CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

This toxicological profile on perfluoroalkyls discusses information on 14 perfluoroalkyl compounds 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. These compounds include:

Perfluorobutyric acid (PFBA)
Perfluorohexanoic acid (PFHxA)
Perfluoroheptanoic acid (PFHpA)
Perfluorooctanoic acid (PFOA)
Perfluorononanoic acid (PFNA)
Perfluorodecanoic acid (PFDeA)
Perfluoroundecanoic acid (PFUA)
Perfluorobutane sulfonic acid (PFBuS)
Perfluorohexane sulfonic acid (PFHxS)
Perfluorooctane sulfonic acid (PFOS)
Perfluorododecanoic acid (PFDoA)
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 toxicological profile is referring to these 14 compounds and the information may not be applicable to other perfluoroalkyl compounds.

1.1 OVERVIEW AND U.S. EXPOSURES

The perfluoroalkyl compounds discussed in this profile primarily consist of perfluorinated aliphatic carboxylic acids (PFCAs), perfluorinated aliphatic sulfonic acids (PFSAs), and some polyfluorinated substances that may degrade or be metabolized to some important perfluorinated substances such as PFOA or PFOS. 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).

1. RELEVANCE TO PUBLIC HEALTH

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 companies began 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 are no longer manufactured or imported into the United States; however, there could be some imported goods containing trace amounts of these substances as impurities. Information 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 processes has not been located. 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).

Due to their chemical structure, perfluoroalkyls are very stable in the environment and are resistant to biodegradation, photoxidation, 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 high 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 of in 45,276 non-occupationally exposed individuals residing in southeastern Ohio and West Virginia who were exposed to PFOA via contaminated drinking water were approximately 6 times greater than the median concentration of the general population when compared to NHANES data (Shin et al. 2011). Serum levels of PFOA and PFOS in the general population of the United States have decreased dramatically in recent years as U.S. production of these substances ceased (CDC 2018). 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, but have decreased to 1.94 ng/mL (PFOA) and 4.99 ng/mL (PFOS) in 2013–2014 (CDC 2018).

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.

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 were 0.360–0.639 and 0.210–0.490 ng/mL, respectively (Kärrman et al. 2007; So et al. 2006b; Völkel et al. 2008). Maximum concentrations of other perfluoroalkyl compounds were <0.18 ng/mL. In most umbilical cord samples, the concentrations of PFOS and PFOA ranged from 4.9 to 11.0 and from 1.6 to 3.7 ng/mL, respectively (Apelberg et al. 2007a, 2007b; Fei et al. 2007; Inoue et al. 2004; Midasch et al. 2007). Other perfluoroalkyls have been detected less frequently.

1.2 SUMMARY OF HEALTH EFFECTS

Perfluoroalkyls are ubiquitous chemicals in the environment; they are readily absorbed following inhalation or oral exposure and are not metabolized in humans or laboratory animals. The toxicity of perfluoroalkyl compounds, particularly PFOA and PFOS, has been extensively evaluated in humans and laboratory animals. 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 epidemiology and experimental studies. Substantial differences in the rate of elimination of perfluoroalkyls exist across species. Table 1-1 lists half-lives for PFOA, PFOS, PFHxS, PFBuS, 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 ranges from 8 years in humans to 1.9 hours in female rats.

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

	Humans	Nonhuman primates	Rats[a]	Mice[a]
PFOA	8 years (Olsen et al. 2007a)	20.1–32.6 days (Butenhoff et al. 2004c)	Males: 44–322 hours Females: 1.9–16.2 hours	
PFOS	5.4 years (Olsen et al. 2007a)	110–170 days (Chang et al. 2012; Seacat et al. 2002)	179–1,968 hours	731–1,027 hours
PFHxS	8.5 years (Olsen et al. 2007a)	87–141 days (Sundström et al. 2012)	Males: 382–688 hours Females: 1.03–41.28 hours	597–643 hours
PFBuS	665 hours (Olsen et al. 2009)	8.0–95.2 hours (Chengelis et al. 2009; Olsen et al. 2009)	2.1–7.42 hours	
PFBA	72 hours (Chang et al. 2008b)	40.3–41.0 hours (Chang et al. 2008b)	1.03–9.22 hours	2.79–13.34 hours

[a]See Section 3.1.4 for citations.

The mechanisms of toxicity of perfluoroalkyl compounds have not been fully elucidated. There is strong evidence that some effects observed in rodents, such as hepatotoxicity, immunotoxicity, and developmental toxicity, involve the activation of peroxisome proliferator-activated receptor-α (PPARα); however, humans and nonhuman primates are less responsive to PPARα agonists than rodents. Additionally, PPARα-independent mechanisms are also involved and it is not known if species

differences exist for these mechanisms. In general, epidemiology 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 comparisons between administered doses in laboratory animals and serum concentrations in humans.

Effects in Humans. Perfluoroalkyl compounds have been detected in the serum of workers, residents living near perfluoroalkyl facilities, and the general population. A large number of epidemiology studies have evaluated possible associations between perfluoroalkyl exposure and a wide range of adverse health outcomes. 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 12 perfluoroalkyls included in this toxicological profile. Most of the epidemiology 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 perfluoroalkyl compounds (referred to as general population studies). Of the three categories of subjects, workers have the highest potential exposure to perfluoroalkyls, followed by the highly-exposed residents in the Mid-Ohio Valley, and then the general population. In one study of workers at the Washington Works facility in West Virginia, the average serum PFOA level in 2001–2004 was 1,000 ng/mL (Sakr et al. 2007a); the 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 geometric mean concentration of PFOA in the U.S. population was 3.92 ng/mL in 2005–2006 (CDC 2013). 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. Based on a number of factors (described in Section 2.1) including the consistency of findings across studies, the available epidemiology studies suggest associations between perfluoroalkyl exposure and several health outcomes:

- Pregnancy-induced hypertension/pre-eclampsia (PFOA, PFOS)
- Liver damage, as evidenced by increases in serum enzymes 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, PFDeA)
- Increased risk of thyroid disease (PFOA, PFOS)
- Decreased antibody response to vaccines (PFOA, PFOS, PFHxS, PFDeA)
- Increased risk of asthma diagnosis (PFOA)
- Increased risk of decreased fertility (PFOA, PFOS)
- Small (<20 g or 0.7 ounces per 1 ng/mL increase in blood perfluoroalkyl 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 the higher degree of 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, PFUA, PFDoA). 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 and not perfluoroalkyl toxicity.

***Effects in Laboratory Animals*.** Most of the information regarding the effects of perfluoroalkyl compounds 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 perfluoroalkyl compounds, with considerably less data for the other compounds. Of the 187 animal studies reviewed in this toxicological profile, 48% examined PFOA, 34% examined PFOS, and 18% examined other perfluoroalkyls (4 studies on PFHxS, 16 studies on PFNA, 1 study on PFUA, 4 studies on PFBuS, 6 studies on PFBA, 6 studies on PFDeA, 2 studies on PFDoA, 1 study on PFOSA, and 3 studies on PFHxA). The primary effects observed in laboratory animals exposed to perfluoroalkyl compounds 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 perfluoroalkyl compounds. Based on limited data, the

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

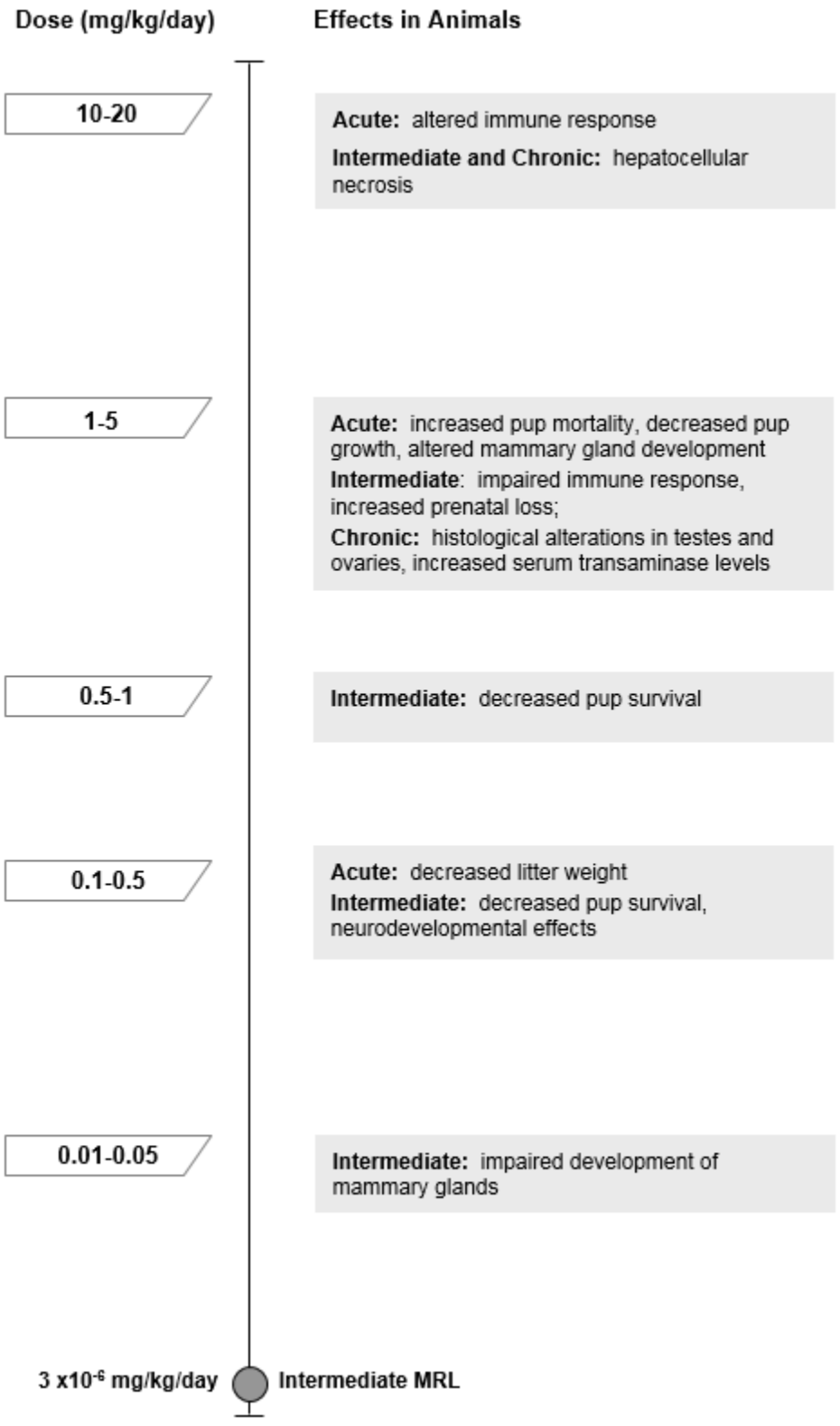


Figure 1-2. Health Effects Found in Animals Following Oral Exposure to PFOS

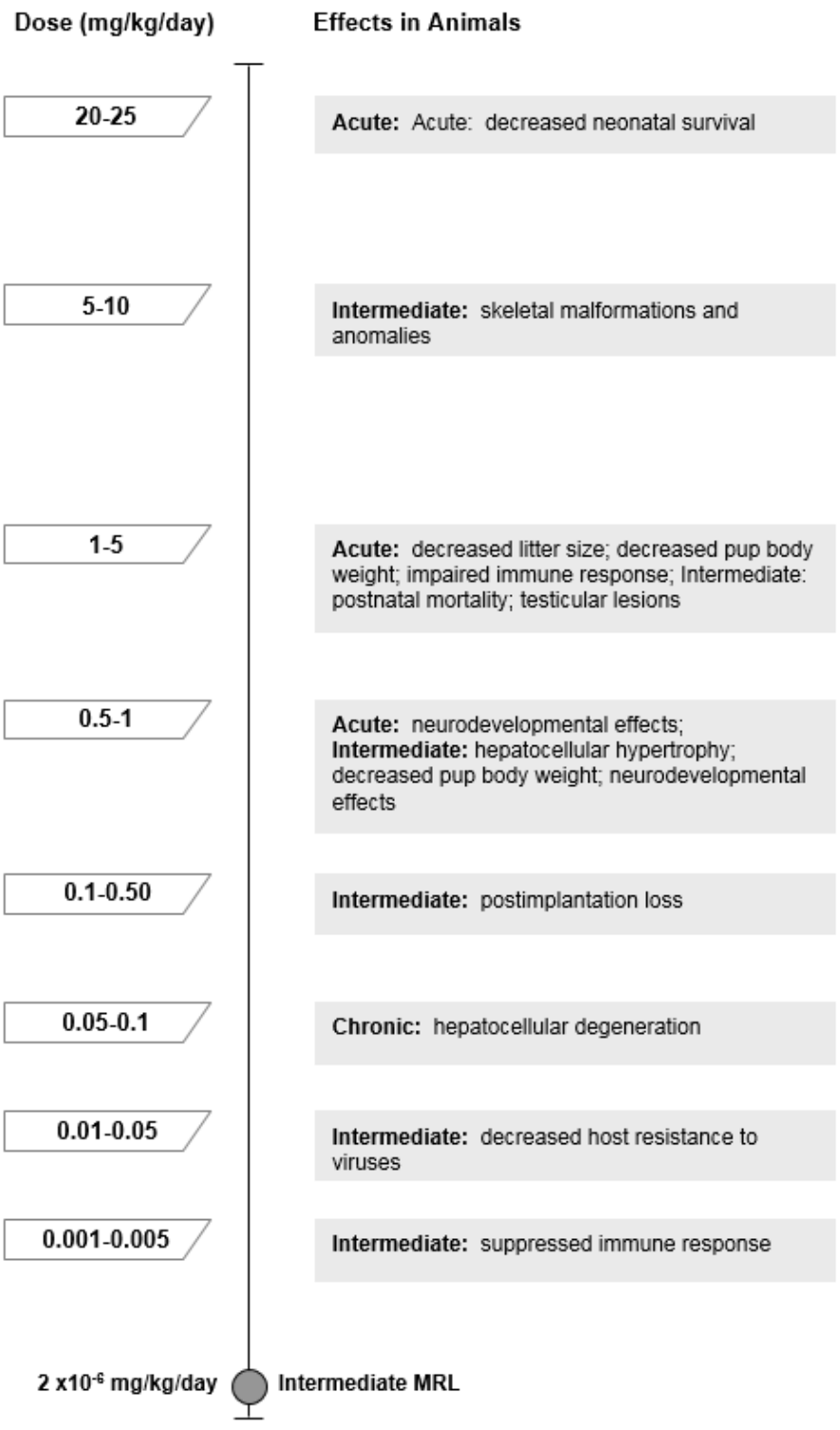
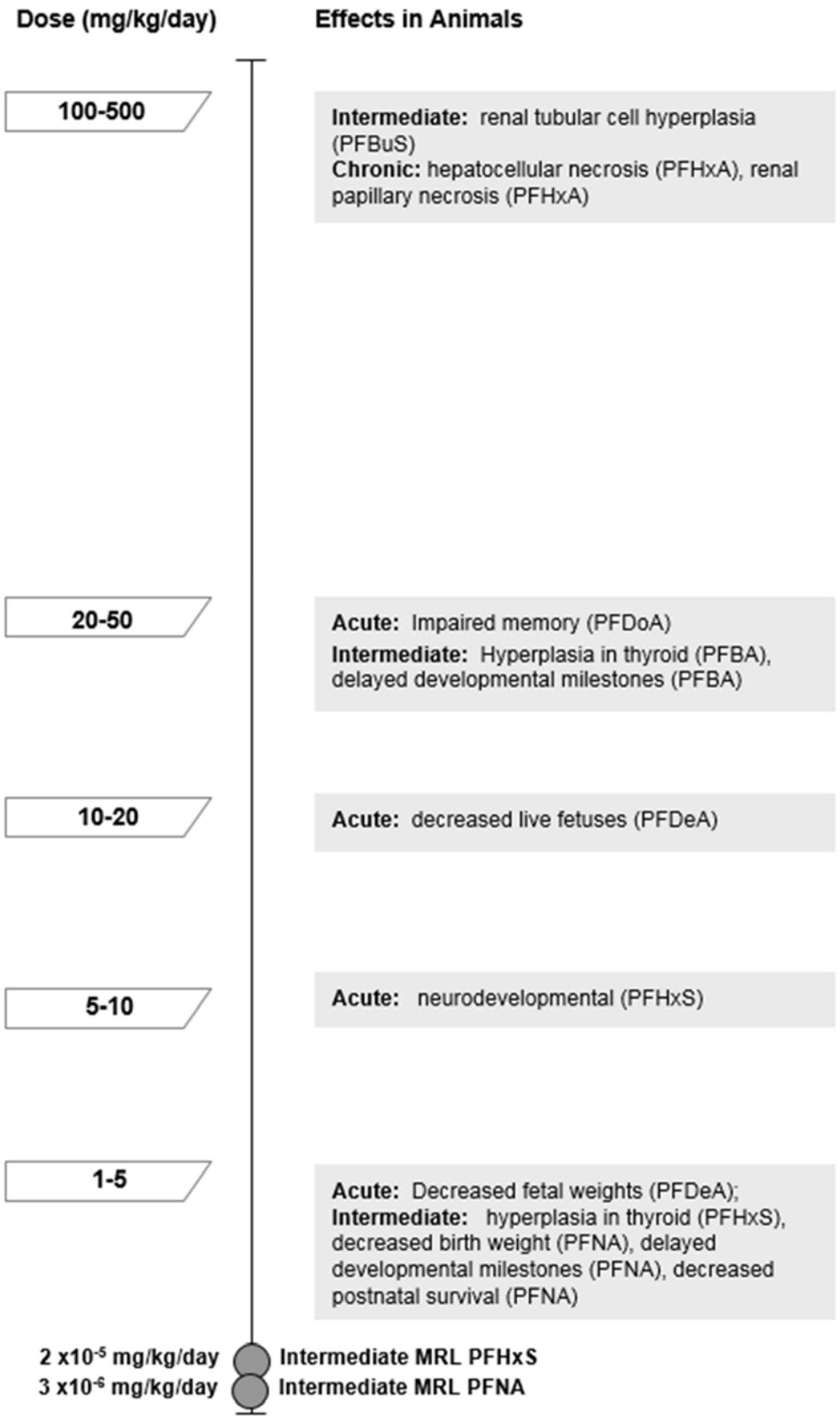


Figure 1-3. Health Effects Found in Animals Following Oral Exposure to Other Perfluoroalkyls



toxicity of perfluoroalkyl compounds 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 perfluoroalkyl compounds 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.

Many of the adverse health effects observed in laboratory animals result from the ability of these compounds (with some structural restrictions) to activate the PPARα, which can mediate a broad range of biological responses (Issemann and Green 1990). Species differences in the response to PPARα agonists have been found; 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 a functional PPARα. Several explanations for these species differences have been suggested (e.g., differences in the ability of PPARα to be induced after exposure to a peroxisome proliferator and differences in the pattern and level of tissue-specific expression of PPARα). Activation of this receptor in rodents initiates a characteristic sequence of morphological and biochemical events, principally, but not exclusively, in the liver (Cattley et al. 1998; Kennedy et al. 2004; Klaunig et al. 2003). The proliferation of peroxisomes has been associated with a variety of effects, including hepatocellular hypertrophy, alterations in lipid metabolism, and decreased pup survival and immune effects. Studies in PPARα-null mice provide evidence that PPARα-independent mechanisms are also involved in PFOA and PFOS toxicity, including liver and immune toxicity. A more complete discussion of the mechanisms of PFOA and PFOS toxicity is presented in Section 2.20.

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). The observed hepatomegaly and hypertrophy are likely due 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 perfluoroalkyl compounds 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; Hoberman and York 2003; 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 mouse COS-1 cells, PFOA had the lowest effective concentration needed for PPARα activation followed by PFNA and PFDeA, 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 PFBuS. Wolf et al. (2008a) also found that carboxylate perfluoroalkyls activated PPARα at lower concentrations than the sulfonate perfluoroalkyls. Studies have shown that the differential activity is also related to differential accumulation of the perfluoroalkyl compound 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).

Developmental Effects. PFOA and PFOS have induced developmental effects in rodents. Most studies with PFOA have been conducted in mice, probably because of the relatively short half-life for PFOA in female rats, which would prevent accumulation of PFOA during the dosing period. Specific effects reported include prenatal loss, reduced neonate weight and viability, neurodevelopment toxicity, 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). 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. 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. 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 PFDeA 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, PFBuS, 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 PFDeA (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), PFDeA (Harris et al. 1989), PFBuS (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). 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 preclude a conclusion regarding the human relevance of PFOA-induced pancreatic tumors (EPA 2016h).

1.3 MINIMAL RISK LEVELS (MRLs)

A summary of the provisional MRLs derived for perfluoroalkyl compounds is presented in Table 1-2. 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 perfluoroalkyl compounds.

Table 1-2. Overview of Provisional Minimal Risk Levels Derived for Perfluoroalkyl Compounds

	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
PFDeA	X	X	X	X	X	X
PFUA	X	X	X	X	X	X
PFHpA	X	X	X	X	X	X

Table 1-2. Overview of Provisional Minimal Risk Levels Derived for Perfluoroalkyl Compounds

	Inhalation MRLs			Oral MRLs		
Compound	Acute	Intermediate	Chronic	Acute	Intermediate	Chronic
PFBuS	X	X	X	X	X	X
PFBA	X	X	X	X	X	X
PFDoA	X	X	X	X	X	X
PFHxA	X	X	X	X	X	X
PFOSA	X	X	X	X	X	X
Me-PFOSA-AcOH	X	X	X	X	X	X
Et-PFOSA-AcOH	X	X	X	X	X	X

[a]X indicates that no MRL was derived.

Et-PFOSA-AcOH = 2-(N-ethyl-perfluorooctane sulfonamide) acetic acid; Me-PFOSA-AcOH = 2-(N-methyl-perfluorooctane sulfonamide); acetic acid; PFBA = perfluorobutyric acid; PFBuS = perfluorobutane sulfonic 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; PFOSA = perfluorooctane sulfonamide; PFUA = perfluoroundecanoic acid

The oral databases were considered adequate for derivation of provisional 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 perfluoroalkyl compounds. 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. The provisional 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.

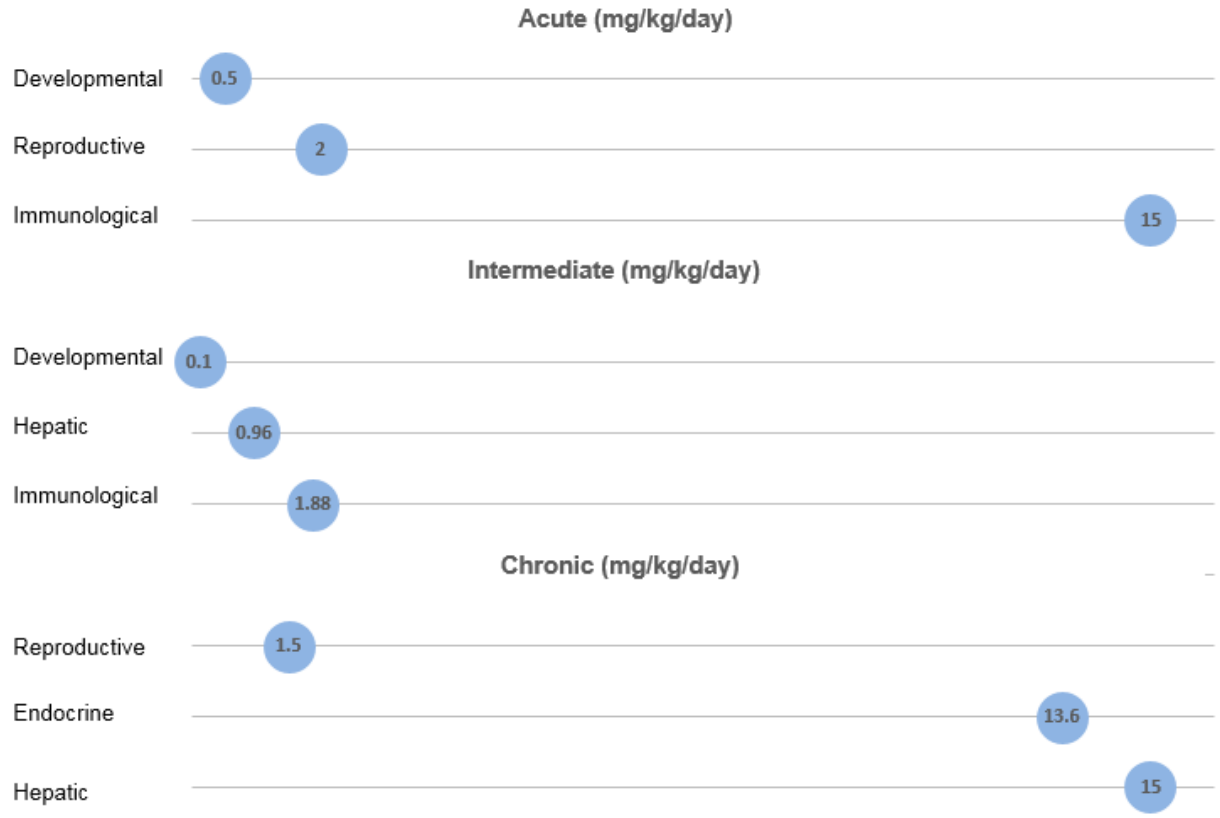


Figure 1-5. Summary of Sensitive Targets of PFOS – Oral

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.

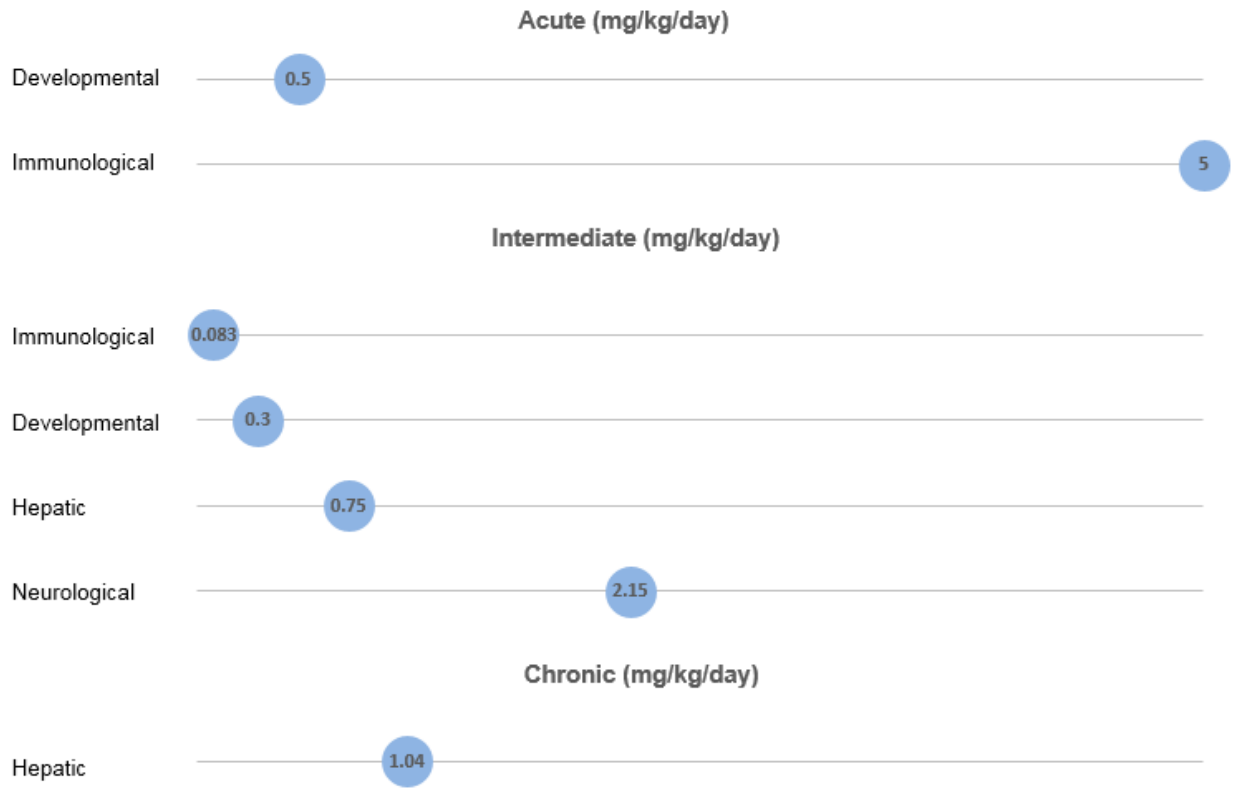


Table 1-3. Provisional Minimal Risk Levels (MRLs) for PFOA[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty factor	Reference
Inhalation exposure					
Acute	Insufficient data for MRL derivation				
Intermediate	Insufficient data for MRL derivation				
Chronic	Insufficient data for MRL derivation				
Oral exposure (mg/kg/day)					
Acute	Insufficient data for MRL derivation				
Intermediate	$3x10^{-6}$	Neurodevelopmental and skeletal effects in mice	0.000821 ($LOAEL_{HED}$)	300	Koskela et al. 2016; Onishchenko et al. 2011
Chronic	Insufficient data for MRL derivation				

[a]See Appendix A for additional information.

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

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

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Insufficient data for MRL derivation				
Intermediate	Insufficient data for MRL derivation				
Chronic	Insufficient data for MRL derivation				
Oral exposure (mg/kg/day)					
Acute	Insufficient data for MRL derivation				
Intermediate	$2x10^{-6}$	Delayed eye opening and decreased pup weight in rats	0.000515 ($NOAEL_{HED}$)[b]	30 10	Luebker et al. 2005a
Chronic	Insufficient data for MRL derivation				

[a]See Appendix A for additional information.

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

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

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Insufficient data for MRL derivation				
Intermediate	Insufficient data for MRL derivation				
Chronic	Insufficient data for MRL derivation				
Oral exposure (mg/kg/day)					
Acute	Insufficient data for MRL derivation				
Intermediate	$2x10^{-5}$	Thyroid follicular cell damage in rats	0.0047 ($NOAEL_{HED}$)	30 10	Butenhoff et al. 2009a
Chronic	Insufficient data for MRL derivation				

[a]See Appendix A for additional information.

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

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

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Insufficient data for MRL derivation				
Intermediate	Insufficient data for MRL derivation				
Chronic	Insufficient data for MRL derivation				
Oral exposure (mg/kg/day)					
Acute	Insufficient data for MRL derivation				
Intermediate	$3x10^{-6}$	Decreased body weight and developmental delays in mice	0.001 ($NOAEL_{HED}$)	30 10	Das et al. 2015
Chronic	Insufficient data for MRL derivation				

[a]See Appendix A for additional information.

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