CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

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

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

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

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

1.1 OVERVIEW AND U.S. EXPOSURES

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

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

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

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

1. RELEVANCE TO PUBLIC HEALTH

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

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

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

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

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

1.2 SUMMARY OF HEALTH EFFECTS

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

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

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

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

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

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

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

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

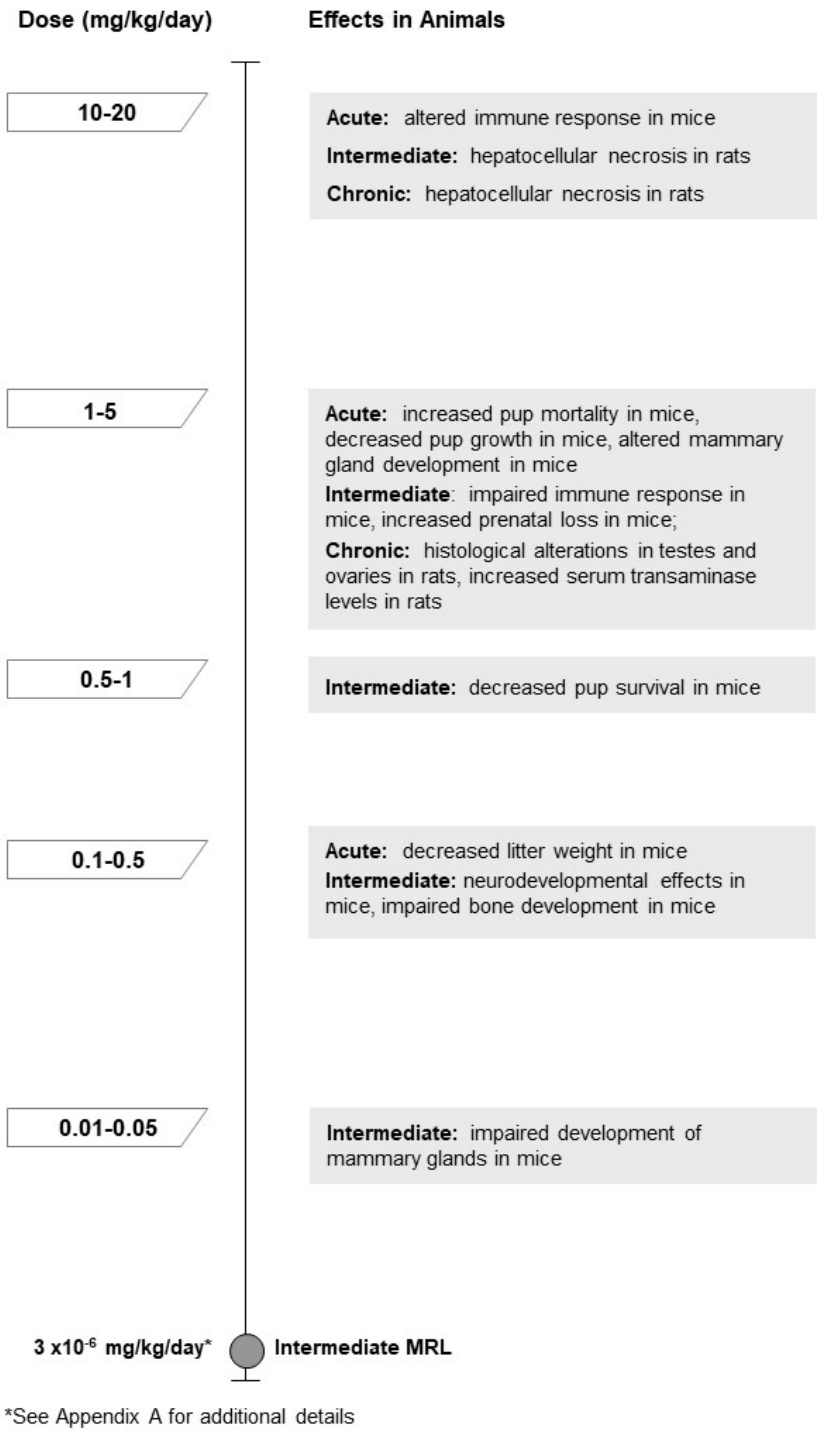
The International Agency for Research on Cancer (IARC 2017) concluded that PFOA is possibly carcinogenic to humans (Group 2B), and EPA (2016e, 2016f) concluded that there was suggestive evidence of the carcinogenic potential of PFOA and PFOS in humans. Increases in testicular and kidney cancer have been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

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

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

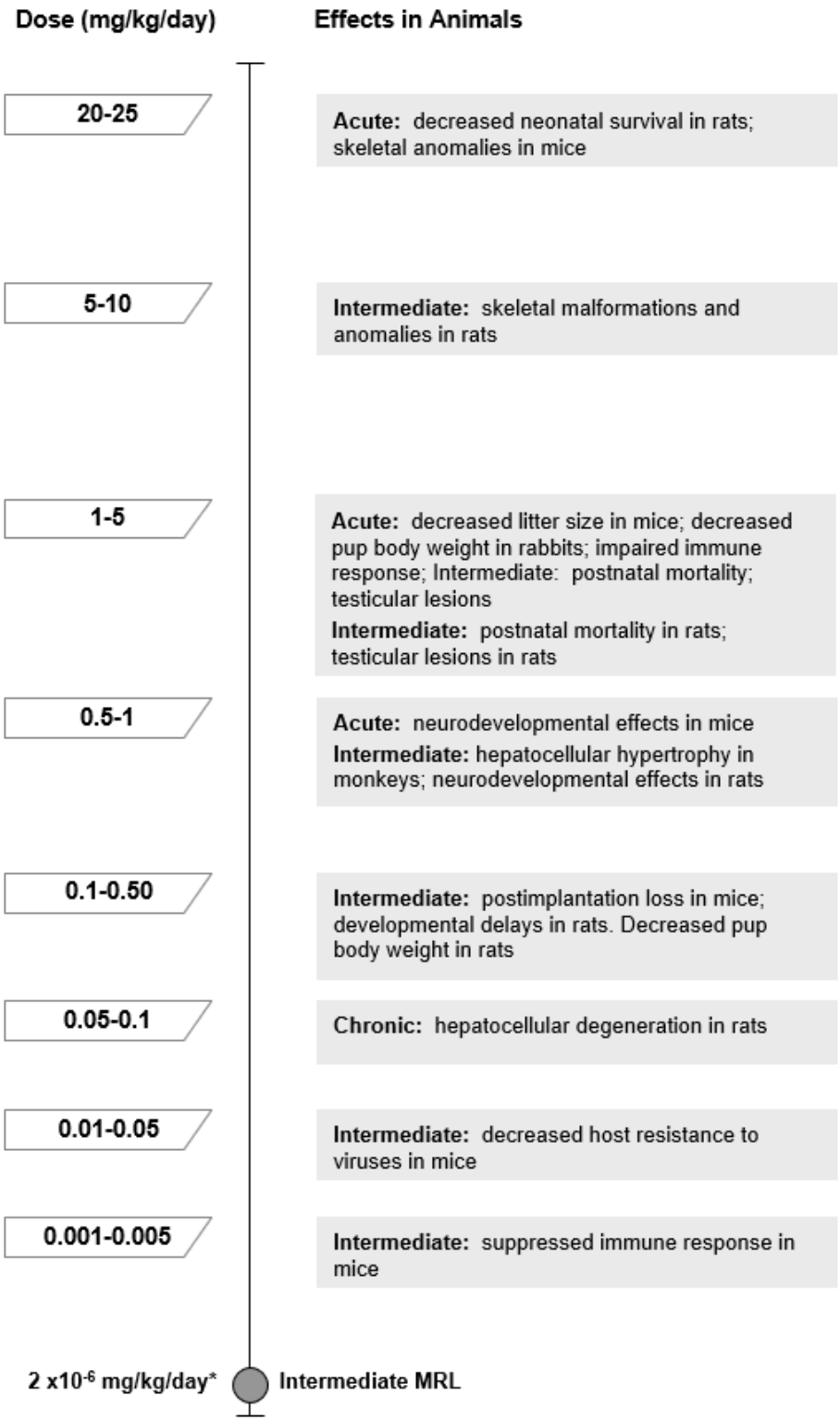
Liver Effects. Many studies have described morphological and biochemical alterations in the liver from rodents following acute and longer-term oral exposure to PFOA. Some of the effects observed in rats include increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol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

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



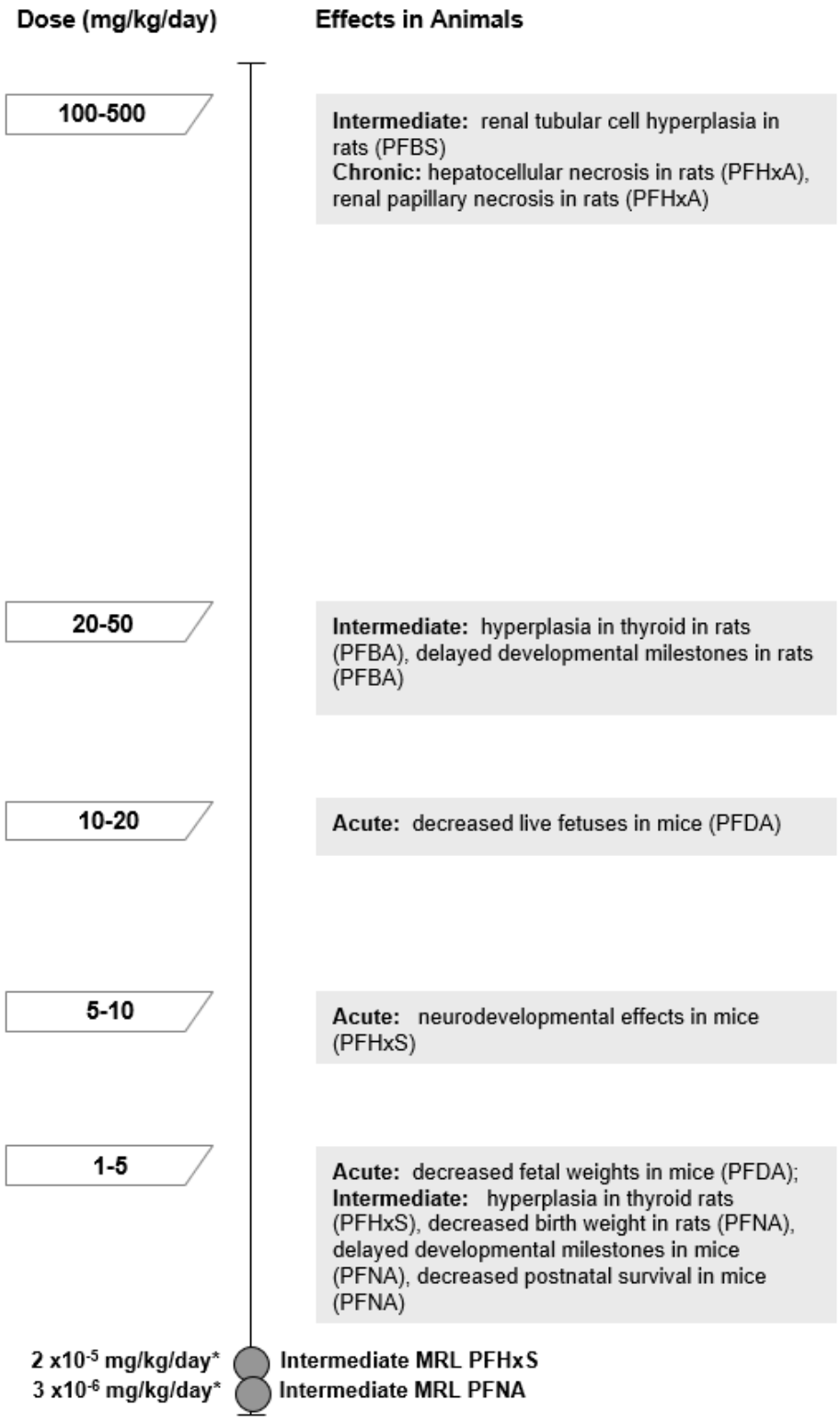
*See Appendix A for additional details

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



*See Appendix A for additional details

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



*See Appendix A for additional details

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

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

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

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

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

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

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

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

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

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

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

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

1.3 MINIMAL RISK LEVELS (MRLs)

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

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

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

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

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

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

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

[a]X indicates that no MRL was derived.

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

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

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

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

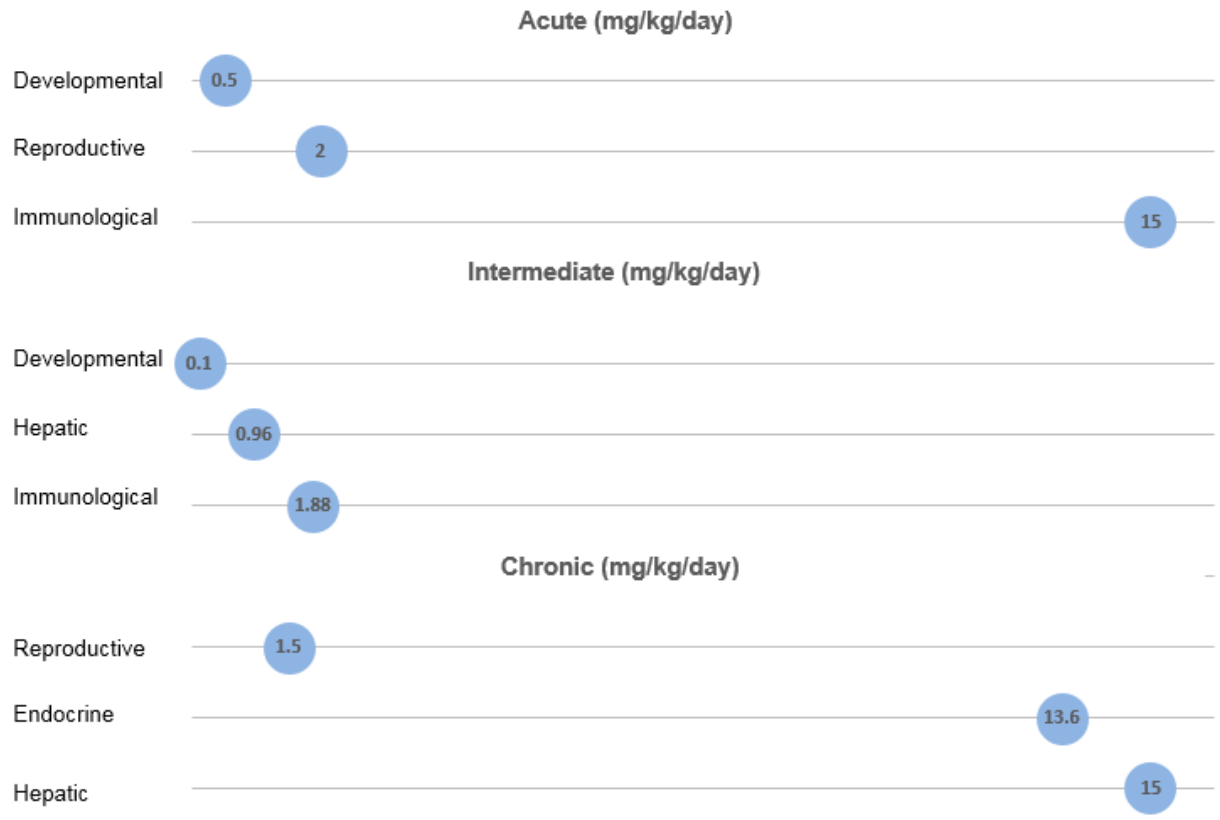


Figure 1-5. Summary of Sensitive Targets of PFOS – Oral

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

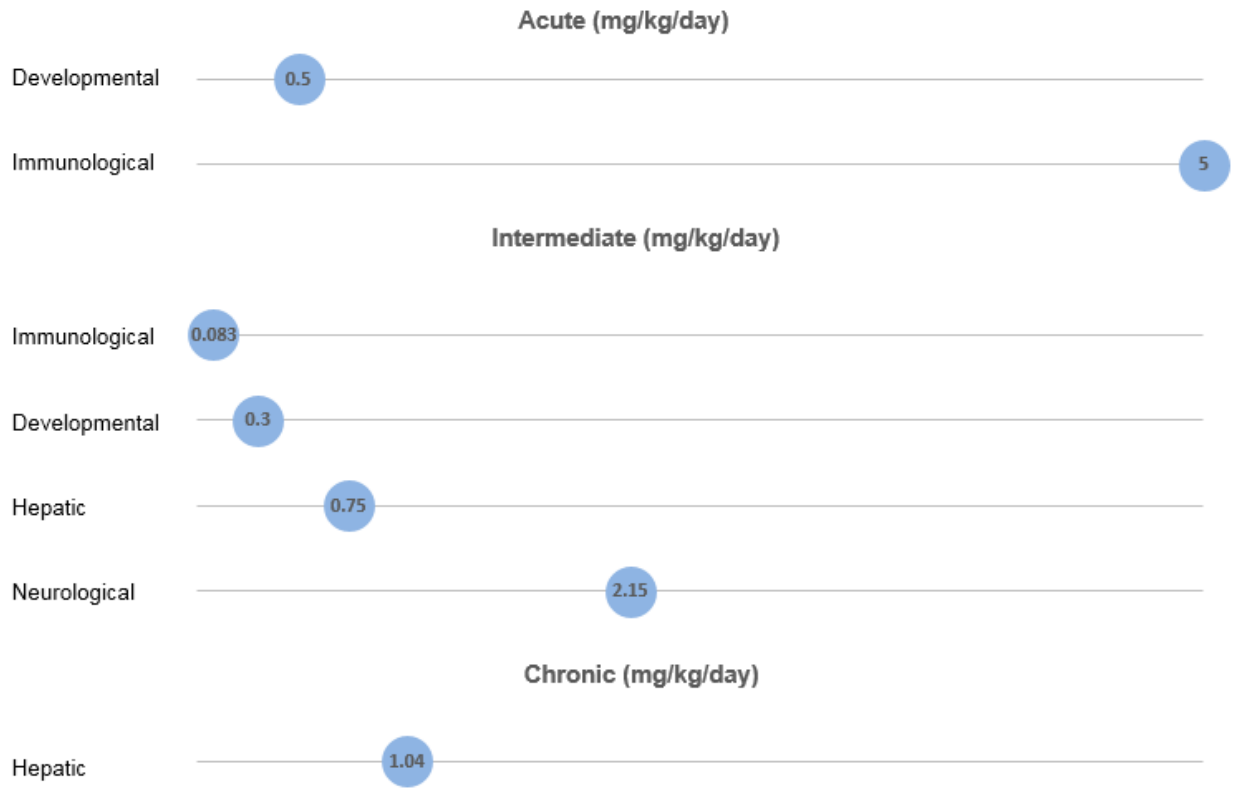


Table 1-3. Minimal Risk Levels (MRLs) for PFOA[a]

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

[a]See Appendix A for additional information.

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

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

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

[a]See Appendix A for additional information.

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

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

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

[a]See Appendix A for additional information.

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

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

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

[a]See Appendix A for additional information.

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