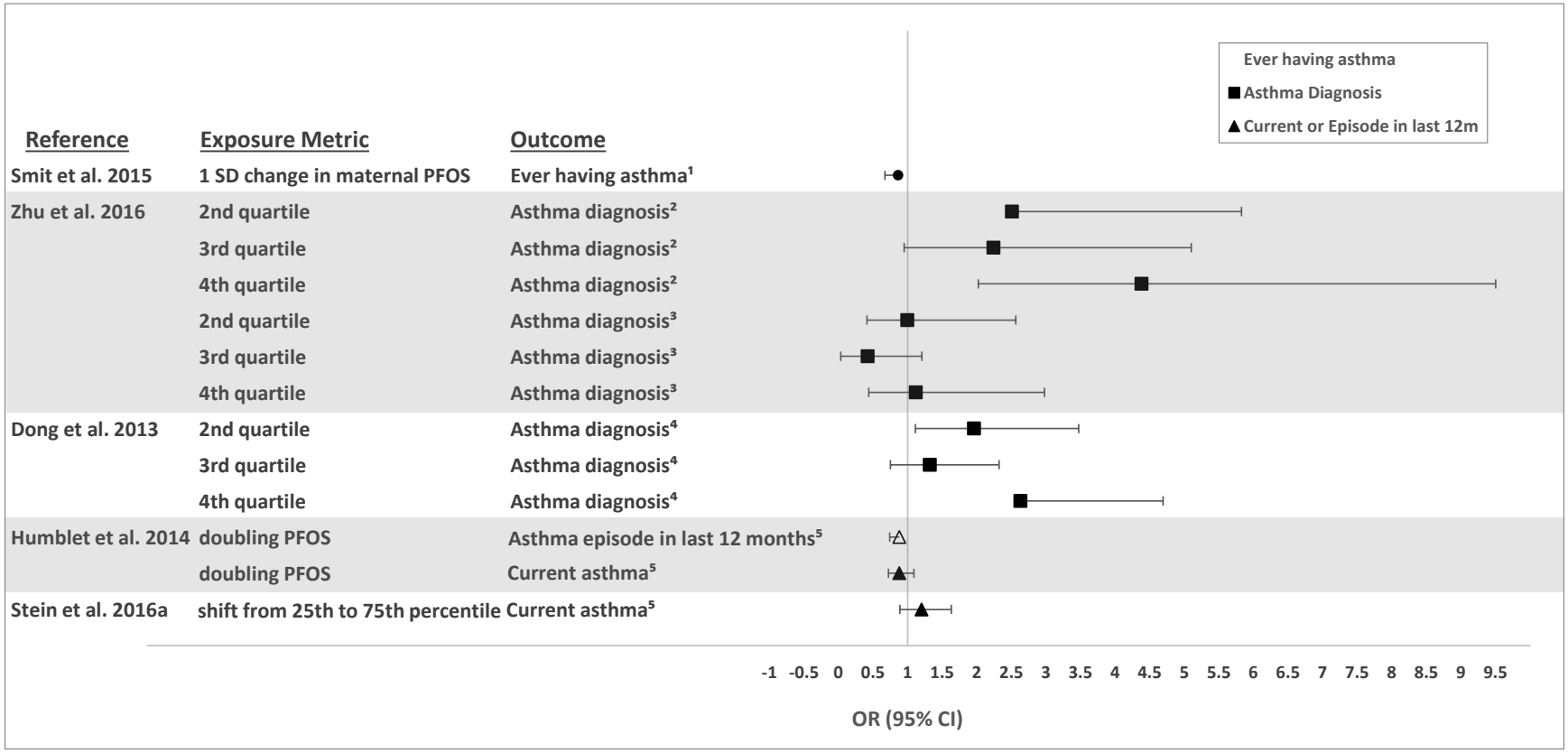
Epidemiology Studies—Hypersensitivity Outcomes. Several studies examined the risk of hypersensitivity associated with serum PFOS in children and adolescents; however, the results are inconsistent. In two case-control studies, increased risks of asthma were observed. Dong et al. (2013) reported an increased 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. 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 olds, between maternal PFOS and asthma diagnosis in children in adolescence (Humblet et al. 2014; Stein et al. 2016a). Data evaluating associations between serum PFOS and the risk of asthma diagnosis are presented in Figure 2-22.

No associations between maternal PFOS and eczema, atopic dermatitis, or wheezing have been found in children (Granum et al. 2013; 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.

Figure 2-22. Risk of Asthma Diagnosis Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



[1]age 5–9 years
[2]males, age 9–16 years
[3]females age 9–16 years
[4]age 10–15 years
[5]age 12–19 years

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.

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 epidemiology 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

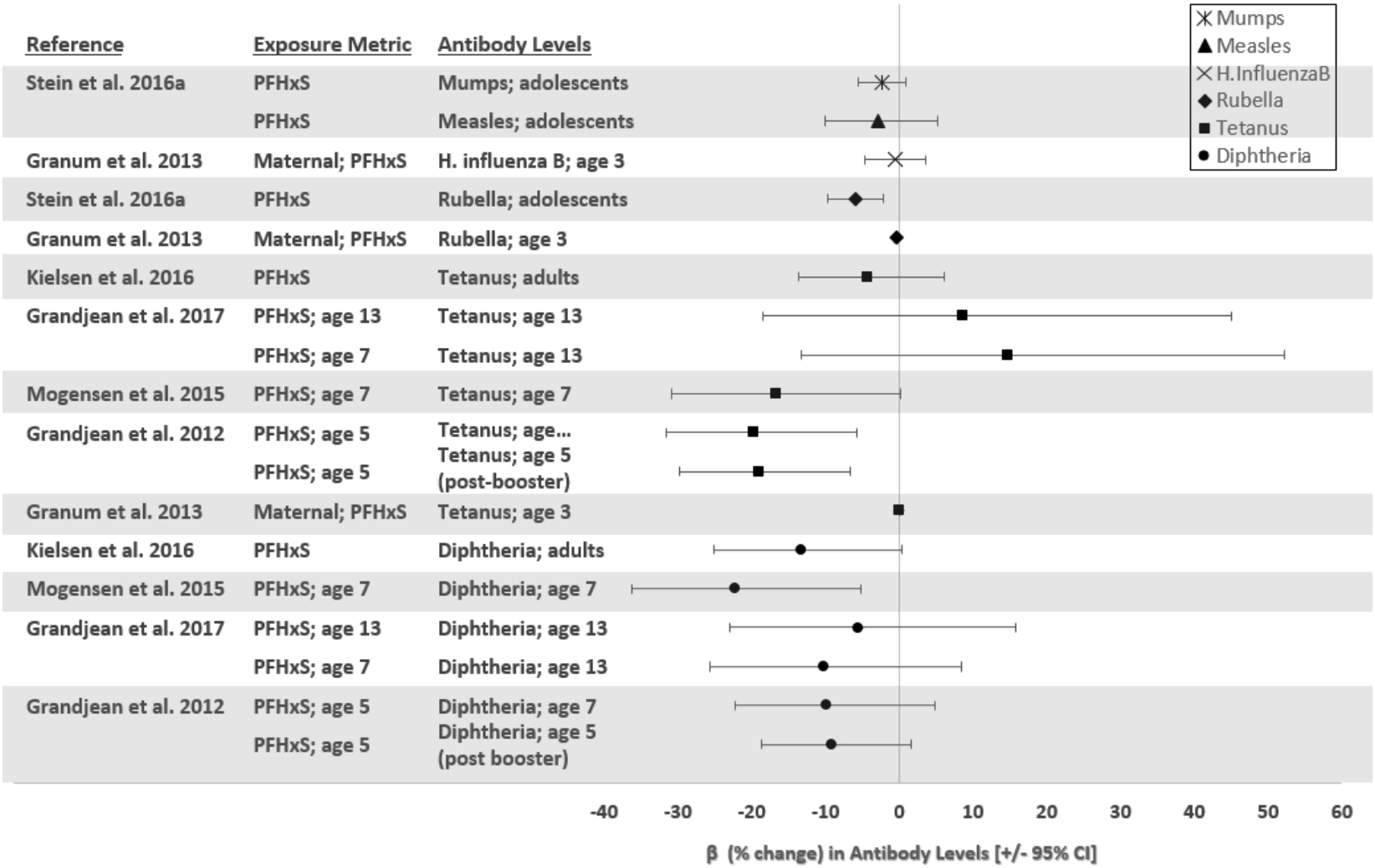
results have been examined in studies evaluating associations between serum PFOS and hypersensitivity outcomes, such as asthma; no associations were found for eczema, dermatitis, food allergies/ sensitizations. Laboratory animal studies, particularly studies in mice, provide strong evidence of the immunotoxicity of PFOS. The strongest evidence comes from studies reported impaired antibody responses resulting in 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

Epidemiology Studies—Immunosuppression Outcomes. Several epidemiology 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 2-23. Inverse associations were observed between tetanus antibody levels in 5 and 7 year olds and serum PFHxS levels at age 5 or 7 years (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); one study found an association between maternal PFHxS levels and the number of episodes of gastroenteritis in children (Granum et al. 2013).

Figure 2-23. Antibody Responses Relative to Serum PFHxS Levels in Epidemiology Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFHxS)



Epidemiology Studies—Hypersensitivity Outcomes. Data evaluating associations between serum PFHxS and the risk of asthma diagnosis are presented in Figure 2-24. No associations were observed between asthma diagnosis, wheezing, and/or eczema in children and maternal serum PFHxS levels (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; Zhu et al. 2016), but no association with asthma severity (Dong et al. 2013). Dong et al. (2013) also reported associations between serum PFHxS levels and 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).

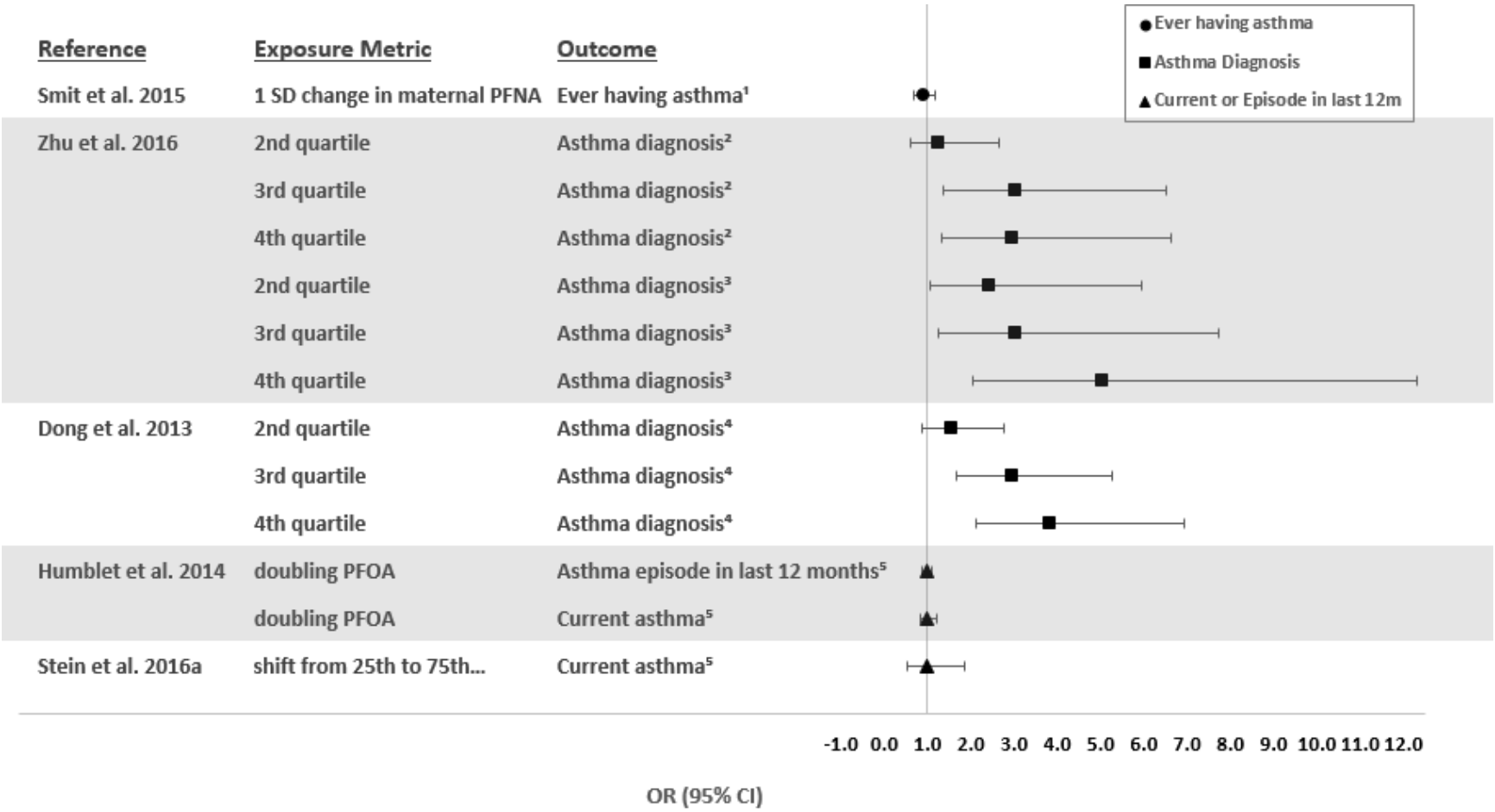
An increased risk of food allergies associated with serum PFHxS levels, but not increased sensitivity to foods, was found in adolescents (Buser and Scinicariello 2016). Another study found no associations between serum PFHxS levels and allergic sensitization to plants, dust mites, pets, cockroaches/shrimp, 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; Hoberman and York 2003) 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

Epidemiology 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

Figure 2-24. Risk of Asthma Diagnosis Relative to PFHxS Levels (Presented as Adjusted Odds Ratios)



[1]age 5–9 years
[2]males, age 9–16 years
[3]females age 9–16 years
[4]age 10–15 years
[5]age 12–19 years

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 2-25.

Overall, the epidemiology data do not suggest an association between decreased infectious disease resistance and serum 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. 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).

Epidemiology Studies—Hypersensitivity Outcomes. Case-control studies of asthmatic children have reported associations between serum PFNA and asthma diagnosis (Dong et al. 2013; Zhu et al. 2016), but no association with asthma severity (Dong et al. 2013). However, cross-sectional or retrospective studies (Humblet et al. 2014; Smit et al. 2015; Stein et al. 2016a) have not found associations. Data evaluating associations between serum PFNA and the risk of asthma diagnosis are presented in Figure 2-26. No associations were found in adolescents between 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).

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).

Figure 2-25. Antibody Responses Relative to Serum PFNA Levels in Epidemiology Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFNA)

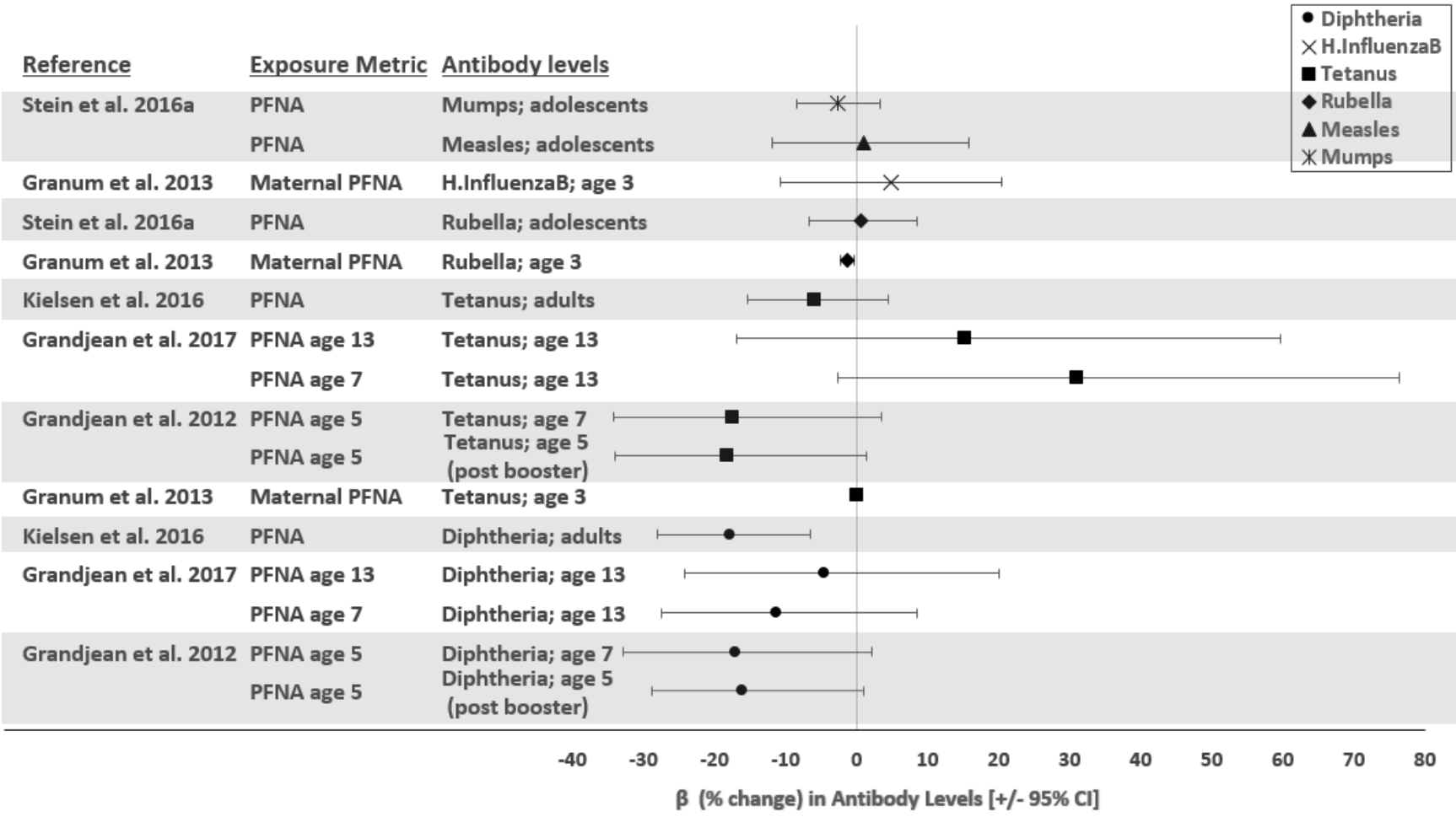
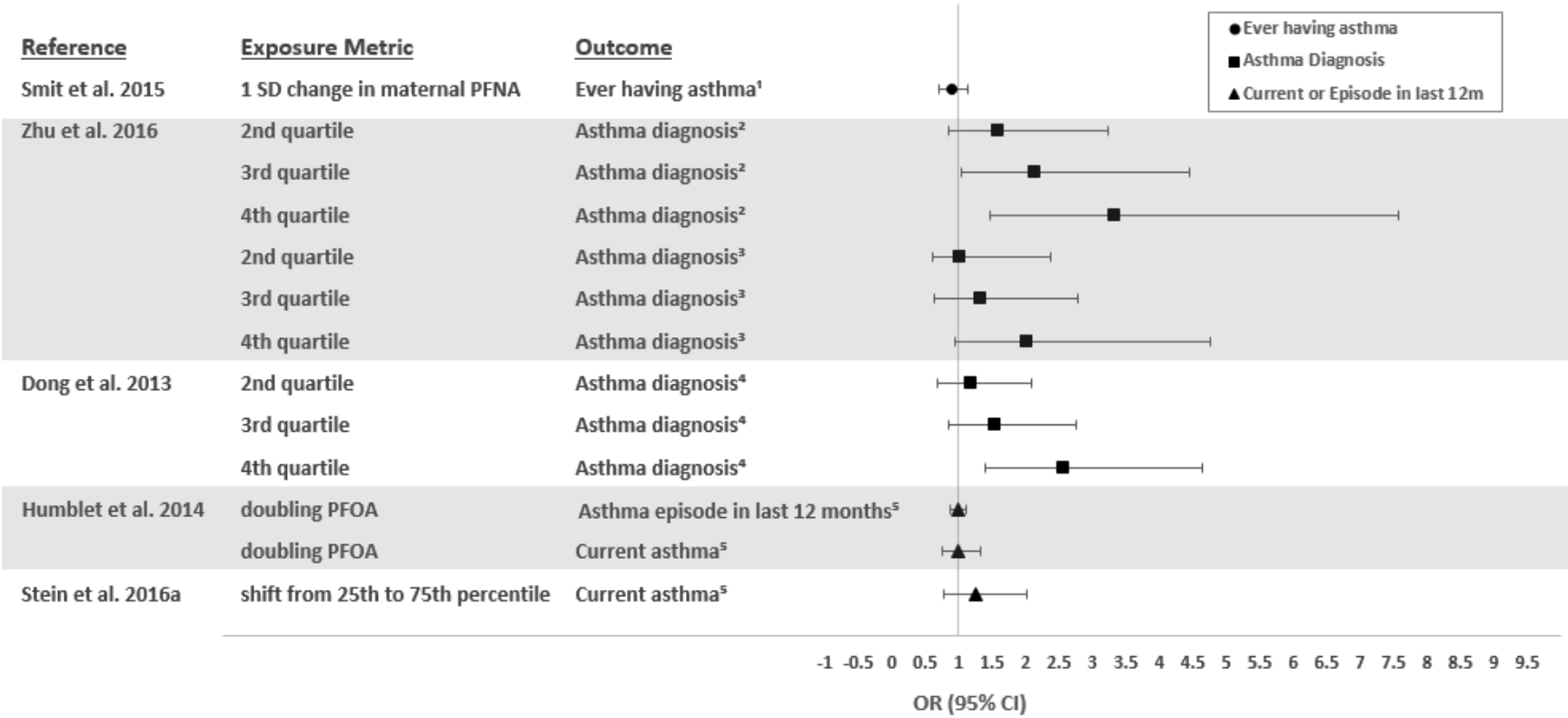


Figure 2-26. Risk of Asthma Diagnosis Relative to PFNA Levels (Presented as Adjusted Odds Ratios)



[1]age 5–9 years
[2]males, age 9–16 years
[3]females age 9–16 years
[4]age 10–15 years
[5]age 12–19 years

No alterations were observed in the response of splenic T lymphocytes to ConA at 5 mg/kg/day (Fang et al. 2008).

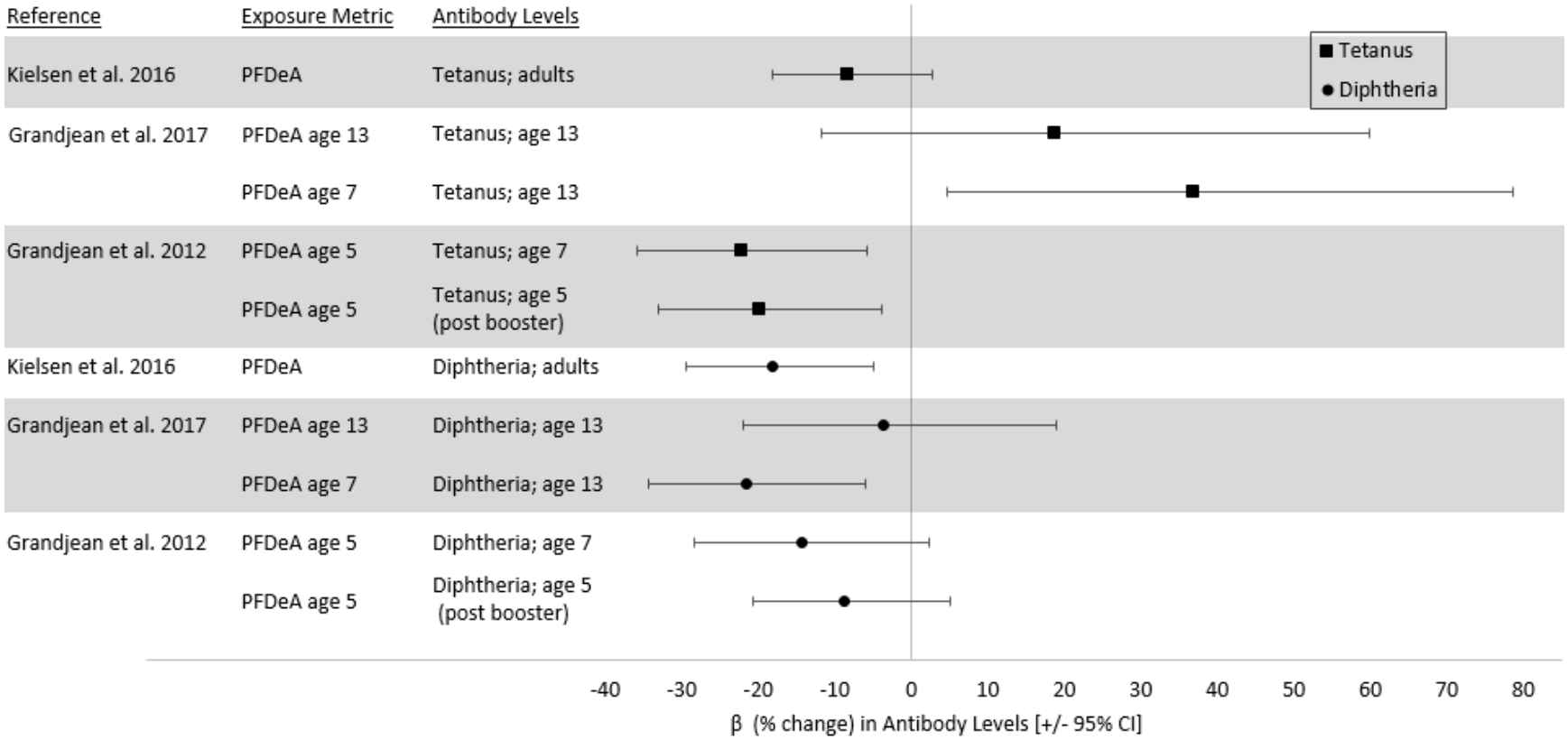
Two weeks after a single intraperitoneal administration of 46 mg/kg PFNA to male and female B57BL/6J mice, a number of immunological alterations included significant decreases relative spleen weight and splenic leukocyte counts, alterations in splenic T-lymphoctye phenotypes (increased ratios of CD4+ 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).

PFDeA

Epidemiology Studies—Immunosuppression Outcomes. Studies examining possible associations between serum PFDeA levels and response to vaccines have reported mixed results; see Figure 2-27 for a graphical presentation of the antibody response relative to PFDeA levels. Inverse associations were observed between serum PFDeA levels at age 5 and tetanus antibody levels at ages 5 and 7 (Grandjean et al. 2012) and serum PFDeA levels at age 7 and antibody levels at age 13 (Grandjean et al. 2017). Similarly, diphtheria antibody levels at age 13 were inversely associated with serum PFDeA levels at age 7 (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 PFDeA levels, but there was no association for tetanus antibody levels (Kielsen et al. 2016); this study did not adjust for potential confounders. In the only study examining the possible association between serum PFDeA levels and infectious disease resistance, no association was found between maternal serum PFDeA levels and symptoms of infection in children aged 1–4 years (Dalsager et al. 2016).

Epidemiology 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

Figure 2-27. Antibody Responses Relative to Serum PFDeA Levels in Epidemiology Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFDeA)



were also observed. A cross-sectional study of children did not find associations between maternal PFDeA 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 PFDeA levels (Okada et al. 2014). Data evaluating associations between serum PFDeA and the risk of asthma diagnosis are presented in Figure 2-28.

Laboratory Animal Studies. A single gavage dose of 80 mg/kg PFDeA 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.

PFUA

Epidemiology Studies. Three epidemiology studies have evaluated the potential immunotoxicity of PFUA in humans. Kielsen et al. (2016) reported inverse associations between serum PFUA (unadjusted for potential confounders) and diphtheria and tetanus antibody levels in adults. No significant associations between maternal PFUA levels and the risk of asthma diagnosis, eczema, or wheezing were observed in children (Smit et al. 2015). The third study (Okada et al. 2012) found inverse associations between maternal serum PFUA and risk of allergies or eczema in female infants, but not in males.

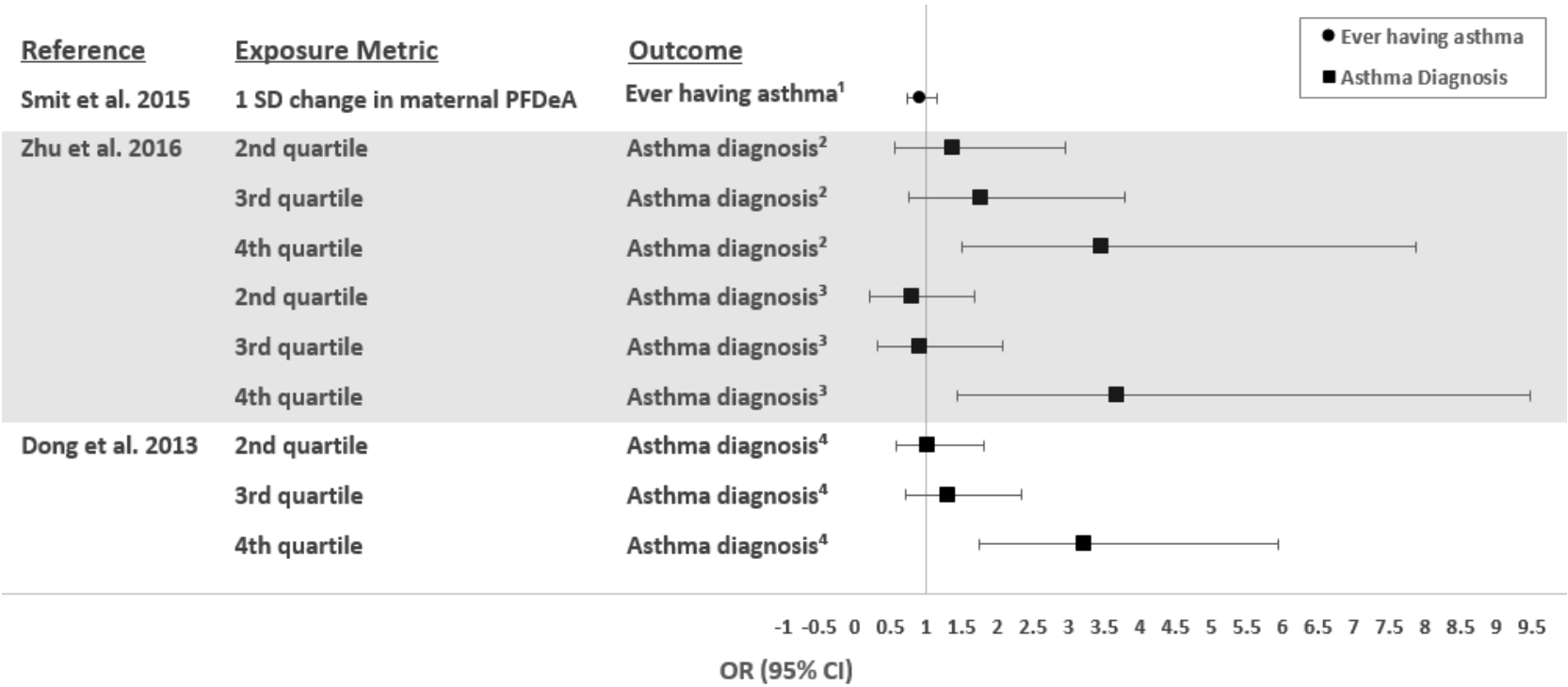
PFHpA

Epidemiology 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.

PFBuS

Epidemiology Studies. The epidemiology database for PFBuS consists of two case-control studies in asthmatic children (Dong et al. 2013; Zhu et al. 2016). Both studies reported increases in asthma diagnosis, but no association with serum IgE levels.

Figure 2-28. Risk of Asthma Diagnosis Relative to PFDeA Levels (Presented as Adjusted Odds Ratios)



[1]age 5–9 years
[2]males, age 9–16 years
[3]females age 9–16 years
[4]age 10–15 years

Laboratory Animal Studies. No significant histological alterations were observed in spleen, thymus, or lymph nodes of rats administered via gavage 900 mg/kg/day 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).

PFDoA

Epidemiology Studies. Four epidemiology studies examining potential immunotoxic endpoints were identified. Kielsen et al. (2016) found inverse associations between recent serum PFDoA levels (not adjusted for potential confounders) and diphtheria and tetanus antibody levels in adults. Associations between serum PFDoA 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 PFDoA levels and risk of asthma diagnosis, eczema, or wheezing (Smit et al. 2015). Another study did not find associations between maternal serum PFDoA levels and the risk of allergic disease or eczema in infants (Okada et al. 2014).

PFHxA

Epidemiology Studies. In the only epidemiology study examining 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.

2.15 NEUROLOGICAL

Overview. There are limited data on the neurotoxicity of perfluoroalkyls in humans or laboratory animals; epidemiology data come from two 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*

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)
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)
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**

Table 2-17. Summary of Neurological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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 compound; 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

Perfluoroalkyls, Table 11. 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. There are limited epidemiology data on the neurotoxicity of perfluoroalkyls. The two available studies found decreases in the risk of memory loss associated with serum PFOA, PFOS, PFHxS, and PFNA.

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 2-4, 2-6, 2-7, and 2-8. No morphological alterations in the brain and nerves were observed in studies of PFOA, PFOS, PFBuS, or PFBA. No alterations in neurological function tests were observed in studies of PFHxS, PFHxA, PFBA, or PFDoA. Impaired learning and memory was observed in a study of PFOS and decreases in grip strength were observed in a study of PFUA.

PFOA

Epidemiology 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. In a second 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). 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

Epidemiology Studies. Two 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).

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

Epidemiology 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; Hoberman and York 2003). 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

Epidemiology 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).

PFUA

Laboratory Animal Studies. In the only study located for PFUA, a decrease in grip strength was observed in male and female rats administered 1.0 mg/kg/day PFUA 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.

PFBuS

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 PFBuS 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 PFBuS 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.

PFDoA

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).

PFHxA

Laboratory Animal Studies. Treatment with 100 or 200 mg/kg/day PFHxA in male and female Sprague-Dawley rats via gavage for 104 weeks had no effect on locomotion or performance in the FOB test (Klaunig et al. 2015).

2.16 REPRODUCTIVE

Overview. A number of epidemiology 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 epidemiology 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, or PFUA 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 PFHxS, PFNA, PFDeA, or PFOSA. Some associations between serum perfluoroalkyls (PFOA, PFOS, PFHxS, PFNA, PFDeA) 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. Epidemiology studies of PFOA and PFOS provide evidence of impaired fertility (increased risks of longer time to pregnancy and infertility); there is also some evidence for PFHxS and PFNA, but the results are not consistent across studies. The small number of studies evaluating fertility for PFDeA, PFUA, PFOSA, Me-PFOSA-AcOH, and Et-PFOSA-AcOH did not find associations.

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

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			
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
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)

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]
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)	SHGB	**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)***
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)

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]
		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	**Inverse association (p=0.0001)***
		Menopausal subgroup	**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
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)

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]
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)	SHGB	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)
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)

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]
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
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)
PFDeA			
Barrett et al. 2015 General population (n=178 women)	0.25 and 0.24 ng/mL (mean PFDoA 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)	0.38 ng/mL (mean PFDeA)	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)
PFUA			
Barrett et al. 2015 General population (n=178 women)	0.40 and 0.42 ng/mL (mean PFUA 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 PFUA)	SHBG	NS (p>0.05)
		FSH	**Inverse association (p<0.05)*, females 12–17 years old**
		Testosterone	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]
PFOSA			
Barrett et al. 2015 General population (n=178 women)	0.25 and 0.23 ng/mL (mean PFOSA 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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; DHEAS = dihydroepiandrosterone sulfate; FSH = follicle stimulating hormone; LH = luteinizing hormone; NS = not significant; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFUA = 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 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 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 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)
PFDeA			
Buck Louis et al. 2015 General population (n=96 Michigan and 366 in Texas)	0.3 and 0.5 ng/mL (median PFDeA 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 PFDeA)	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)
Me-PFOSA-AcOH			
Buck Louis et al. 2015 General population (n=96 Michigan and 366 in Texas)	0.4 and 0.25 ng/mL (median Me-PFOSA-AcOH 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 neck/midpiece abnormalities	**Association (p<0.05)***
		↑ immature sperm	**Association (p<0.05)***
		Other parameters	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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

Me-PFOSA-AcOH = 2-(N-methyl-perfluorooctane sulfonamide) acetic acid; NS = not significant; PFDeA = 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 (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 (cumulative PFOA exposure 5th quintile) >80.8 ng/mL (measured 5th PFOA quintile)	Menopause age (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
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**

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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**
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**
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
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**

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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**
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
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)
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**

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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
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)
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**
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

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDeA			
Buck Louis et al. 2012 General population (n=473)	0.20 and 0.18 ng/mL (geometric mean PFDeA 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

[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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; OR = odds ratio; NS = not significant; PFDeA = 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. 2015b, 2015c 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. 2015b, 2015c (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
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**
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)
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)***

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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	**OR 1.6 (1.1–2.3)*, 2nd quartile**
		Parous subgroup	**OR 2.4 (1.4–4.1)*, 3rd quartile**
		Primiparous subgroup	OR 0.5 (0.2–1.2), 4th quartile
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. 2015b, 2015c General population (n=440 pregnant women)	36.3–103.8 ng/mL (4th PFOS quartile)	Fecundability	FR 0.96 (0.75–1.24), 4th quartile
		Parous subgroup	FR 1.04 (0.70–1.55), 4th quartile
		Nulliparous subgroup	FR 0.97 (0.62–1.51), 4th quartile
		Infertility	OR 1.03 (0.54–2.00), 4th quartile
		Parous subgroup	OR 0.70 (0.16–3.11), 4th quartile
		Nulliparous subgroup	OR 1.23 (0.452–3.39), 4th quartile
Bach et al. 2015b, 2015c (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	**FR 0.79 (0.66–0.95)*, 2nd quartile**
		Parous subgroup	FR 0.90 (0.70–1.14), 4th quartile
		Nulliparous subgroup	**FR 0.68 (0.52–0.91)*, 3rd quartile**
		Infertility	**OR 1.65 (1.01–2.68)*, 2nd quartile**
		Parous subgroup	OR 1.60 (0.78–3.28), 4th quartile
		Nulliparous subgroup	**OR 2.71 (1.38–5.30)*, 3rd quartile**
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)
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)
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
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
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)***

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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
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)***
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)
PFDeA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.3 ng/mL (median maternal PFDeA)	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
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.10 and 0.11 ng/mL (median PFDeA 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)
PFUA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.3 ng/mL (median maternal PFUA)	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
PFOSA			
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.10 and 0.11 ng/mL (median PFOSA 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)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Me-PFOSA-AcOH			
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.45 and 0.39 ng/mL (median Me-PFOSA-AcOH in women with no pregnancy and pregnant)	Fecundability	OR 0.99 (0.82-–1.18)
		Not becoming pregnant within first six cycles	OR 1.23 (0.68–2.20)
Et-PFOSA-AcOH			
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	2.12 and 1.79 ng/mL (median Et-PFOSA-AcOH in women with no pregnancy and pregnant)	Fecundability	OR 0.79 (0.62–1.00)
		Not becoming pregnant within first six cycles	OR 1.53 (0.85–2.77)

[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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

Et-PFOSA-AcOH = 2-(N-ethyl-perfluorooctane sulfonamide) acetic acid; FOR = fecundability odds ratio; FR = fecundability ratio (probability of conceiving during a given menstrual cycle); OR = odds ratio; Me-PFOSA-AcOH = 2-(N-methyl-perfluorooctane sulfonamide) acetic acid; NS = not significant; PFDeA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFUA = perfluoroundecanoic acid

are presented in Tables 2-1, 2-3, 2-4, 2-5, and 2-6 and in Figures 2-4, 2-6, 2-7, and 2-8. Multigeneration studies on PFOA, PFOS, and PFBuS have not found alterations in reproductive parameters in animals; similarly, no effect on fertility was observed for PFHxS. An increase in the incidence of Leydig cell hyperplasia (reclassified as gonodal stromal hyperplasia) has been observed in animals exposed to PFOA; one study for PFDoA reported ultrastructural alterations in the testes. Studies on PFOS, PFHxS, PFBuS, 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).

PFOA

Epidemiology Studies—Reproductive Hormone Levels. Two studies have evaluated potential effects of PFOA exposure on reproductive hormone levels in workers (Olsen et al. 1998b; Sakr et al. 2007b). Sakr et al. (2007b) found associations between serum PFOA and estradiol and testosterone levels male workers at the Washington Works 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.

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.

Epidemiology 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 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).

Epidemiology Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. One study examined possible associations between serum PFOA levels and alterations in menstrual cycle length (Lyngsø et al. 2014). 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. No alterations in the risk of having a short menstrual cycle (≤24 days) or irregular menstrual cycles (≥7 days difference between cycles) were observed. 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. An analysis by Taylor et al. (2014) of possible reverse causality showed that serum PFOA levels increased after menopause. In contrast, no alterations in the risk of early menopause or age of menopause were associated with 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). 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 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 have evaluated a possible link between maternal PFOA levels and breastfeeding duration. Both 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). When the women were segregated by parity, the associations were only found in multiparous women (Fei et al. 2010). This finding may be due to reverse causality since shorter breastfeeding would likely result in lower maternal transfer of PFOA.

Epidemiology 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 2-29 and 2-30, respectively. With the exception of the Vestergaard et al. (2012) study, all of the women were pregnant; thus, couples with unresolved infertility are underrepresented in these analyses. No studies were identified that used paternal serum PFOA levels as the biomarker 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 ≥3.91 ng/mL (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 fecudability risk among the nulliparous women. In another set of women participating in the Danish National Birth Cohort study (Bach et al. 2015b), no alterations in fecudability 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 fecudability (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 2-29. Fecundability Relative to PFOA Levels (Presented as Adjusted Fecundability Ratios)

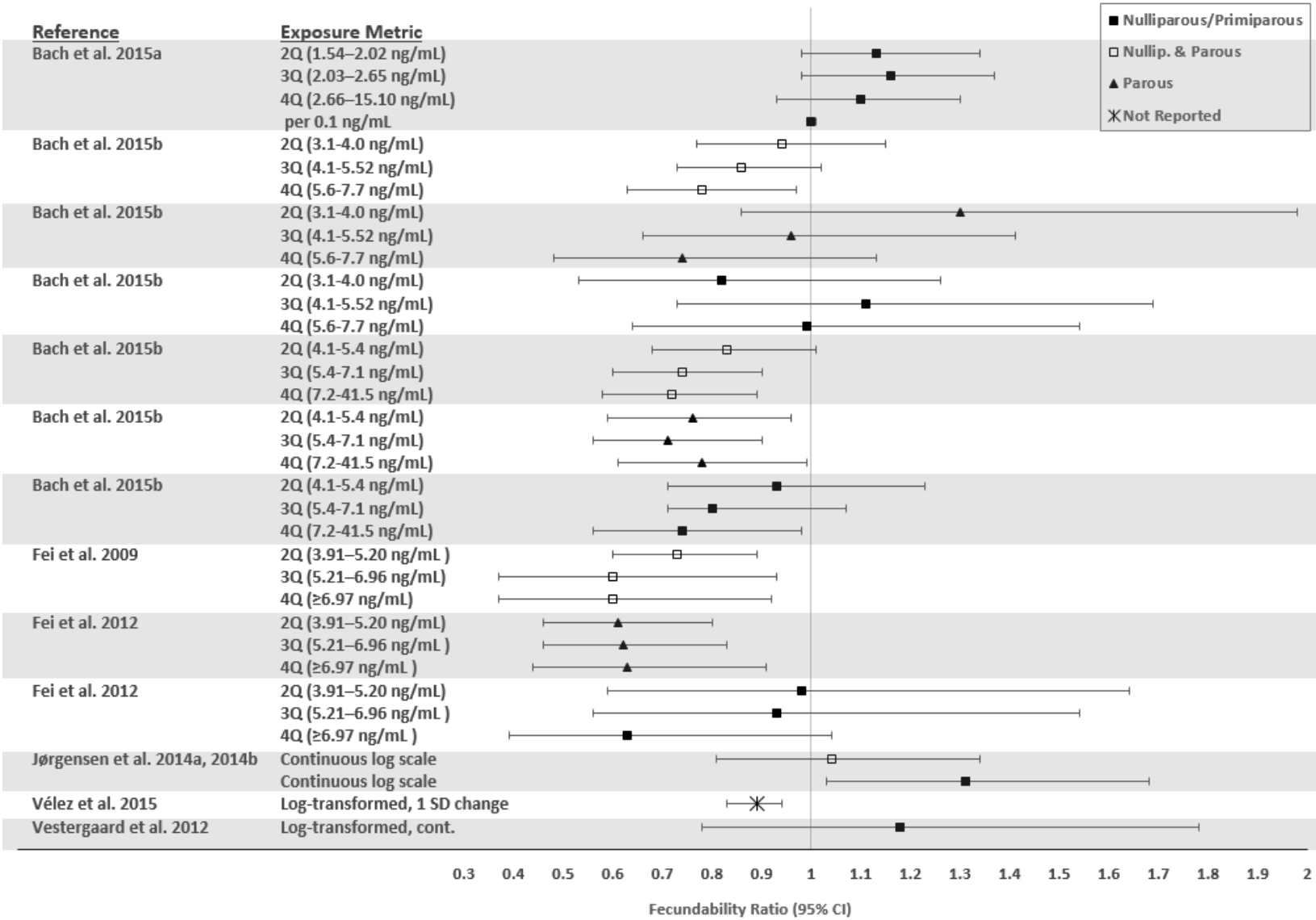
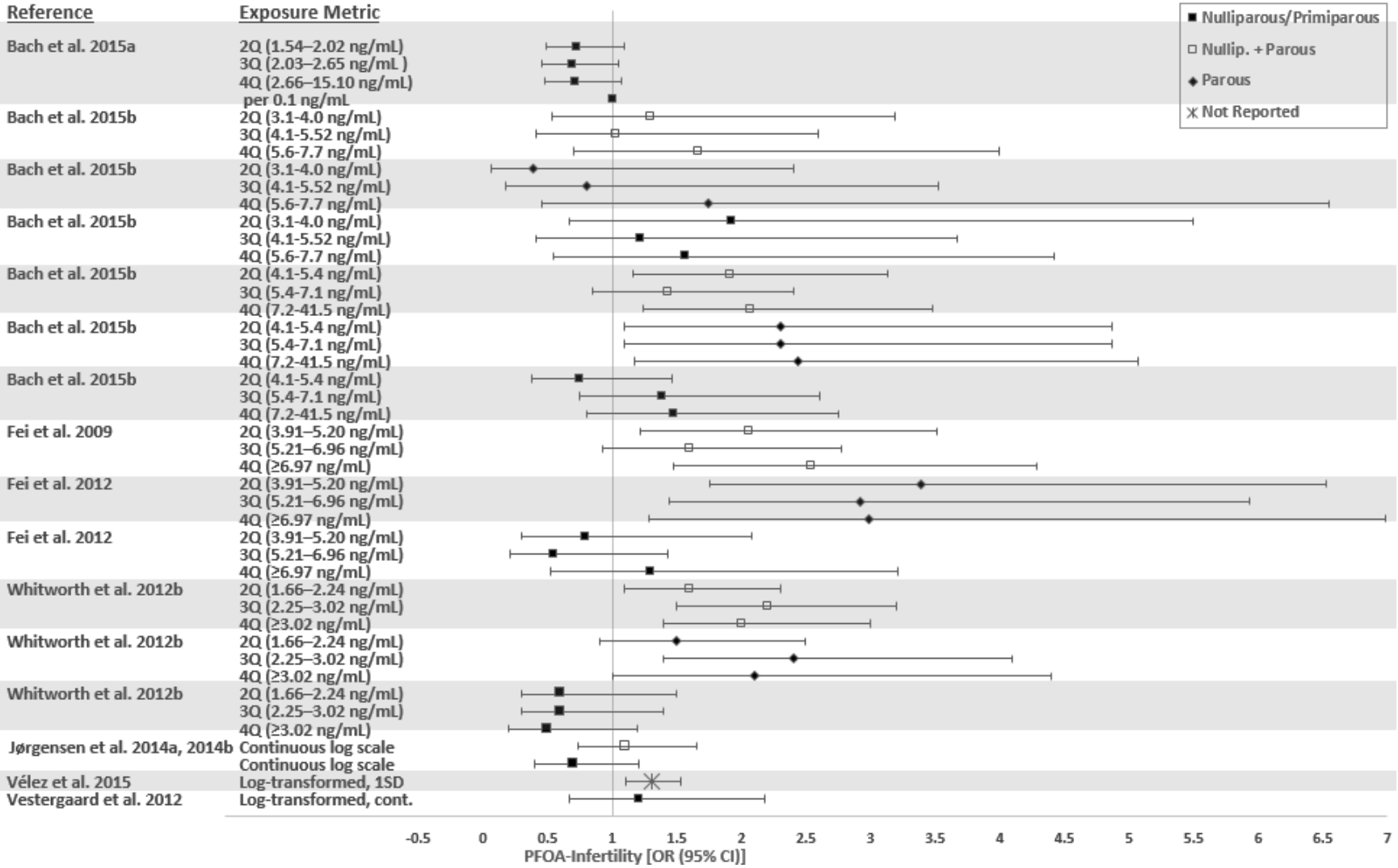


Figure 2-30. Infertility Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



Other studies have not found alterations in fecudability and/or infertility (Bach et al. 2015a; Jørgensen et al. 2014a; Vestergaard et al. 2012).

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 cells 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). 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). A peer review of the histological slides from 3M (1983) 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).

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.

No gross or microscopic alterations were reported in the testes from rats dermally exposed to 2,000 mg/kg/day APFO (Kennedy 1985).

Summary. Epidemiology 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). Several general population studies found associations between serum PFOA and impaired fertility (increased time to pregnancy and/or infertility). 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

Epidemiology 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).

Epidemiology 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.

Epidemiology 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). 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). 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 2nd and 3rd tertiles (Taylor et al. 2014) and the risk of having polycystic ovary syndrome in women with serum PFOS levels in the 3rd 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.

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). When the women were segregated by parity, the associations were only found in multiparous women (Fei et al. 2010). This finding may be due to reverse causality since longer breastfeeding would likely result in lower maternal PFOS levels.

Epidemiology Studies—Effects on Fertility. Several general population studies have evaluated whether there is a possible link 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 2-31 and 2-32, 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 using a different statistical approach (Bach et al. 2015b) resulted in the same findings of 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, 2015b; Jørgensen et al. 2014a; Vélez et al. 2015; Vestergaard et al. 2012).

Laboratory Animal Studies. A significant decrease in serum testosterone levels and epididymal sperm count was observed in mice administered 10 mg/kg/day PFOS for 21 days (Wan et al. 2011). 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. 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. Multigeneration studies with PFOS in

Figure 2-31. Fecundability Relative to PFOS Levels (Presented as Adjusted Fecundability Ratios)

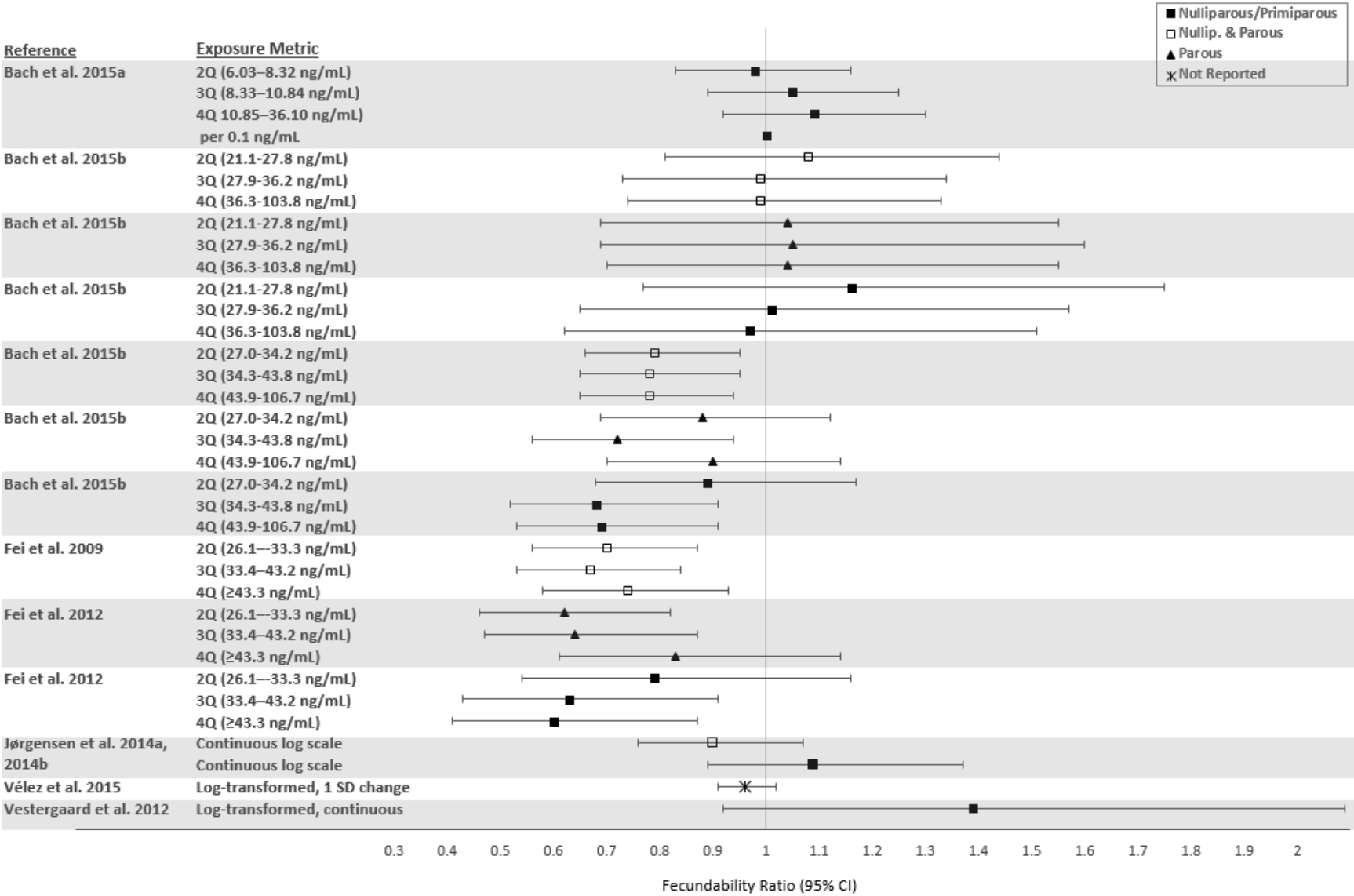
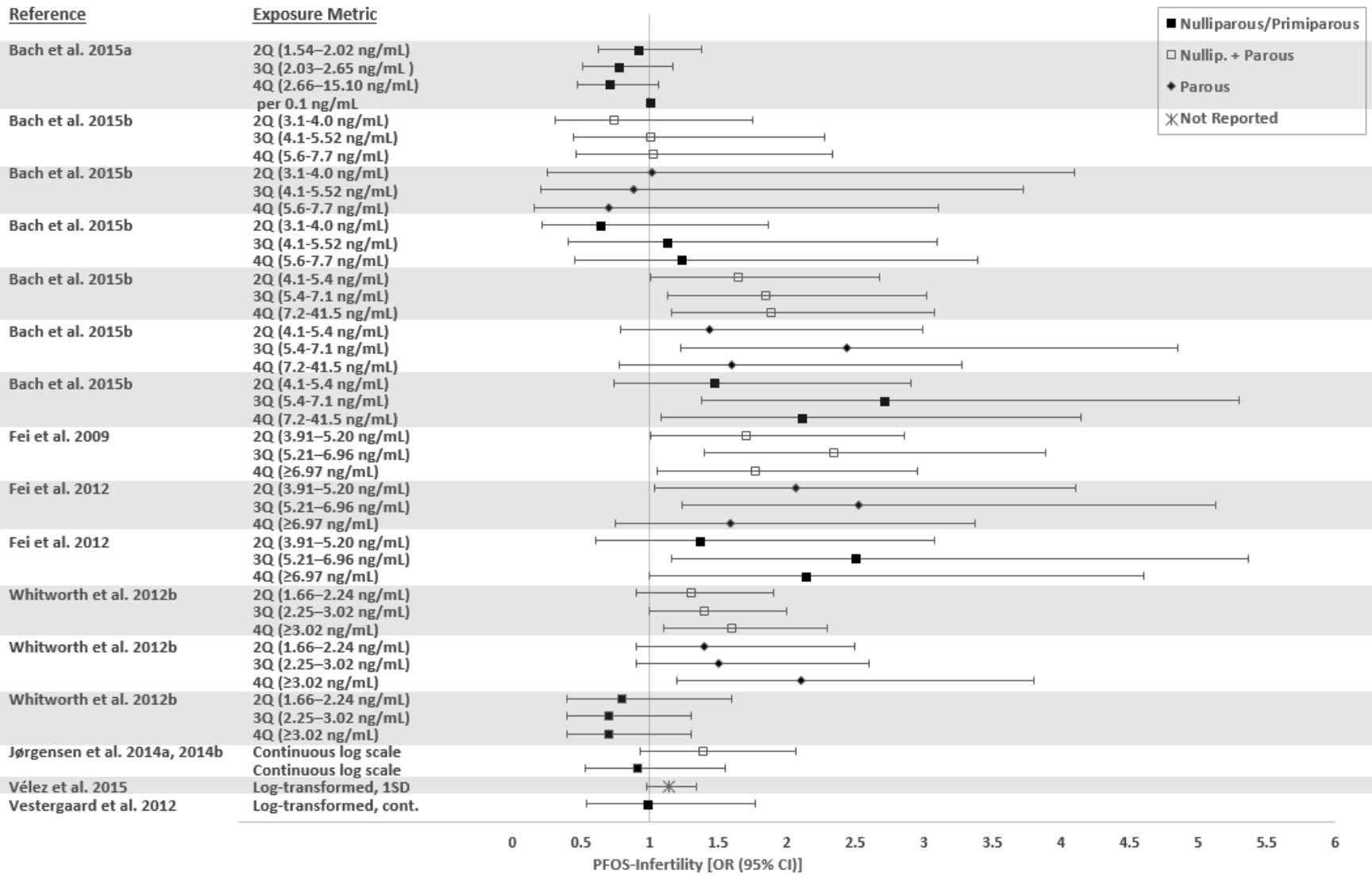


Figure 2-32. Infertility Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



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 have been observed.

PFHxS

Epidemiology Studies—Reproductive Hormone Levels. Two 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).

Epidemiology 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.

Epidemiology Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. Four 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. Other studies did not find associations with

the risk and severity of endometriosis (Buck Louis et al. 2012) 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.

Epidemiology Studies—Effects on Fertility. Four 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 fecudability 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. Studies by Bach et al. (2015a) and Jørgensen et al. (2014a) did not find alterations in time to pregnancy 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; Hoberman and York 2003). 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.

PFNA

Epidemiology 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). A third study did not find alterations in follicular estradiol or luteal progesterone levels in women (Barrett et al. 2015).

Epidemiology 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).

Epidemiology Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. 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. Vagi et al. (2014) did not find an increased risk of polycystic 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).

Epidemiology 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). Studies by Bach et al. (2015a) and Vestergaard et al. (2012) did not find associations between serum PFNA levels and fecundability ratio; Bach et al. (2015a) also did not find an increase in the 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; 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).

PFDeA

Epidemiology Studies—Reproductive Hormone Levels. No associations were found between serum PFDeA 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).

Epidemiology Studies—Effects on Sperm. Two general population studies evaluated potential effects of PFDeA exposure on sperm parameters. Buck Louis et al. (2015) found associations between serum PFDeA 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).

Epidemiology Studies—Effects on Menstrual Cycle Length, Menopause Onset, and Endometriosis. Only one study examined alterations in female reproductive outcomes associated with serum PFDeA levels. In this study, no associations between serum PFDeA levels and the risk or severity of endometriosis were found (Buck Louis et al. 2012).

Epidemiology Studies—Effects on Fertility. Two studies have examined the potential for PFDeA 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). Another study (Vestergaard et al. 2012) also found no association between female serum PFDeA levels and time to pregnancy.

PFUA

Epidemiology Studies—Reproductive Hormone Levels. An inverse association between serum PFUA 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).

Epidemiology Studies—Effects on Fertility. Only one study evaluated possible associations between maternal serum PFUA levels and fertility; no alterations in time to pregnancy (measured as a fecundability ratio) or infertility risk were observed in pregnant women (Bach et al. 2015a).

PFBuS

Laboratory Animal Studies. Administration of up to 900 mg/kg/day PFBuS 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 PFBuS 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, 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).

PFDoA

Laboratory Animal Studies. Treatment of male rats with 1, 5, or 10 mg/kg/day PFDoA 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.

PFOSA

Epidemiology Studies. One study examined reproductive hormone levels and did not find an association between serum PFOSA and follicular estradiol or luteal progesterone levels in women (Barrett et al. 2015). The only epidemiology study evaluating fertility 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 (Vestergaard et al. 2012).

Me-PFOSA-AcOH

Epidemiology Studies. In the only study identified that examined sperm parameters associated with Me-PFOSA-AcOH, increases in the percentage of sperm with neck/midpiece abnormalities and increases in the number of immature sperm were found (Buck Louis et al. 2015). No associations with sperm viability, count, or motility were found. One study examining fertility did not find associations between serum Me-PFOSA-AcOH levels in women and time to pregnancy (Vestergaard et al. 2012).

Et-PFOSA-AcOH

Epidemiology Studies. No associations between serum Et-PFOSA-AcOH levels in females and the time to pregnancy were observed in a study of nulliparous couples (Vestergaard et al. 2012).

2.17 DEVELOPMENTAL

Overview. A large number of epidemiology 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. The discussion of these developmental outcomes is divided into four categories: pregnancy outcome, birth outcome, neurodevelopment, and sexual maturation. The epidemiology 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 epidemiology studies examining birth outcomes, which include birth weight, birth size, low birth weight, small for gestational age, birth defects, and sex ratio. Epidemiology studies examining neurodevelopmental endpoints, particularly risks for ADHD, are summarized in Table 2-24. Studies evaluating possible links 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*

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)
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)
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**
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**
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)
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**

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
PFNA			
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)***
PFDeA			
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.27 ng/mL (maternal median PFDeA)	Miscarriage before gestation week 12	**OR 2.30 (1.18–4.47)***
PFUA			
Chen et al. 2012a General population (n=429 infants)	10.26 ng/mL (cord blood geometric mean PFUA)	Preterm birth	OR 0.87 (0.64–1.16)

[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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

OR = odds ratio; NS = not significant; PFDeA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUA = 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
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**
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
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

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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 gestation 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)
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)

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)	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)
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**
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)
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
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)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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)
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**

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)	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
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)
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)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		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)
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)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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)
Monroy et al. 2008 General population (n=101 pregnant women)	14.54 mg/mL 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
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)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
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
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
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)
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)

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)	2.05, 1.56, and 2.28 ng/mL (maternal median PFHxS for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.801)
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)
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)
Monroy et al. 2008 General population (n=101 pregnant women)	1.62 mg/mL (maternal median PFHxS)	Birth weight	NS (p>0.05)
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
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

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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)
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)
Monroy et al. 2008 General population (n=101 pregnant women)	0.69 mg/mL (maternal median 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]
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
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)
PFDeA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.3 ng/mL (PFDeA median)	Birth weight	NS, investigators noted no consistent alterations across PFDeA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFDe quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFDeA 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 PFDeA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.07 (0.81–1.42), maternal PFDeA OR 1.02 (0.78–1.34), paternal PFDeA
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.523 and 0.298 ng/mL (mean PFDeA 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 PFDeA)	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)	0.40, 0.16, and 0.22 ng/mL (maternal median PFDeA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.158)
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 PFDeA in boy and girl cases) 0.17 and 0.16 ng/mL (maternal median PFDeA in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.1 (0.7–1.7) RR 0.6 (0.3–1.1)
Robledo et al. 2015a, 2015b General population (n=234 couples)	0.402 and 0.458 ng/mL (maternal and paternal geometric mean PFDeA)	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
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 PFDeA 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**
PFUA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.3 ng/mL (PFUA median)	Birth weight	NS, investigators noted no consistent alterations across PFUA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFUA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFUA quartiles
Chen et al. 2012a General population (n=429 infants)	10.26 ng/mL (cord blood geometric mean PFUA)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	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]
		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 PFUA 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 PFUA)	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 PFUA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.275)
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 PFUA 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)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
PFDoA			
Lee et al. 2016 General population (n=85 infants)	0.14 ng/mL (cord blood mean PFDoA)	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 PFDoA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.440)
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 PFDoA 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)
PFOSA			
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 PFOSA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.07 (0.81–1.41), maternal PFOSA OR 1.14 (0.86–1.51), paternal PFOSA

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 PFOSA)	Birth weight Boys Girls	 **Inverse association (p<0.05)*, maternal only** 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
Me-PFOSA-AcOH			
Bae et al. 2015 General population (233 couples)	0.29 and 0.28 ng/mL and 0.28 and 0.32 ng/mL (geometric mean Me-PFOSA-AcOH in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 0.98 (0.75–1.28), maternal Me-PFOSA-AcOH OR 0.80 (0.60–1.06), paternal Me-PFOSA-AcOH
		Female birth	**OR 0.34 (0.13–0.89)*, paternal Me-PFOSA-AcOH 3rd tertile**
Robledo et al. 2015a, 2015b General population (n=234 couples)	0.301 and 0.324 ng/mL (maternal and paternal geometric mean Me-PFOSA-AcOH)	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
Et-PFOSA-AcOH			
Bae et al. 2015 General population (233 couples)	0.11 and 0.10 ng/mL and 0.10 and 0.11 ng/mL (geometric mean Et-PFOSA-AcOH in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.22 (0.92–1.60), maternal Et-PFOSA-AcOH OR 0.98 (0.75–1.29, paternal Et-PFOSA-AcOH

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)	0.109 and 0.105 ng/mL (maternal and paternal geometric mean Et-PFOSA-AcOH)	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	**β -0.09 (-0.16 to -0.02)*, maternal** NS (p>0.05), 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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BMI = body mass index; Et-PFOSA-AcOH = 2-(N-ethyl-perfluorooctane sulfonamide) acetic acid; FSH = follicle stimulating hormone; HR = hazard ratio; LH = luteinizing hormone; Me-PFOSA-AcOH = 2-(N-methyl-perfluorooctane sulfonamide) acetic acid; NS = not significant; NR = not reported; OR = odds ratio; PFDeA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFUA = 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

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		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 5–18 years old	 OR 0.96 (0.73–1.26), 4th quartile OR 0.90 (0.76–1.06), 4th quartile
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

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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. 2016 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)
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**

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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
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)
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 olds)	IQ score	
		Age 5 years	NS
		Age 8 years	NS
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	**OR 0.83 (0.70–0.98)*, 2nd quartile**
		12–15 years old	**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	**Association (p=0.041 for trend)***
		Earlier use of word-like sounds	**Association (p=0.039 for trend)***
		Delays in using 2-word sentences	**Association (p=0.050 for trend)***
		Other milestones	NS (p>0.05)
		Motor and mental development at 6 months	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]
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. 2016 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)
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
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)
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)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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
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 Males Females	NS (p=0.19), 3rd tertile NS (p=0.35), 3rd tertile NS (p=0.43), 3rd tertile
		Scores on test evaluating externalizing problem Males Females	NS (p=0.31), 3rd tertile NS (p=0.74), 3rd tertile 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)
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)***
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 olds)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)
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

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)	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**
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)
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)***
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 olds)	IQ score Age 5 years Age 8 years	 NS (p>0.05) 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**

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)	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
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)
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)
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 olds)	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)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDeA			
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.26 ng/mL (mean PFDeA)	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 PFDeA 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**
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	0.2 ng/mL (maternal median PFDeA)	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 PFDeA for 5 and 8 year olds)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)
PFUA			
Lien et al. 2016 General population (n=282 children aged 7 years)	7.96 ng/mL (cord blood weighted average PFUA)	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 PFUA for 5 and 8 year olds)	IQ score	NS (p>0.05)
		IQ scores, age 8 years Full scale IQ Visual IQ Performance IQ	 NS (p>0.05) NS (p>0.05) **Inverse 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]
PFDoA			
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 PFDoA for 5 and 8 year olds)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)
PFOSA			
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.75 ng/mL (mean PFOSA)	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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

ADHD = attention deficit hyperactivity disorder; MDI/PDI = mental and psychomotor development indices; NHANES = National Health and Nutrition Examination Survey; NS = not significant; OR = odds ratio; PFDeA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFUA = 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 SHGB	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)
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**
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**

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]
		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 SHGB	NS (p>0.05)
		Cord insulin-like factor 3	NS (p>0.05)
		Cord inhibin Males Females	 **Association (p<0.001)*** 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 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)
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)***
		Androstendione	**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)
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**
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
PFOSA			
Christensen et al. 2011 General population (n=448 girls)	0.2 ng/mL (maternal median PFOSA)	Earlier age of menarche	OR 0.91 (0.67–1.24)
Me-PFOSA-AcOH			

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]
Christensen et al. 2011 General population (n=448 girls)	0.4 ng/mL (maternal median Me-PFOSA-AcOH)	Earlier age of menarche	OR 0.86 (0.66–1.12)
Et-PFOSA-AcOH			
Christensen et al. 2011 General population (n=448 girls)	0.6 ng/mL (maternal median Et-PFOSA-AcOH)	Earlier age of menarche	OR 1.03 (0.75–1.43)

[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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; DHEAS = dihydroepiandrosterone sulfate; Et-PFOSA-AcOH = 2-(N-ethyl-perfluorooctane sulfonamide) acetic acid; FSH = follicle stimulating hormone; LH = luteinizing hormone; Me-PFOSA-AcOH = 2-(N-methyl-perfluorooctane sulfonamide) acetic acid; NS = not significant; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SHBG = sex hormone binding globulin

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 epidemiology studies did not find associations between perfluoroalkyl exposure and adverse pregnancy outcomes (miscarriage, preterm birth, or gestational age) for PFOA, PFOS, PFHxS, PFNA, or PFUA. Mixed results have been found for birth outcomes, particularly birth weight. Some epidemiology 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 15–19 g or 5 g decreases in birth weight, respectively; accounting for maternal glomerular filtration rates attenuated these results by about 50%. No consistent associations for alterations in birth weight were found for other perfluoroalkyls (PFHxS, PFNA, PFDeA, PFUA, PFDoA, Me-PFOSA-AcOH, Et-PFOSA-AcOH). Overall, no associations were found between serum PFOA, PFOS, PFHxS, PFNA, or PFUA and increases in the risk of low birth weight or small for gestational age infants. No consistent results for risks of birth defects have been found; these potential endpoints were only examined for a few perfluoroalkyls. The available epidemiology data do not suggest associations between perfluoroalkyls and IQ or scholastic achievement for PFOA, PFOS, PFHxS, PFNA, PFDeA, PFUA, or PFDoA. Similarly, no associations were found between PFOA, PFOS, PFHxS, PFNA, or PFDeA and increased risk of ADHD; several studies have 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 2-4, 2-6, 2-7, and 2-8. Laboratory animal studies provide strong evidence of the developmental toxicity of a number of perfluoroalkyl compounds. Prenatal losses and decreases in pup survival were observed following exposure to PFOA, PFOS, PFNA, and PFDeA; no deaths were observed in a single study of PFBuS. Decreases in fetal weights, birth weight, and pup weight were observed in studies of PFOA, PFOS, PFNA, PFDeA, and PFUA; no effects on weight were observed in studies on PFHxS or PFBuS. 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 PFDeA. Studies in laboratory animals have examined a number of developmental endpoints, including pup survival, malformations, birth weight, mammary gland development, and neurodevelopment.

PFOA

Epidemiology Studies—Pregnancy Outcomes. The results of available epidemiology 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), or pre-term birth (Chen et al. 2012a; Darrow et al. 2013; Hamm et al. 2010; Stein et al. 2009; Whitworth et al. 2012a) were found; the Whitworth et al. (2012a) general population studies reported a decrease in the risk of preterm births among women with serum PFOA levels in the 4th quartile. Additionally, Apelberg et al. (2007b) and Chen et al. (2012a) did not find associations between maternal serum PFOA levels and gestational age.

Epidemiology 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), Lenters et al. (2016a), and Maisonet et al. (2012) found an inverse association between maternal serum PFOA and birth weight. However, 12 other general population studies did not find associations (Bach et al. 2016; Chen et al. 2012a; Govarts et al. 2016; Hamm et al. 2010; Kim et al. 2011; Lee et al. 2013, 2016; Monroy et al. 2008; Robledo et al. 2015a; Wang et al. 2016; Washino et al. 2009; Whitworth et al. 2012a). As illustrated in Figure 2-33, 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; 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, there were no increases in the risk for small for gestational age (Chen et al. 2012a; Fei et al. 2007, 2008a; Hamm et al. 2010; Savitz et al. 2012b; Wang et al. 2016; Whitworth et al. 2012a); these data are presented in Figure 2-34. 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). Two 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) (Apelberg et al. 2007b; Fei et al. 2007, 2008a). However, most studies did not find

Figure 2-33. Risk of Low Birth Weight Infant Relative to PFOA Levels (Presented as Adjusted Odds Ratios)

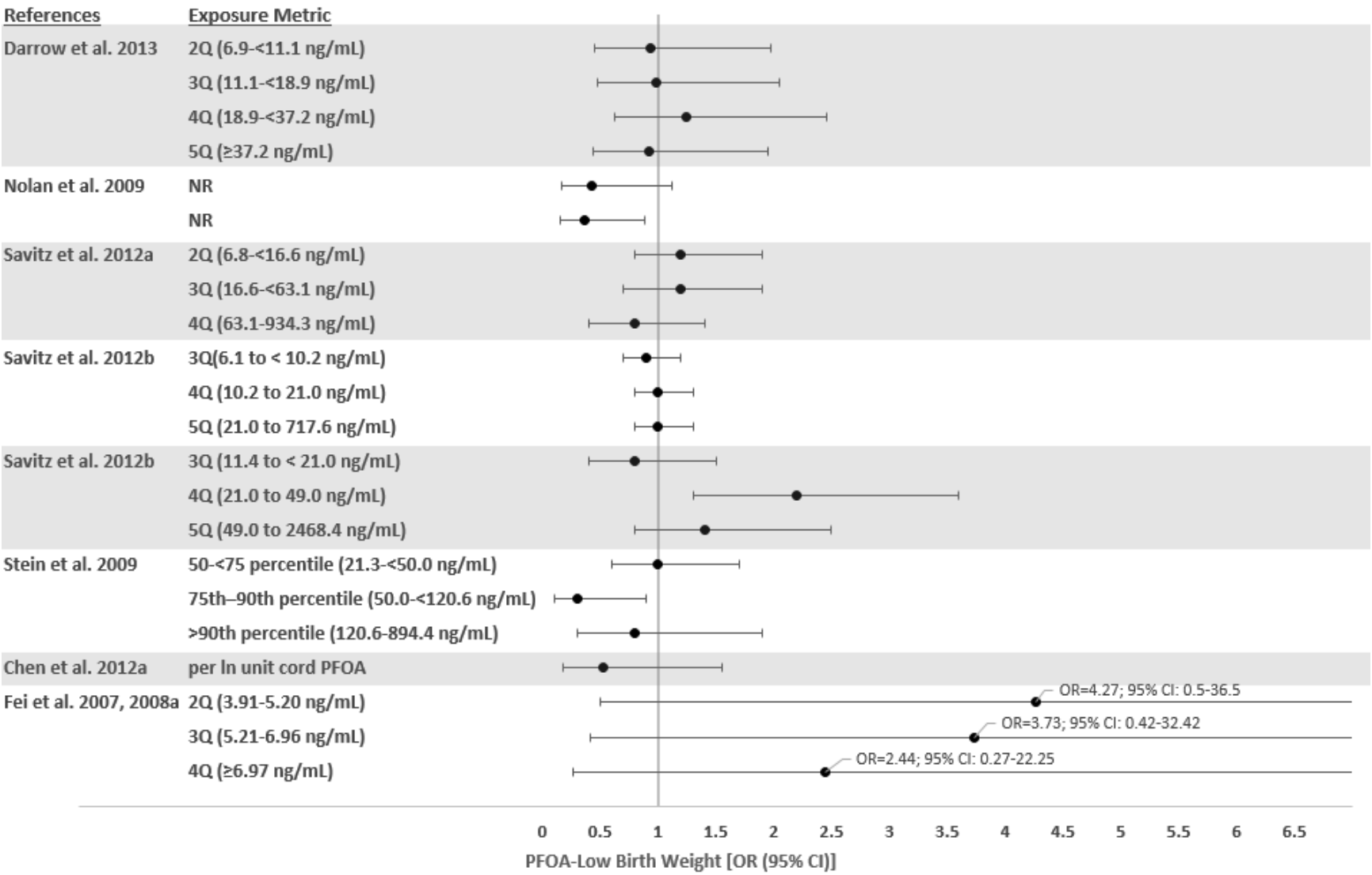
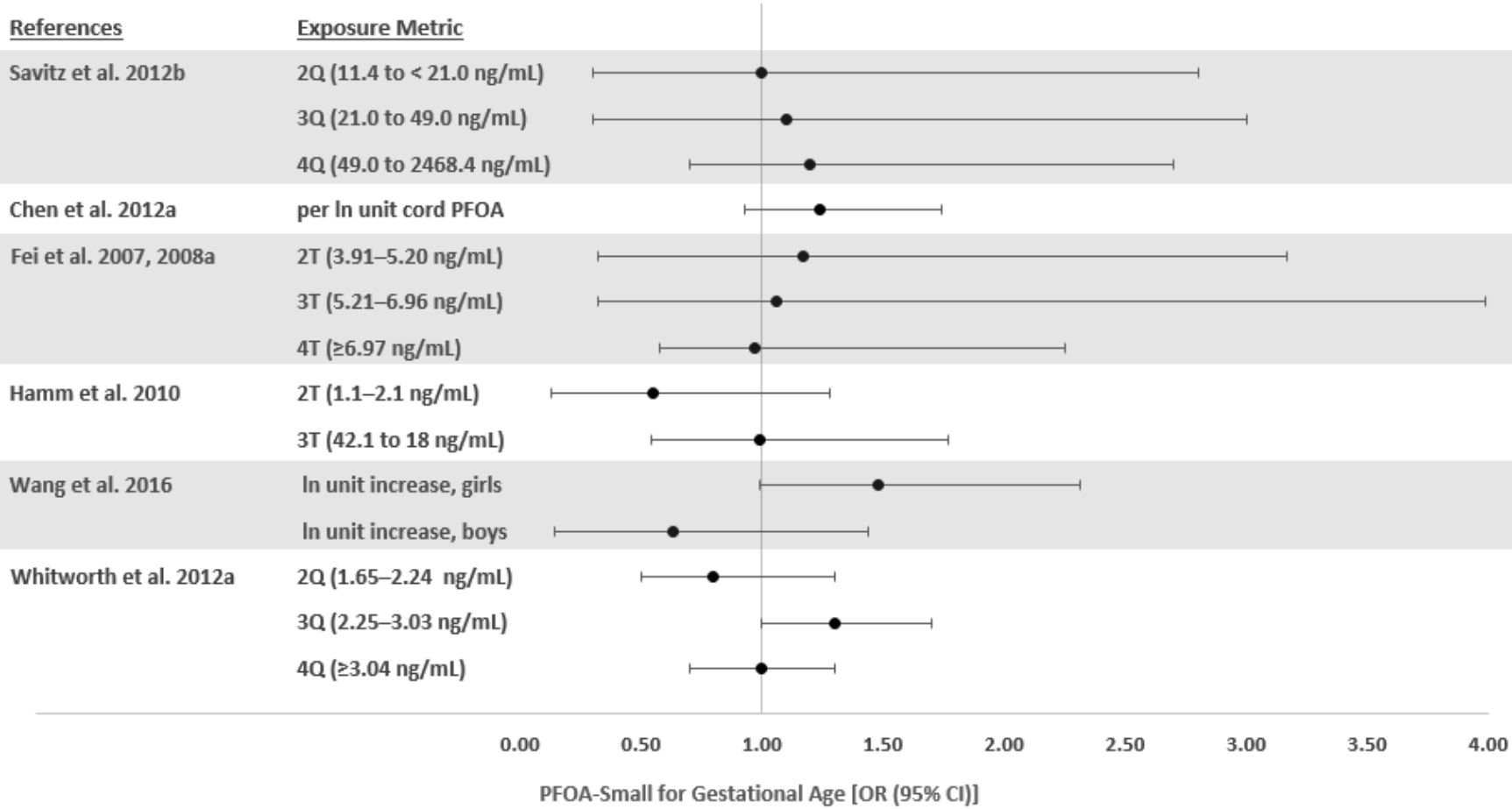


Figure 2-34. Risk of Small For Gestational Age Infant Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



associations between maternal serum PFOA levels and birth length; head, chest, or abdominal circumference; or ponderal index (Bach et al. 2016; Chen et al. 2012a; Lee et al. 2013; Maisonet et al. 2012; Robledo et al. 2015a; Wang et al. 2016).

In a systematic review of 19 epidemiology 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), and Maisonet 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. 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 evaluate the influence of glomerular filtration rate on the association between maternal PFOA and birth weight. Utilizing simulated maternal PFOA levels at delivery, 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 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).

Epidemiology Studies—Neurodevelopmental Outcomes. A number of epidemiology 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. 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. (2016) 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. (2016) 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 links 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. 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 olds or motor skills in 5–9 year olds.

Several studies have examined possible associations between maternal or child PFOA levels and scores on tests/surveys that assess behavioral problems. No associations between maternal PFOA levels and behavioral problems in 7 year olds (Fei and Olsen 2011) or behavioral regulation problems in 5 or 8 year olds (Vuong et al. 2016). Similarly, no associations between serum PFOA levels and performance on tasks requiring behavioral inhibition were observed in 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.

Ten studies have looked for a possible link 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 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).

Epidemiology Studies—Development of the Reproductive System. Studies exploring possible links 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, 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).

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 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; Li et al. 2016). 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 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.

A consistent finding in the four 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. 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).

Although not investigated in mammalian species, studies in chicken embryos and hatchlings demonstrate the developmental cardiotoxicity of PFOA (Jiang et al. 2012, 2013, 2016). 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. Epidemiology studies have examined a number of potential developmental outcomes in communities living near a PFOA facility and in the 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

Epidemiology 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). Two 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) 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). Two other studies did not find associations for preterm birth (Fei et al. 2007, 2008a; Hamm et al. 2010).

Epidemiology Studies—Birth Outcomes. Occupational, community, and general population exposure studies have examined the possible links 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 (Apelberg et al. 2007b; Ashley-Martin et al. 2016; 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; Lee et al. 2013, 2016; Lenters et al. 2016a; Maisonet et al. 2012; Monroy et al. 2008; Robledo et al. 2015a; 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). Three 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. 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). 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; Stein et al. 2009) or small for gestational age infants (Chen et al. 2012a; Fei et al. 2007, 2008a; Hamm et al. 2010; Whitworth et al. 2012a). The ORs for low birth weight and small for gestational age risks are presented in Figures 2-35 and 2-36. Maternal PFOS was not associated with birth length (Apelberg et al. 2007b; Bach et al. 2016; Chen et al. 2012a; Fei et al. 2007, 2008a; Lee et al. 2013; Robledo et al. 2015a; Washino et al. 2009) with the exception of the finding of a small decrease in birth length (-0.63 cm) that was associated with 3rd tertile maternal serum PFOS levels. Two studies reported inverse associations between ponderal index and cord blood PFOS levels (Apelberg et al. 2007b) and maternal serum PFOS levels (Lee et al. 2013); other studies did not find this effect (Chen et al. 2012a; Maisonet et al. 2012; Robledo et al. 2015a). 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; de Cock et al. 2014; Fei et al. 2007, 2008a; Lee et al. 2013; Robledo et al. 2015a; Washino et al. 2009). 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.

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.

Epidemiology Studies—Neurodevelopmental Outcomes. Epidemiology 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. (2016) 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).

Figure 2-35. Risk of Low Birth Weight Infant Relative to PFOS Levels (Presented as Adjusted Odds Ratios)

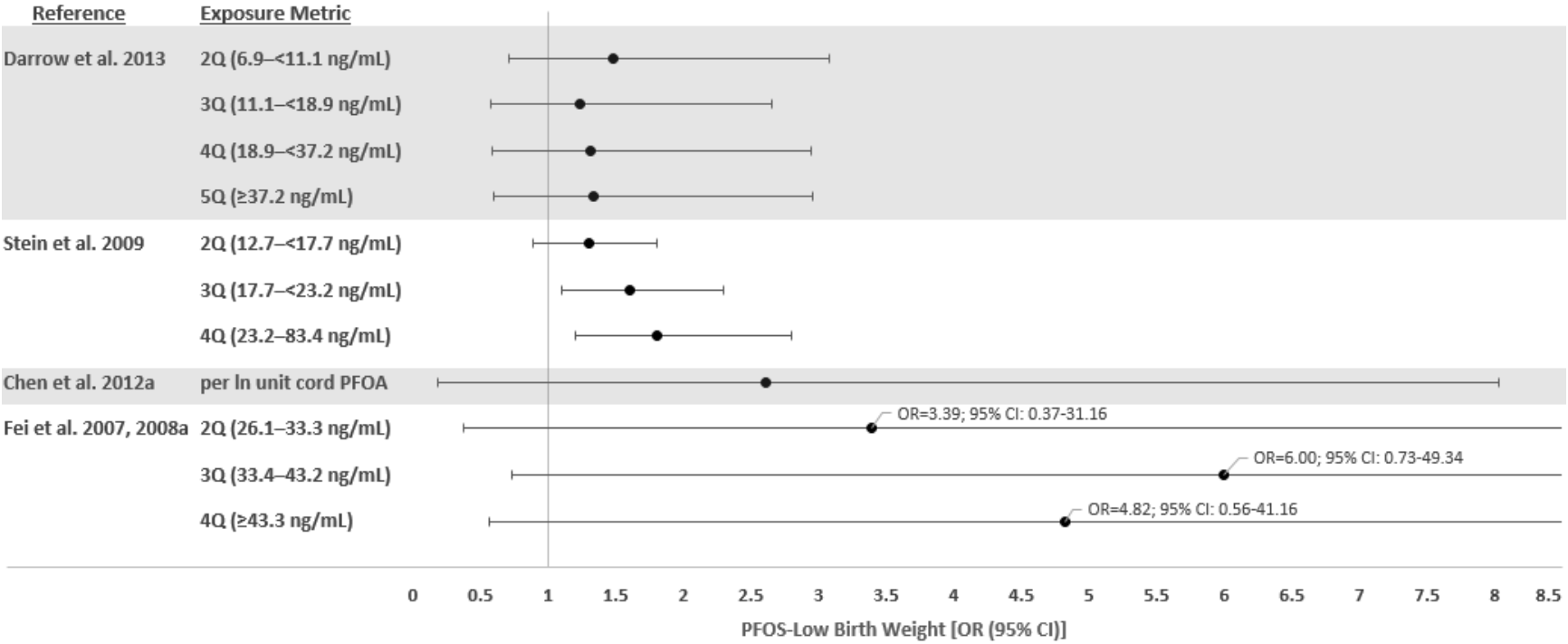
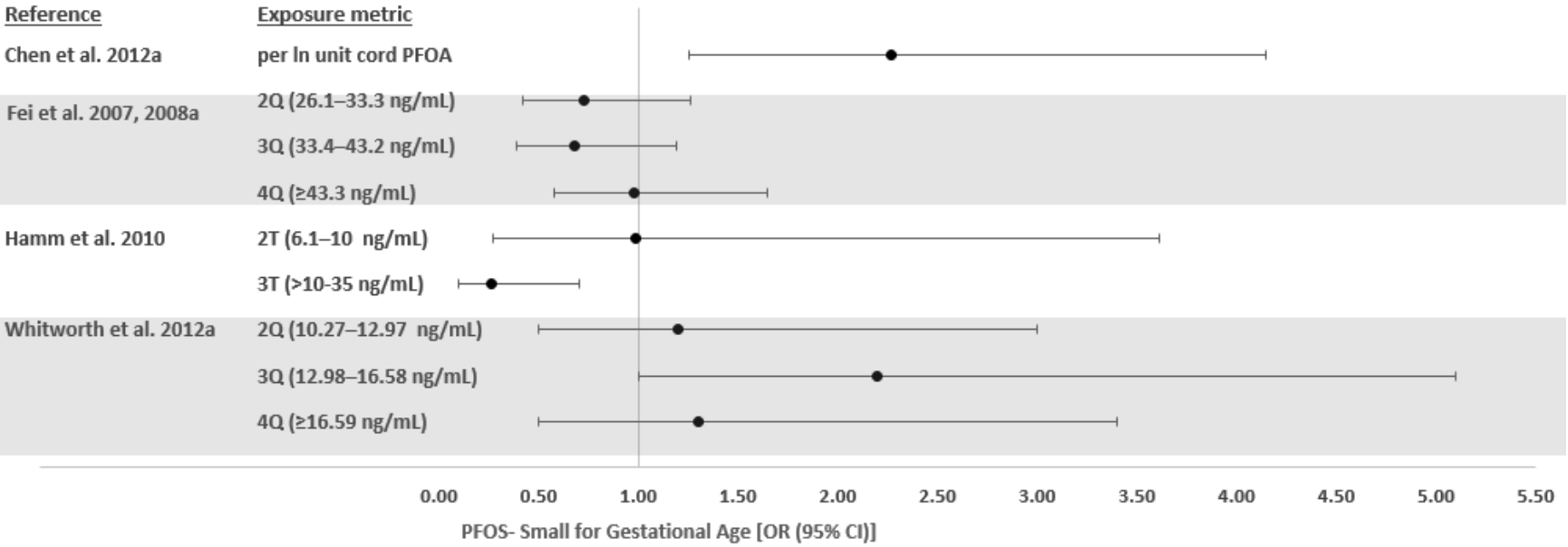


Figure 2-36. Risk of Small for Gestational Age Infant Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



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. 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. Three studies have not found links between maternal PFOS levels and behavioral health and motor coordination/skills in children (Fei and Olsen 2011; Høyer et al. 2015a) or between breast milk PFOS levels and behavioral development in 6- and 24-month-old infants (Forns et al. 2015). A third 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.

Epidemiology Studies—Development of Reproductive System. Several epidemiology 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. 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; 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.

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 epidemiology 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

Epidemiology Studies—Pregnancy Outcomes. Two studies, summarized in Table 2-22 have evaluated possible links 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.

Epidemiology 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 (Hamm et al. 2010; Kim et al. 2011; Lee et al. 2013, 2016; Lenters et al. 2016a; Monroy et al. 2008). The risk of low birth weight infants was not examined in the available epidemiology studies; Hamm et al. (2010) did not find an association between maternal PFHxS level and the relative risk of small for gestational age. Several studies did not find associations between maternal PFHxS levels and birth length, head circumference, or ponderal index (Bach et al. 2016; Lee et al. 2013). Maisonet et al. (2012) found an inverse association for birth length, but no association for ponderal index. 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.

Epidemiology Studies—Neurodevelopmental Outcomes. Epidemiology studies, summarized in Table 2-24, have examined PFHxS-related alterations in risks of ADHD, autism, IQ, and behavior. Wang et al. (2015b) did not find associations between maternal PFHxS levels and IQ in 5- or 8-year-old children. However, Vuong et al. (2016) found a higher risk of performing poorly on tests of global executive function with increasing maternal PFHxS levels. 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) did not find alterations in behavioral regulation. 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.

Epidemiology 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. Christensen et al. (2011) did not find an association between maternal PFHxS levels and risk of an earlier menarche. Summaries of these epidemiology 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. Another study 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 for 14 days prior to mating and throughout gestation and lactation (Butenhoff et al. 2009a; Hoberman and York 2003).

PFNA

Epidemiology Studies—Pregnancy Outcomes. Two studies (summarized in Table 2-22) have examined pregnancy outcome. 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. No alterations in the risk of preterm birth was found in a study conducted by Chen et al. (2012a).

Epidemiology Studies—Birth Outcomes. Several studies have examined the possible link 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; Chen et al. 2012a; Lee et al. 2016; Lenters et al. 2016a; Monroy et al. 2008; Robledo et al. 2015a). No alterations in the risk of low birth weight or small for gestational age were found in a study conducted by Chen et al. (2012a). Wang et al. (2016) did find an inverse association between maternal PFNA levels and birth weight in girls only. 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; Robledo et al. 2015a; Wang et al. 2016). Most studies did not find alterations in ponderal index or head circumference (Bach et al. 2016; Chen et al. 2012a; Robledo et al. 2015a; 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.

Epidemiology Studies—Neurodevelopmental Outcomes. Several potential neurodevelopmental outcomes have been examined in epidemiology 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 olds (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. Vuong et al. (2016) did not find an association between maternal PFNA levels and metacognition or global executive functioning in 5 or 8 year olds. 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. 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).

Epidemiology 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) and in male offspring of mice administered ≥3 mg/kg/day PFNA on GDs 1–17 (Das et al. 2015). 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).

PFDeA

Epidemiology Studies—Pregnancy Outcomes. In the only identified epidemiology study examining pregnancy outcomes, Jensen et al. (2015) found an increased risk of miscarriage that was associated with maternal PFDeA levels.

Epidemiology Studies—Birth Outcomes. A small number of epidemiology studies examined risks of adverse birth outcomes associated with maternal PFDeA exposure; these studies are summarized in Table 2-23. Wang et al. (2016) found an inverse association between maternal PFDeA 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; Lee et al. 2016; Lenters et al. 2016a; Robledo et al. 2015a). Epidemiology studies have not found associations between birth length, ponderal index, and/or head circumference and maternal PFDeA levels (Bach et al. 2016; Robledo et al. 2015a; 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 PFDeA levels. Additionally, Kim et al. (2016b) did not find associations between serum PFDeA levels and thyroid parameters.

Epidemiology Studies—Neurodevelopmental Outcomes. Several studies have evaluated the potential of PFDeA to adversely affect neurodevelopment; see Table 2-24 for a summary of the studies. Wang et al. (2015b) did not find associations between maternal PFDeA 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. Liew et al. (2015) found decreases in the risk of ADHD and autism in children.

Laboratory Animal Studies. An increase in fetal mortality was observed in mice exposed to 12.8 mg/kg/day PFDeA 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 PFDeA 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.

PFUA

Epidemiology Studies—Pregnancy Outcomes. Limited epidemiology studies evaluated pregnancy outcomes. Jensen et al. (2015) did not find an alteration in the risk of miscarriage before gestation week 12.

Epidemiology Studies—Birth Outcomes. The results from a study conducted by Wang et al. (2016) found an inverse association between maternal PFUA levels and birth weight and an increased risk of small for gestation age among female infants. The remaining epidemiology studies have not found alterations in infant size (birth weight, birth length, ponderal index, head circumference) (Bach et al. 2016; Chen et al. 2012a; Lee et al. 2016; Lenters et al. 2016a) or the risks of low birth weight (Chen et al. 2012a) or small for gestational age (Chen et al. 2012a). No association between serum PFUA levels and thyroid parameters were observed in infants (Kim et al. 2016a). The results of the epidemiology studies examining associations between birth outcome and PFUA are presented in Table 2-23.

Epidemiology Studies—Neurodevelopmental Outcomes. The results of two studies examining possible associations between neurodevelopmental outcome and PFUA are summarized in Table 2-24. Wang et al. (2015b) found no association between maternal PFUA levels and IQ score in 5 and 8 year olds; the study did find an inverse association with scores on tests assessing performance IQ. Lien et al. (2016) found no associations between cord blood PFUA levels and performance on behavioral tests.

Laboratory Animal Studies. One study was identified that examined the potential developmental toxicity of PFUA (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 PFUA.

PFBuS

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 PFBuS (Lieder et al. 2009b).

PFBA

Epidemiology Studies—Birth Outcomes. In the only available epidemiology studies examining birth outcomes, no associations were found between serum PFBA levels and thyroid parameters in infants (Kim et al. 2016a).

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).

PFDoA

Epidemiology Studies—Birth Outcomes. General population studies conducted by Lee et al. (2016) and Lenters et al. (2016a) did not find associations between cord blood PFDoA or maternal PFDoA levels and birth weight. Wang et al. (2016) found an inverse association between maternal PFDoA levels and birth weight and head circumference in female infants; no alteration in the risk of small for gestation age was found. The results of these three studies are summarized in Table 2-23.

Epidemiology Studies—Neurodevelopmental Outcomes. As summarized in Table 2-24, only one study examined neurodevelopmental outcomes. In this study, maternal PFDoA levels were not associated with IQ scores in 5- or 8-year-old children (Wang et al. 2015b).

PFOSA

Epidemiology Studies. Robledo et al. (2015a) found an inverse association between maternal PFOSA levels and birth weight in boys, but not in girls; paternal PFOSA 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 PFOSA levels. As summarized in Table 2-24, only one study evaluated possible associations between PFOSA and neurodevelopmental outcomes. Gump et al. (2011) reported an inverse association between serum PFOSA 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 PFOSA levels and the risk of an earlier age of menarche in girls (see Table 2-25).

Me-PFOSA-AcOH

Epidemiology Studies. Bae et al. (2015) found decreased odds of a female birth that was associated with paternal Me-PFOSA-AcOH levels; there were no alterations in the odds of male births associated with maternal or paternal Me-PFOSA-AcOH levels. Robledo et al. (2015a) found no associations between maternal or paternal Me-PFOSA-AcOH levels and birth weight, birth length, head circumference, or ponderal index. The results of these studies are presented in Table 2-23. Christensen et al. (2011) did not find an association between maternal Me-PFOSA-AcOH levels and an earlier age of menarche; the results of the study are summarized in Table 2-25.

Et-PFOSA-AcOH

Epidemiology Studies. Two epidemiology studies (summarized in Table 2-23) evaluated birth outcomes. No associations were found between paternal or maternal Et-PFOSA-ACOH levels and the odds of male birth (Bae et al. 2015) or birth weight, birth length, head circumference, or ponderal index (Robledo et al. (2015a). An earlier age of menarche was not associated with maternal Et-PFOSA-AcOH levels were found in a study conducted by Christensen et al. (2011).

2.18 OTHER NONCANCER

Overview. A number of epidemiology 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 epidemiology 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, PFDeA, PFUA, PFHpA, or PFOSA. Additionally, results of studies on PFOA, PFOS, PFHxS, and Me-PFOSA-AcOH do not suggest a link between perfluoroalkyls and gestational diabetes. Only one laboratory animal study examined other noncancer endpoints (Table 2-5).

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)	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)***
Karnes et al. 2014 Community (n=32,254 C8 participants)	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) (10th decile)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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)
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)
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)
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)
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)***

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Glycated hemoglobin	**Inverse association (p=0.04 for trend)***
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			
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
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)
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)
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)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Su et al. 2016 General population (n=571)	>4.8 ng/mL (4th 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)***
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)
PFHxS			
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)
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)
		B-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)
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)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
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)
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 adolescent 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)
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)
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)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDeA			
Zhang et al. 2015a General population (n=258)	0.41 and 0.40 ng/mL ng/mL (geometric mean PFDeA in women with or without gestational diabetes)	Gestational diabetes	OR 1.04 (0.70–1.53)
PFUA			
Lind et al. 2014 General population (n=1,016)	0.3 ng/mL (median PFUA)	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 PFUA 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)
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)
PFOSA			
Lind et al. 2014 General population (n=571)	0.11 ng/mL (median PFOSA)	Diabetes	OR 1.07 (0.75–1.53, p=0.71)
		HOMA	NS (p=0.070)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Me-PFOSA-AcOH			
Zhang et al. 2015a General population (n=258)	0.30 and 0.29 ng/mL ng/mL (geometric mean Me-PFOSA-AcOH in women with or without gestational diabetes)	Gestational diabetes	OR 1.05 (0.71–1.54)

[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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

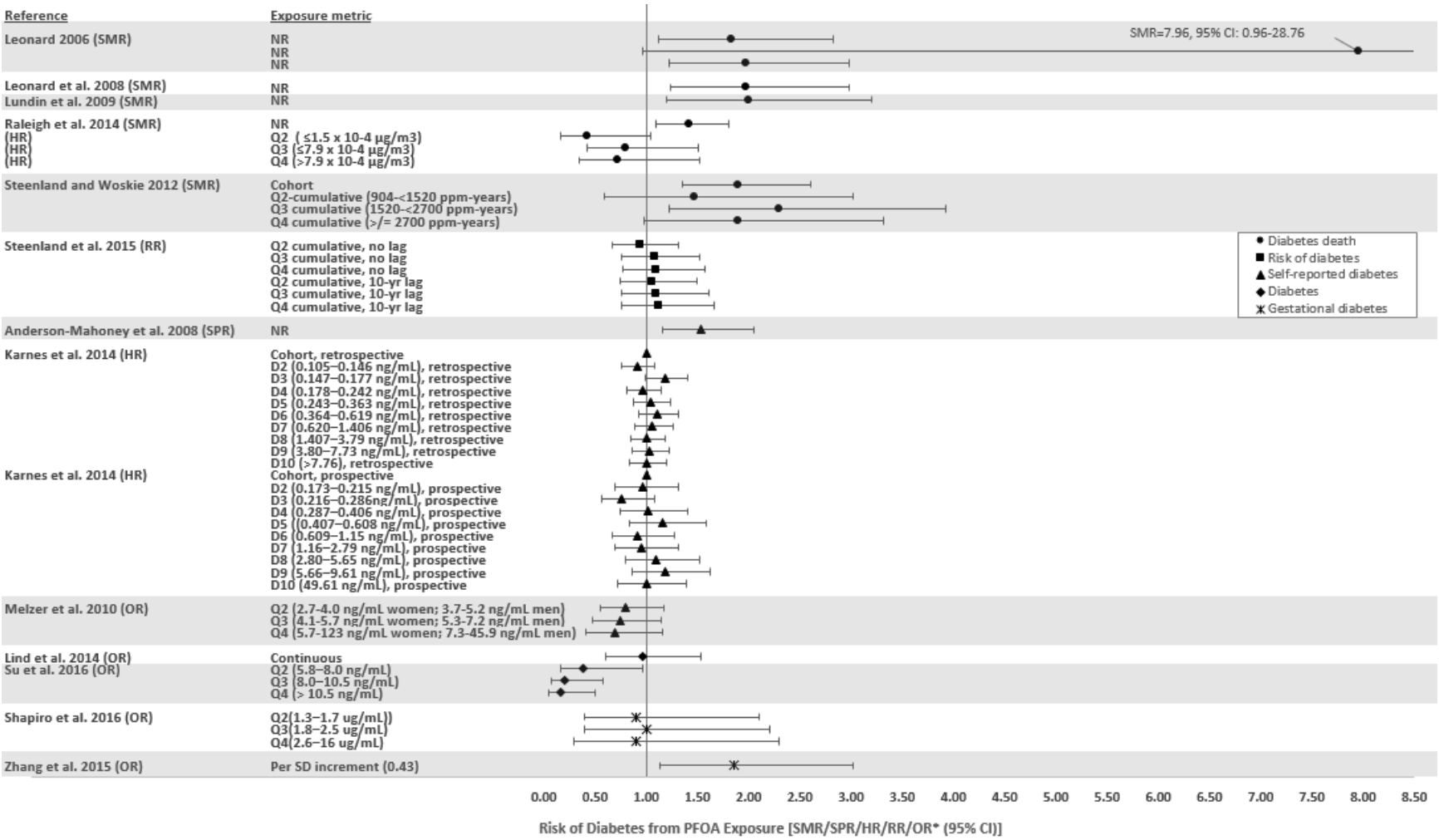
(F) = females; HOMA = homeostatic model assessment; HR = hazard ratio; IR = insulin resistance; (M) = males; Me-PFOSA-AcOH = 2-(N-methyl-perfluorooctane sulfonamide) acetic acid; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFDeA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFUA = perfluoroundecanoic acid; SMR = standardized mortality ratio; SPR = standardized prevalence ratio

PFOA

Epidemiology 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 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 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. In contrast, Karnes et al. (2014) did not find an increased risk of self-reported diabetes associated with 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) or no association (Lind et al. 2014; Melzer et al. 2010). Additionally, general population studies have not found associations between serum PFOA levels and insulin (Fisher et al. 2013; Lin et al. 2009), blood glucose levels (Fisher et al. 2013; Lin et al. 2009; Su et al. 2016), homeostatic model assessment for insulin resistance (HOMA-IR) (Fisher et al. 2013; Lin et al. 2009; Lind et al. 2014; Nelson et al. 2010), or glucose tolerance (Su et al. 2016). Two 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) did not find associations between serum PFOA and gestational diabetes or impaired glucose tolerance. The ORs for the risk of diabetes and gestational diabetes are graphically presented in Figure 2-37.

Figure 2-37. Diabetes Risk Relative to Serum PFOA Levels (Presented as Adjusted Ratios)



PFOS

Epidemiology Studies. 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. Two other general population studies did not find increased risks of diabetes (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; Nelson et al. 2010). Lin et al. (2009) found associations between serum PFOS and insulin and HOMA-IR in a study of NHANES adult participants; these associations were not found in adolescent participants. No alterations in the risk of gestational diabetes were observed in two general population studies (Shapiro et al. 2016; Zhang et al. 2015a); the Shapiro et al. (2016) study also found no association between serum PFOS and glucose tolerance. The ORs for the risk of diabetes and gestational diabetes are graphically presented in Figure 2-38.

PFHxS

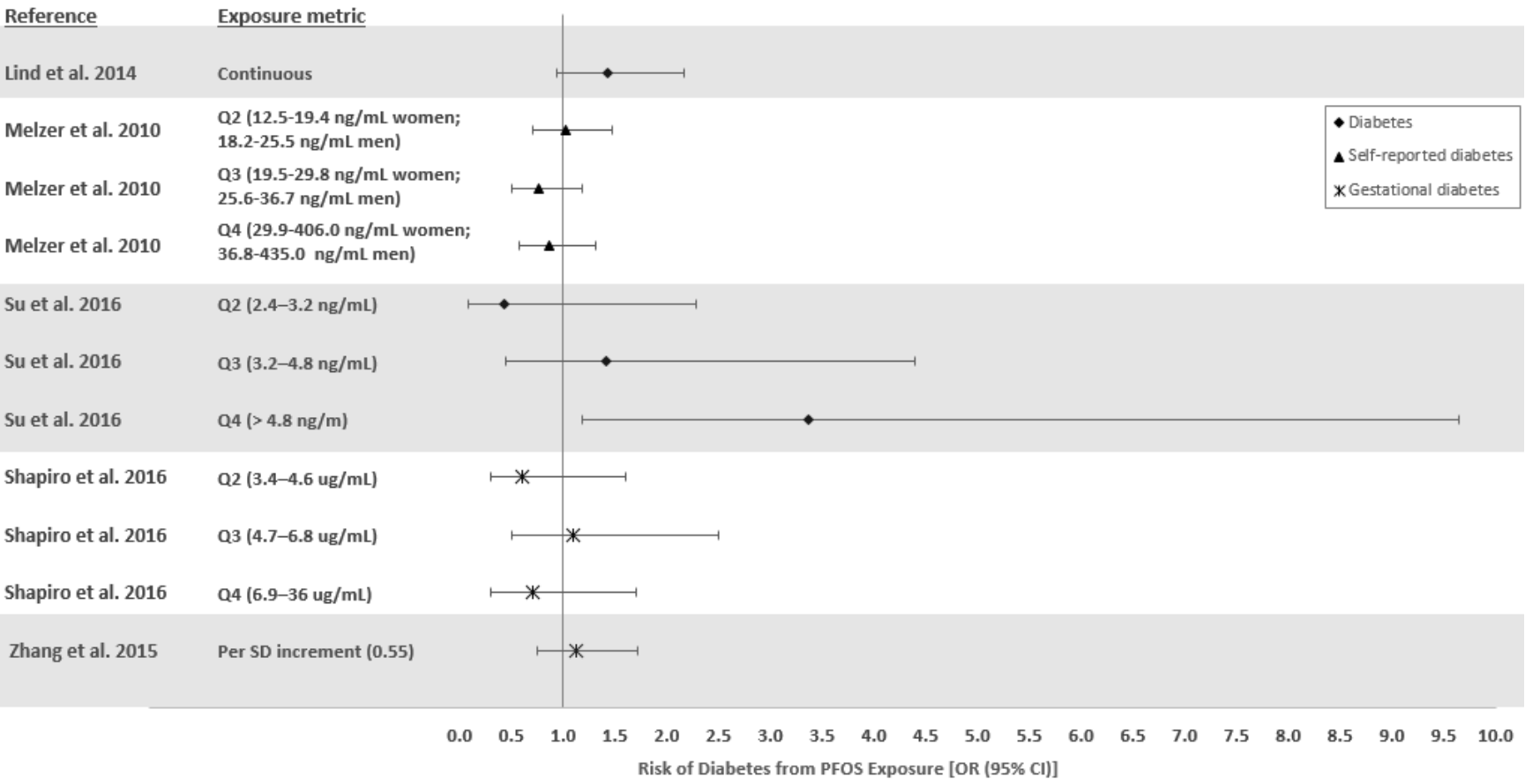
Epidemiology Studies. Five general population studies have examined diabetes-related outcomes and have not found associations between serum PFHxS levels and diabetes risk (Lind et al. 2014), gestational diabetes (Shapiro et al. 2016) or insulin, blood glucose, or HOMA-IR levels (Fisher et al. 2013; Lin et al. 2009; Lind et al. 2014; Nelson et al. 2010).

PFNA

Epidemiology Studies. An inverse association between serum PFNA levels and the risk of diabetes was observed in a general population study (Su et al. 2016). Two other studies did not find associations for diabetes (Lind et al. 2014) or gestational diabetes (Zhang et al. 2015a). The Su et al. (2016) study also reported an inverse association between PFNA levels and response on a glucose tolerance test. 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 HOMA-IR (Lin et al. 2009; Lind et al. 2014; Nelson et al. 2010).

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).

Figure 2-38. Diabetes Risk Relative to Serum PFOS Levels (Presented as Adjusted Odds Ratios)



PFDeA

Epidemiology Studies. In the only study identified, Zhang et al. (2015a) did not find an association between serum PFDeA levels and the risk of gestational diabetes.

PFUA

Epidemiology Studies. The two epidemiology studies evaluating associations between PFUA and diabetes-related outcomes have found conflicting results. Su et al. (2016) found inverse associations between serum PFUA levels and diabetes risk, fasting blood glucose levels, and glucose tolerance test results, whereas Lind et al. (2014) found no alterations in the risk of diabetes or HOMA.

PFHpA

Epidemiology Studies. Lind et al. (2014) did not find associations between serum PFHpA levels and the risk of diabetes or HOMA alterations.

PFOSA

Epidemiology Studies. In the one epidemiology study identified, no associations between serum PFOSA levels and the risk of diabetes or HOMA were found (Lind et al. 2014).

Me-PFOSA-AcOH

Epidemiology Studies. In a case-control study, Zhang et al. (2015a) did not find alterations in the risk of gestational diabetes associated with serum Me-PFOSA-AcOH levels.

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

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
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)	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 (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)	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**
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 ($p<0.05$)
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)***

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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**
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)***
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**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
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
PFDeA			
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.338 and 0.291 ng/mL (mean PFDeA 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 PFDeA above the median**
PFUA			
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.308 and 0.285 ng/mL (mean PFUA 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 PFUA 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]
PFOSA			
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	3.5 ng/mL (mean PFOSA)	Breast cancer	**RR 1.89 (1.01–3.54)*, among women with serum PFOSA >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 compound; unless otherwise specified, values in parenthesis are 95% confidence intervals.

AOR = adjusted odds ratio; CI = confidence interval; HR = hazard ratio; IRR = incidence rate ratio; NIOSH = National Institute for Occupational Safety and Health; NR = not reported; NS = not significant; OR = odds ratio; PFDeA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFUA = 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

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. Epidemiology studies examining other perfluoroalkyl compounds consisted of two case-control studies. No increases in breast cancer risk were observed for PFHxS or PFNA; an increased breast cancer risk was observed for PFOSA. Another case-control study did not find increases in prostate cancer for PFOA, PFOS, PFHxS, PFNA, PFDeA, or PFUA. However, among men with a first-degree relative with prostate cancer, associations were found for PFOA, PFOS, PFHxS, PFDeA, and PFUA, but not for PFNA.

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 and pancreatic acinar cell 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 1 year and allowed to recover for an additional year.

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

Epidemiology 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). Steenland et al. (2015) found an inverse association between 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); 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 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 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 epidemiology 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), 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 the 3M (1983) 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

Epidemiology 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). 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

Epidemiology Studies. Two 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. 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 men 20–49 or 50–69 years of age participating in the C8 Health Studies (Ducatman et al. 2015b).

PFNA

Epidemiology Studies. The carcinogenic potential of PFNA has been examined in two case-control studies. No associations between serum PFNA levels and breast cancer (Bonefeld-Jorgensen et al. 2014) 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).

PFDeA

Epidemiology Studies. Hardell et al. (2014) examined the possible association between the serum PFDeA level and risk of prostate cancer and only found an association in men with a heredity risk factor and PFDeA levels above the median.

PFUA

Epidemiology Studies. An increased risk of prostate cancer was found in men with first-degree relatives with prostate cancer and serum PFUA levels above the median (Hardell et al. 2014).

PFOSA

Epidemiology Studies. Bonefeld-Jorgensen et al. (2014) reported an increased risk of breast cancer among women with serum PFOSA levels >5.75 ng/mL.

2.20 MECHANISM OF TOXICITY

The primary effects observed in laboratory animals exposed to perfluoroalkyl compounds 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 perfluoroalkyl compounds 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 perfluoroalkyl compounds 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 perfluoroalkyl compounds.

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. Proliferation of peroxisomes in laboratory animals exposed to perfluoroalkyl compounds 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 PFDeA.

Many, but not all, of the adverse effects induced by perfluoroalkyl compounds are mediated through activation of the PPARα. Ligands, including perfluoroalkyl compounds, 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 that 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 perfluoroalkyl compounds, including PFOA, PFOS, PFUA, PFHpA, PFDoA, and perfluoropentanoic acid 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. Perfluoroalkyl compounds have been shown to induce changes in the expression of genes under the regulation of PPARα. The expression of PPARα target genes involved in fatty acid metabolism, cell cycle control, peroxisome biogenesis, and proteasome structure and organization were upregulated, while inflammatory response genes 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 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 perfluoroalkyl compounds, and the diminished expression of inflammatory response genes may be involved in immunosuppression induced by perfluoroalkyl compounds.

Gene expression changes induced by perfluoroalkyl compounds 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 perfluoroalkyl compounds, demonstrate the following:

- A majority of the gene expression changes induced in rodents by perfluoroalkyl compounds tested to date, especially PFOA and PFNA, are dependent on activation of PPARα.
- Perfluoroalkyl compounds 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.
- PPARα-independent gene expression changes induced by perfluoroalkyl compounds are similar to those induced by compounds that activate PPARγ, constitutive androstane receptor (CAR), and estrogen receptor alpha (ERα) (discussed further under PPARα-independent mechanisms).

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 perfluoroalkyl compounds 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 perfluoroalkyl compounds 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, PFUA, PFDeA, PFDoA), 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 Perfluoroalkyl Compounds (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

Table 2-28. Transactivation of Human and Mouse PPARα in Transfected Cos-1 Cells Exposed to Perfluoroalkyl Compounds (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
PFUA	11	50	75	5	10	86	8
PFHpA	7	<0.5	0.5	3	5	15	11
PFDeA	10	100	>100[c]	<5	5	–	20
PFDoA	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
PFBuS	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 = perfluorobutyric acid; PFBuS = perfluorobutane sulfonic acid; PFDeA = perfluorodecanoic acid; PFDeA = perfluorodecanoic acid; PFDoA = 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; PFUA = 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 perfluoroalkyl compounds 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 perfluoroalkyl compounds 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 perfluoroalkyl compounds 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 perfluoroalkyl compounds 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 Perfluoroalkyl Compounds

	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	0.1% in diet	2	+	–	–	–	+	–	–	–

+ = significant (p<0.0001) similarity to gene expression changes induced by prototypical receptor agonist as assessed by running Fisher test; 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 perfluoroalkyl compounds 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 perfluoroalkyl compounds. 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. Perfluoroalkyl compounds 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 perfluoroalkyl compounds 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 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. Thus, these data demonstrated increased sensitivity of younger male mouse pups, compared with older pups or female pups, to oxidative damage by PFOS.

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. Perfluoroalkyl compounds also have been shown to inhibit GJIC both *in vivo* and *in vitro* (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 perfluoroalkyl compounds. 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 PFBuS and PFHxA) (Wallace et al. 2013).

2.20.2 Hepatic Toxicity Mechanisms

Hepatic effects of perfluoroalkyl compounds 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 Perfluoroalkyl Compounds 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	50	+++	–	+++	–	–	–	+++	–	+	–

+ = 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 perfluoroalkyl compounds affect both sides of this balance, but a growing body of evidence indicates that fatty acid accumulation induced by perfluoroalkyl compounds tips the balance in favor of hepatic steatosis (Das et al. 2017). As discussed above, perfluoroalkyl compounds 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 perfluoroalkyl compounds 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 perfluoroalkyl compounds (Das et al. 2017; Minata et al. 2010; Nakagawa et al. 2012), but not in those exposed to 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

perfluoroalkyl compounds (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 perfluoroalkyl compounds 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 perfluoroalkyl compounds induce hepatic steatosis by perturbing lipid homeostasis in favor of the accumulation of fatty acids and triglycerides in the liver.

2.20.3 Developmental Toxicity Mechanisms

Developmental effects observed in laboratory rodents exposed to perfluoroalkyl compounds 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 perfluoroalkyl compounds 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).

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 perfluoroalkyl compounds were not located. However, other molecular- and cellular-level effects of perfluoroalkyl compounds, 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 perfluoroalkyl compounds may modulate cell-signaling responses critical to antibody production, including c-Jun, NF-κB, and IL-6.

2.20.5 Endocrine Mechanisms

Perfluoroalkyl compounds 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 perfluoroalkyl compounds 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 perfluoroalkyl compounds 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 perfluoroalkyl compounds may interact with the estrogen and androgen receptors. PFOA, PFOS, PFHxS, PFNA, and PFDeA 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

Like many other PPARα agonists, 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, PFDeA, and PFUA were potent inducers of vitellogenin, an estrogen-responsive biomarker protein. *In vitro*, PFOA, PFOS, PFNA, and PFDeA 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 PFDeA enhanced liver carcinogenesis 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 perfluoroalkyl compounds, 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 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).

2.21 GENOTOXICITY

No studies of genotoxicity in humans exposed to perfluoroalkyl compounds were located.

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; the same dose of PFBA had no effect on liver or kidney DNA (Takagi et al. 1991). Oral administration of approximately 20 mg/kg/day PFOA or 10 mg/kg/day PFDeA 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). These findings led the authors to conclude that induction of peroxisome proliferation also leads to organ-specific oxidative DNA damage. Unpublished studies summarized by Butenhoff et al. (2014) and Kennedy et al. (2004) did not find increases in the occurrence of polychromatic erythrocytes in the bone marrow of mice orally exposed to PFOA. PFOS induced micronuclei frequency and decreased the ratio of polychromatic erythrocytes to normochromatic erythrocytes in bone marrow of rats following oral exposure to 0.6–2.5 mg/kg for 30 days (Celik et al. 2013; Eke and Celik 2016).

In vitro studies provide evidence that PFOA and PFOS are not mutagenic at noncytotoxic concentrations. Butenhoff et al. (2014) and Kennedy et al. (2004) summarized the results of various unpublished mutagenicity studies with PFOA. Negative results were found in reverse mutation assays using *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538), *Saccharomyces cerevisiae*, and *Escherichia coli* (WP2uvrA strain) with or without metabolic activation. 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. Similarly, two *in vivo* assays found no alterations in the occurrence of micronuclei in mice. PFOA also was not mutagenic in *S. 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).

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 reactive oxygen species (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 the mutagenic potential, indicating that ROS may play an important role in mediating the genotoxic effects of PFOA. PFOA induced DNA damage in *Paramecium caudatum* following exposure to 100 μM for 12 and 24 hours (Kawamoto et al. 2010). Intracellular ROS was significantly increased and 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. PFOS did not induce DNA damage in this study. In contrast, no increases in DNA damage or micronuclei formation were found in human hepatoma HepG2 cells following a 24-hour exposure to PFOA concentrations as high as 800 μM (Florentin et al. 2011); cytotoxicity was observed at ≥200 μM. Eriksen et al. (2010) also found no evidence of DNA damage in HepG2 cells incubated with 100 or 400 μM PFOA for 24 hours.

OECD (2002) summarized unpublished mutagenicity studies conducted with PFOS. PFOS was negative in all tested assays. It 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). 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. In addition, PFOS did not induce micronuclei in the bone marrow of CD-1 mice in an *in vivo* assay. 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 micronuclei formation in human hepatoma HepG2 cells following a 24-hour exposure to PFOA 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).

Limited *in vitro* data on the genotoxicity of other perfluoroalkyls were located. No DNA damage was found in HepG2 cells incubated with 100 or 400 μM PFHxS or PFBuS for 24 hours (Eriksen et al. 2010). A “modest” increase in DNA damage was observed at 400 μM PFNA, a cytotoxic concentration (Eriksen et al. 2010).