

Notification Level Recommendations

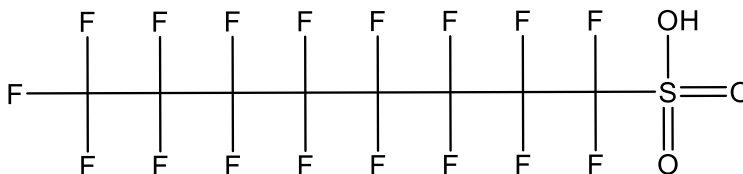
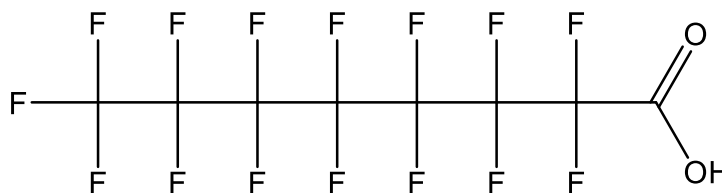
Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Drinking Water

August 2019



Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

**Notification Level Recommendations for
Perfluorooctanoic Acid (PFOA) and
Perfluorooctane Sulfonate (PFOS)
in Drinking Water**



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August 2019

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SUMMARY

This document presents final notification level (NL) recommendations by the Office of Environmental Health Hazard Assessment (OEHHA) for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in drinking water. This supersedes the underlying scientific analysis (OEHHA, 2018) that supported the interim NLs that were adopted in 2018 (SWRCB, 2018a,b).

Based on the current evaluation of recent human and animal toxicity data, and applying OEHHA's risk assessment methodology and the US Environmental Protection Agency's (US EPA) human clearance factors (US EPA, 2016a,b) to account for the chemical half-life differences between rodents and humans, OEHHA developed PFOA and PFOS reference levels (RLs) for cancer effects. These levels represent concentrations of the chemicals in drinking water that would not pose more than a one in one million cancer risk over a lifetime:

- 0.1 ng/L (nanogram/liter) or parts per trillion (ppt) for PFOA, based on pancreatic and liver tumors in male rats (NTP, 2018c);
- 0.4 ng/L (or ppt) for PFOS, based on liver tumors in male rats (Butenhoff et al. 2012a) and the structural and biological similarity of PFOS to PFOA.

OEHHA also developed RLs for noncancer effects as follows:

- 2 ng/L (or ppt) for PFOA, based on liver toxicity in female mice (Li et al., 2017);
- 7 ng/L (or ppt) for PFOS, based on immunotoxicity in male mice (Dong et al., 2009).

The cancer RLs cited above are lower than the levels of PFOA and PFOS that can be reliably detected in drinking water using currently available technologies. In light of this, OEHHA recommends that the State Water Resources Control Board (SWRCB) set the NLs at the lowest levels at which PFOA and PFOS can be reliably detected in drinking water using available and appropriate technologies.

INTRODUCTION

At SWRCB's request, OEHHA has developed recommendations for drinking water NLs for PFOA and PFOS. Health and Safety Code Section 116455 defines an NL as the level of a drinking water contaminant that SWRCB has determined, based on available scientific information, does not pose a significant health risk but, when exceeded, warrants notification to a water system's governing body and other specified entities. NLs are nonregulatory, health-based advisory levels that SWRCB establishes as a precautionary measure for contaminants for which regulatory standards have not been set but that may be considered candidates for the establishment of maximum contaminant levels.

As a first step, OEHHA in June 2018 presented recommended interim NLs for PFOA and PFOS to SWRCB. OEHHA performed an expedited review of health-based values developed by several federal and state government agencies (US EPA, 2016a; US EPA, 2016b; New Jersey DWQI, 2017; ATSDR, 2018; New Jersey DWQI, 2018) and found the process used by New Jersey to be sufficient for establishing the interim NLs for PFOA and PFOS. Thus, OEHHA recommended that SWRCB adopt the following interim NLs based on New Jersey's evaluation:

- 14 ng/L (or ppt) for PFOA, based on liver toxicity in mice (Loveless et al., 2006) and carcinogenicity in rats (Butenhoff et al., 2012b);
- 13 ng/L (or ppt) for PFOS, based on immunotoxicity in mice (Dong et al., 2009).

In July 2018, SWRCB adopted these interim NLs for PFOA and PFOS, based on OEHHA's recommendations.

OEHHA has now completed a focused review, primarily evaluating studies that have been published since the above-cited reviews. This review evaluated human and animal toxicity studies published since 2016 and focused on hepatotoxicity, immunotoxicity, thyroid toxicity, reproductive toxicity, and cancer. These endpoints are known hazards of PFOA and PFOS exposure, and were readily observed in recent studies.

OEHHA recommends that SWRCB set the final NLs at the lowest levels at which PFOA and PFOS can be reliably detected in drinking water using currently available and appropriate technologies. OEHHA has developed RLs of 0.1 ppt for PFOA and 0.4 ppt for PFOS based on cancer endpoints, which are below levels that can be reliably detected with current technologies. RLs for noncancer endpoints are 2 ppt for PFOA based on liver toxicity and 7 ppt for PFOS based on immunotoxicity.

While OEHHA reviewed human epidemiology studies focusing on liver toxicity, immunotoxicity, and thyroid toxicity, an epidemiological analysis is not presented in this document because there were no studies that could be used for point of departure (POD) determination and dose-response assessment. Nonetheless, the epidemiology data suggest that there are associations between PFOA and/or PFOS and suppressed antibody response and increased liver enzymes. These epidemiological data are supportive of the animal toxicology data used to derive the RLs for noncancer effects. Use of data on immunotoxicity for noncancer RLs is further supported by the National Toxicology Program's (NTP) immunotoxicity review of PFOA and PFOS, which concluded that these chemicals are presumed to be an immune hazard to humans (NTP, 2016). The epidemiology data on thyroid hormone levels are inconsistent and, at times, contradictory.

TOXICOLOGICAL REVIEW

Liver Toxicity – PFOA

In vivo studies

PFOA exposure has consistently induced liver toxicity in experimental animals, and as with PFOS, a thorough examination of the literature was previously conducted by other agencies (US EPA, 2016a; New Jersey DWQI, 2017; ATSDR, 2018). In general, increases in absolute and/or relative liver weight, increased liver histopathology, increased biomarkers of liver damage, and changes in liver lipid content were observed.

OEHHA's review of recent animal studies that were not included in the above-cited reviews by other agencies is summarized in Table 1. Notable studies are described in greater detail below.

Table 1. Summary of recent animal toxicity studies of PFOA reporting liver effects

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male Balb/c mice (n not specified)	0, 1, or 5 mg/kg-day orally for 7 days	↑ absolute liver weight; hepatocyte cytoplasmic vacuolization; ↑ serum ALT; changes in serum and liver lipid levels	LOAEL: 1 mg/kg-day for ↑ serum ALT levels	Hui et al. (2017)
Male Balb/c mice (n=20/dose)	0 or 1.25 mg/kg-day for 28 days	↑ relative liver weight; altered glucose metabolism	NA ^a	Zheng et al. (2017)
Male Balb/c mice (n=5/dose)	0, 0.5, or 2.5 mg/kg-day via oral infusion for 28 days	↑ absolute and relative liver weight; changes in lipid metabolism; altered glucose metabolism	NOAEL: 0.5 mg/kg-day for increased liver weight	Yu et al. (2016)
Male Balb/c mice (n not specified)	0, 0.08, 0.31, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	hepatocyte swelling; lipid deposits	Not provided ^b	Yan et al. (2017)
C57BL/6 mice (n=4/dose, sex not specified)	0 or 20 mg/kg-day i.p. for 1 or 3 days	↑ relative liver weight	NA ^a	Abe et al. (2017)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male Sprague Dawley rats (n=5/dose)	Single dose of 0 or 150 mg/kg intragastrically	↑ relative liver weight	NA ^a	Cavallini et al. (2017)
Male Kunming mice (n not specified)	Single oral dose of 0 or 5 mg/kg	↑ hepatic cytoplasmic vesicles; ↑ inflammatory cells around the hepatic portal area; changes in hepatic cholesterol level	NA ^a	Wu et al. (2017)
Male and female Balb/c mice (n=30/sex/group)	0, 0.05, 0.5, or 2.5 mg/kg-day via oral gavage for 28 days	↑ absolute liver weight; hepatocellular hypertrophy and apoptosis; lipid accumulation in cytoplasm of hepatocytes; mitochondrial morphology changes; changes in mitochondrial membrane potential; oxidative DNA damage (ROS generation)	LOAEL: 0.05 mg/kg-day for hepatic mitochondrial membrane potential changes, apoptosis, oxidative DNA damage	Li et al. (2017)
Male Kunming mice (n=8/dose)	0, 1, or 5 mg/kg-day intragastrically for 21 days	↑ absolute and relative liver weight; ↑ serum ALT and AST; elevated blood insulin; ↓ serum triglycerides and H-LDL; elevated triglycerides in liver; ↑ L-LDL in serum; ↑ hepatic vacuoles	NOAEL: 1 mg/kg-day for ↑ liver enzymes and triglyceride levels	Wu et al. (2018)
Pregnant Kunming mice (n=8/dose)	0 or 5 mg/kg-day intragastrically throughout gestation	↑ ALT, AST, triglycerides, and cholesterol in pup serum on PND 21 (although the changes were not statistically significant)	NA ^a	Qin et al. (2018)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male and female Sprague Dawley rats (n=10/sex/dose)	0, 0.625, 1.25, 2.5, 5 or 10 mg/kg-day via oral gavage for males and females for 28 days	Hepatocyte hypertrophy, hepatocyte cytoplasmic alteration, ↑ absolute and relative liver weight, changes in cholesterol and triglyceride levels, ↑ serum ALT and ALP Males: ↑ serum AST and bilirubin	LOAEL: 0.625 mg/kg-day for hepatocyte cytoplasmic alteration and ↑ liver weight in males	NTP (2018b)
Female Sprague Dawley rats (n=10/dose)	0, 300, or 1,000 ppm (0, 27.7, or 92.7 mg/kg-day, calculated by OEHHA) in feed for 16 weeks	↑ absolute and relative liver weight, hepatocyte cytoplasmic alteration, hepatocyte hypertrophy, ↑ serum ALT and ALP	NOAEL: 300 ppm (27.7 mg/kg-day) for all liver endpoints	NTP (2018c)
Male Sprague Dawley rats (n=10/dose)	0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed for 16 weeks	↑ relative liver weight, liver necrosis, liver pigment, hepatocyte hypertrophy, hepatocyte cytoplasmic alteration, hepatocyte single cell death, ↑ serum ALT and ALP, ↑ bile salts	LOAEL: 150 ppm (14.7 mg/kg-day) for all liver endpoints	NTP (2018c)
Male Sprague Dawley rats (n=10/dose)	0, 20, 40, or 80 ppm (0, 1.8, 3.7, or 7.5 mg/kg-day) in feed for 16 weeks	↑ absolute and relative liver weight, liver necrosis, liver pigment, hepatocyte cytoplasmic alteration, hepatocyte hypertrophy, hepatocyte single cell death, ↑ serum ALT and ALP	LOAEL: 20 ppm (1.8 mg/kg-day) for ↑ liver weight, ↑ ALT and ALP, and hepatocyte necrosis, cytoplasmic alteration, and single cell death	NTP (2018c)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Female Sprague Dawley rats (n=50/dose)	0, 300, or 1,000 ppm (0, 18, or 63 mg/kg-day) in feed for 107 weeks	Liver necrosis, liver pigment, bile duct hyperplasia, hepatocyte cytoplasmic alteration, hepatocyte hypertrophy, hepatocyte single cell death, hepatocyte ↑ mitoses	LOAEL: 300 ppm (18 mg/kg-day) for hepatocyte cytoplasmic alteration and hepatocyte hypertrophy	NTP (2018c)
Male Sprague Dawley rats (n=50/dose)	0, 20, 40, or 80 ppm (0, 1, 2.2, or 4.5 mg/kg-day) in feed for 107 weeks	Liver cystic degeneration, liver eosinophilic and mixed cell focus, liver focal inflammation, liver necrosis, liver pigment, hepatocyte hypertrophy, hepatocyte cytoplasmic alteration, hepatocyte single cell death	LOAEL: 20 ppm (1 mg/kg-day) for liver necrosis, hepatocyte hypertrophy, and hepatocyte cytoplasmic alteration	NTP (2018c)

^a LOAEL/NOAEL not applicable for single dose studies.

^b Histology data are presented in the supplementary materials, but specific doses for which hepatocyte swelling and lipid deposits become significant are not provided.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GD, gestation day; i.p., intraperitoneal; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PND, postnatal day

Recently, the NTP released toxicity data from subacute (28 days) and chronic (16 or 107 weeks) bioassays for PFOA conducted in male and female rats. Animals were given PFOA in feed (concentrations are provided in Table 1). For the chronic studies, an additional cohort of animals was exposed to PFOA during gestation and lactation (perinatal exposure; 150 or 300 parts per million [ppm] for males and females). The toxicity data obtained from this additional cohort were examined to provide supportive evidence of toxicity (when compared with non-perinatally exposed animals), but were not evaluated specifically for NL development. Although the initial chronic study in male rats with concentrations of 0, 150, or 300 ppm (0, 14.7, or 29.5 milligrams per kilogram of bodyweight per day [mg/kg-day]) in feed was ended at 21 weeks due to overt toxicity, it appears a subset of animals receiving these doses were examined at 16 weeks, and the study was repeated with lower doses. Liver toxicity was observed in all of the studies, regardless of sex or duration. Common liver effects include increased weight, increased alanine aminotransferase (ALT) and alkaline phosphatase (ALP), necrosis, liver pigment, hepatocyte cytoplasmic alteration and hypertrophy, and hepatocyte single

cell death (NTP, 2018b,c). Liver toxicity lowest-observed-adverse-effect levels (LOAELs) of 0.625 mg/kg-day and 1 mg/kg-day for the 28-day and 107-week studies in male rats, respectively, were identified. This corresponds to plasma concentrations of 50.7 and 81.4 milligrams per liter (mg/L) respectively. Plasma/serum concentration is the most appropriate dose metric for extrapolating toxicity data from rodent studies to humans because of the large difference in the chemical's half-life between rodents (1-3 weeks) and humans (2-3 years). This accounts for the accumulation of PFOA in humans due to the chemical's long half-life. Plasma concentration in the chronic male rat study was determined at 16 weeks, but because the serum half-life of PFOA is estimated to be 4-6 days in male rats (New Jersey, 2017; Lau et al., 2006), it is anticipated that by 16 weeks, a steady-state concentration would have been reached. Thus, the plasma concentration would remain relatively stable over the 107-week period of continuous dosing.

Male Kunming mice administered 0, 1, or 5 mg/kg-day PFOA intragastrically for 21 days displayed increased absolute and relative liver weight, increased serum ALT and aspartate aminotransferase (AST), elevated hepatic triglycerides, decreased serum triglycerides, increased hepatic vacuoles, changes in serum cholesterol levels, and increased blood insulin (Wu et al., 2018). OEHHA identified a no-observed-adverse-effect level (NOAEL) of 1 mg/kg-day based on these effects.

Li et al. (2017) administered 0, 0.05, 0.5 or 2.5 mg/kg-day PFOA via oral gavage to male and female Balb/c mice for 28 days. The authors reported decreased body weight, increased absolute liver weight, hepatocellular hypertrophy and apoptosis, lipid accumulation in hepatocyte cytoplasm, changes to mitochondrial morphology and membrane potential, and oxidative DNA damage (increased 8-hydroxydeoxyguanosine formation) in the liver. Toxicity endpoint data and PFOA serum concentrations were quantified using GetData graph digitizer software (version 2.26), and are presented in Table 2. Female mice were more sensitive to apoptosis than male mice. The administered dose of 0.05 mg/kg-day corresponds to a serum concentration of approximately 1 microgram per milliliter ($\mu\text{g/ml}$) (for both sexes), which was measured at the end of the exposure period. OEHHA identified a LOAEL of 0.05 mg/kg-day (serum concentration of 1 $\mu\text{g/ml}$) for changes in mitochondrial membrane potential, increases in biomarkers of apoptosis (caspase-9 and p53), and increased oxidative DNA damage (Li et al., 2017).

Table 2. Dose metrics and endpoints in female mice from Li et al. (2017)

Administered dose (mg/kg-day)	Reported serum concentration (mg/L)	Cells with mitochondrial membrane potential changes (%)	Caspase-9 levels (iU/g)	p53 levels (iU/g)	8-OHdG (ng/g)
0	0	1.2 ± 0.5	71.3 ± 4.2	28.9 ± 3.5	22.9 ± 7.3
0.05	0.97	12.3 ± 1.2**	130.2 ± 9.0**	46.8 ± 5.1**	68.6 ± 6.2**
0.5	2.7	17.6 ± 1.1**	157.9 ± 3.5**	58.3 ± 4.5**	87.9 ± 9.3**
2.5	9.5	39.3 ± 14.6**	220.9 ± 1.1**	69.0 ± 3.2**	96.8 ± 2.6**

8-OHdG, 8-hydroxydeoxyguanosine; iU/g, international units/gram

**p<0.01, statistical analysis by OEHHA

Reduced body weight and increased absolute and/or relative liver weight were also reported in several other studies using Balb/c mice and Sprague Dawley rats with higher doses (ranging from 0 to 10 mg/kg-day) for 7-28 days (Du et al., 2018; Hui et al., 2017; Zheng et al., 2017; Yu et al., 2016; NTP, 2018b). Sprague Dawley rats also displayed hepatocyte hypertrophy and cytoplasmic alteration, changes in cholesterol and triglyceride levels, and increased serum ALT, AST, ALP, and bilirubin levels following oral exposure to PFOA for 28 days (NTP, 2018b).

Hepatotoxicity was also observed in frogs (Tang et al., 2018).

In vitro studies

Human liver HL-7702 cells treated with 0, 1, 2.5, or 7.5 micromoles/L (µM) PFOA had elevated levels of apoptosis and oxidative DNA damage (Li et al., 2017). Increased apoptosis was also observed in the mouse liver AML12 cell line (Wu et al., 2017). PFOA increased apoptosis, decreased mitochondrial membrane integrity, and increased anti-inflammatory interleukin 10 (IL-10) levels in rat and/or human organotypic multi-culture hepatocellular models (Orbach et al., 2018). Impaired proteolysis and autophagosome accumulation were observed in HepG2 cells treated with 0, 50, 100, or 200 µM PFOA (Yan et al., 2017). Liu et al. (2017) reported increased oxidative stress in primary rat hepatocytes treated with ≥6.25 µM PFOA.

Mechanistic studies

Mechanisms of hepatotoxicity have been previously reviewed (US EPA, 2016a; New Jersey DWQI, 2017). It has been established that PFOA can induce toxicity via activation of the nuclear receptor peroxisome proliferator-activated receptor alpha

(PPAR α). However, PPAR α activation does not explain all of the observed toxicity, and studies in PPAR α knockout mice clearly demonstrate PPAR α -independent toxicity (reviewed by US EPA, 2016a; New Jersey DWQI, 2017). Furthermore, there is evidence that PFOA activates other nuclear receptors, including constitutive androstane receptor (CAR), pregnane X receptor (PXR), and estrogen receptor alpha (ER α) (New Jersey DWQI, 2017). Recently, it was demonstrated that PFOA indirectly activates CAR, differently from the prototypical CAR activator phenobarbital (Abe et al., 2017).

Additional recent studies examining mechanisms of hepatotoxicity are briefly summarized here.

In mouse liver and human hepatocytes, PFOA administration decreased hepatocellular hepatocyte nuclear factor 4 alpha (HNF4 α), which has an important role in hepatocyte differentiation (Beggs et al., 2016). Additionally, PFOA increased levels of the anti-inflammatory cytokine interleukin-10 (IL-10) in human and rat organotypic cell culture models (Orbach et al., 2018).

Several studies have examined hepatic transcriptomic/proteomic changes in mice (Hui et al., 2017; Li et al., 2017; Abe et al., 2017; Zheng et al., 2017), and in mammalian liver cells (Beggs et al., 2016; Song et al., 2016; Zhang et al., 2016a; Liu et al., 2017; Yan et al., 2017) following PFOA administration. In general, PFOA exposure altered the levels of mRNA transcripts and/or proteins involved with apoptosis, lipid metabolism, cell proliferation, autophagy and vesicular trafficking, and the Krebs cycle. These data suggest that PFOA induces significant gene expression changes in the liver, and are supportive of the observed hepatotoxicity in animals.

Critical Study Selection

Li et al. (2017) generated a LOAEL of 0.05 mg/kg-day (administered dose) for changes in mitochondrial membrane potential, increases in biomarkers of apoptosis, and increased oxidative DNA damage in the liver of female mice. This LOAEL corresponds to a serum concentration of 0.97 mg/L, which is lower than the POD of 4.35 mg/L based on increased relative liver weight in male mice (Loveless et al., 2006) that formed the basis for the interim NL. Therefore, the Li et al. (2017) study is more appropriate than the Loveless et al. (2006) study as a critical study for POD derivation.

Liver Toxicity – PFOS

In vivo studies

PFOS exposure has consistently induced liver toxicity in experimental animals, and a thorough examination of the literature was previously conducted by other agencies (US EPA, 2016b; New Jersey DWQI, 2018). In general, increases in absolute and/or relative liver weight, increased liver histopathology, increased biomarkers of liver damage, and changes in liver lipid content were observed.

Several animal studies published after 2016 reported various hepatotoxic endpoints following oral exposure to PFOS. These studies are summarized in Table 3.

Table 3. Summary of recent animal toxicity studies of PFOS reporting liver effects

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant mice (strain not specified) (n=3-5/dose)	0, 1, 10, or 20 mg/kg-day orally from GD1 to GD14	fetal liver enlargement	Doses that caused effect were not specified	Mehri et al. (2016)
Male and female Cynomolgus monkeys (n=6/sex/dose)	0 or 14 mg/kg orally on three separate occasions over 422 days; maximum PFOS serum concentrations of 165 µg/mL for females and 160.8 µg/mL for males on day 365	no toxicologically significant effects reported	NOAEL: 165 µg/mL serum PFOS	Chang et al. (2017)
Male and female Sprague Dawley rats (n=12/sex/dose)	0 or 100 ppm in diet; equivalent to 6 mg/kg-day for males and 6.6 mg/kg-day for females	Both sexes: ↑ absolute and relative liver weight; hepatocellular hypertrophy Males: ↓ serum cholesterol and triglyceride levels; cytoplasmic vacuolization; ↑ lipid content Females: ↓ free fatty acids and triglycerides	NA ^a	Bagley et al. (2017)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male Sprague Dawley rats (n=6/dose)	0, 1, or 10 mg/kg-day orally for 4 weeks (males only)	hepatocellular hypertrophy; cytoplasmic vacuolization; ↑ serum ALT and AST; ↑ oxidative stress and apoptosis	LOAEL: 1 mg/kg-day for ↑ liver enzymes, oxidative stress, and apoptosis	Han et al. (2018a)
Male Sprague Dawley rats (n=6/dose)	0, 1, or 10 mg/kg-day orally for 4 weeks (males only)	↑ absolute liver weight; hepatocyte degeneration; cytoplasmic vacuolization; ↑ serum ALT and AST	NOAEL: 1 mg/kg-day for increased liver enzymes	Han et al. (2018b)
Male Sprague Dawley rats (n=7/dose)	0, 1, or 10 mg/kg-day orally for 4 weeks (males only)	↑ absolute and relative liver weight; hepatocellular hypertrophy; cytoplasmic vacuolization; ↑ serum ALT and AST; inflammatory cellular infiltration; ↑ apoptosis	LOAEL: 1 mg/kg-day for increased liver enzymes	Wan et al. (2016)
Male C57BL/6 mice (n=10/dose)	0, 2.5, 5, or 10 mg/kg-day via oral gavage for 30 days	↑ absolute liver weight; ↑ serum ALT and AST; hepatocyte vacuolization and necrosis; ↑ oxidative stress and apoptosis	LOAEL: 2.5 mg/kg-day for ↑ liver enzymes, oxidative stress, and apoptosis	Xing et al. (2016)
Male wild-type or ERβ knock-out mice (n=8/dose/group)	0 or 5 mg/kg-day via oral gavage for 28 days	Hepatocyte degeneration and vacuolization; ↓ hepatic cholesterol and bile acids	NA ^a	Xu et al. (2017)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male C57BL/6 mice (n=5-6/dose)	0, 0.003, 0.006, or 0.012% (0, 30, 60 or 120 mg/kg-day) in diet for 21 or 23 days	↑ relative liver weight; ↑ ALT, bile acids and triglycerides; hepatocyte vacuolization and necrosis; altered lipid metabolism	LOAEL: 30 mg/kg-day for increased liver weight and triglycerides	Zhang et al. (2016b)
Male and female Sprague Dawley rats (n=10/sex/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via oral gavage for males and females for 28 days	Hepatocyte hypertrophy, ↑ absolute/relative liver weight, ↓ cholesterol and triglycerides, ↑ ALT, ALP, bile salt/acid, albumin, and direct bilirubin; Males: ↑ AST, ↓ globulin, hepatocyte cytoplasmic vacuolization; Females: hepatocyte cytoplasmic alteration, ↑ total bilirubin	LOAEL: 0.312 mg/kg-day for ↑ relative liver weight in males and females	NTP (2018a)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ERβ, estrogen receptor beta; GD, gestation day; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level

Briefly, several recent studies in Sprague Dawley rats reported various hepatotoxic endpoints following 3-4 weeks of oral exposure to PFOS, including increased absolute and/or relative liver weight (Han et al. 2018b; Bagley et al., 2017; Wan et al. 2016; NTP, 2018a), increased serum ALT and AST (Han et al., 2018a; Wan et al., 2016; NTP, 2018a), altered cholesterol and triglyceride levels (Bagley et al., 2017; NTP, 2018a), hepatocellular hypertrophy (Han et al., 2018a; Bagley et al., 2017; Wan et al., 2016; NTP, 2018a), cytoplasmic vacuolization (Han et al., 2018a; Han et al., 2018b; Wan et al., 2016; NTP, 2018a), and hepatocyte degeneration/necrosis (Han et al., 2018b; Bagley et al., 2017). Additionally, increased levels of oxidative stress markers (Han et al., 2018a), apoptosis (Han et al., 2018a; Wan et al., 2016), and hepatic cell proliferation

(Han et al., 2018b) were observed. OEHHA identified a LOAEL of 0.312 mg/kg-day, based on increased relative liver weight in rats (NTP, 2018a).

Similar endpoints (decreased body weight, increased liver weight, increased ALT, AST, and bile acids, hepatocyte vacuolization and necrosis, and increased oxidative stress) were observed in mice given PFOS orally (doses from 2.5-120 mg/kg-day) for 3-4 weeks (Xing et al., 2016; Xu et al., 2017; Zhang et al., 2016b). OEHHA identified a LOAEL of 2.5 mg/kg-day for increases in liver enzymes, markers of oxidative stress, and apoptosis in C57BL/6 mice (Xing et al., 2016).

Choline supplementation reduced PFOS-induced hepatic oxidative stress and changes in lipid metabolism in male C57BL/6 mice (Zhang et al., 2016b), but had no impact on steatosis in Sprague Dawley rats (Bagley et al., 2017). Additionally, ER β (estrogen receptor beta) knockout mice did not show the hepatotoxic effects (hydropic degeneration and vacuolization of hepatocytes, decreased hepatic cholesterol and bile acid levels) that were present in wild-type mice (Xu et al., 2017). Furthermore, hepatocyte vacuolization, fatty degeneration, lipid accumulation, and ultrastructural changes in the liver were observed in zebrafish exposed to PFOS (Cheng et al., 2016; Cui et al., 2017).

In vitro studies

Cytotoxicity, oxidative stress, mitochondrial dysfunction, impaired proteolysis, autophagosome formation, and lysosomal membrane permeabilization were observed in HepG2 cells exposed to up to 200 μ M PFOS (Wan et al., 2016; Yao et al., 2016; Yan et al., 2017). Primary hepatocytes from Sprague Dawley rats (that were depleted of glutathione prior to PFOS exposure) showed increased oxidative stress, decreased mitochondrial membrane potential, lysosomal membrane damage, and proteolysis following exposure to PFOS (Khansari et al., 2017).

Mechanistic studies

Mechanisms of hepatotoxicity have been previously reviewed (US EPA, 2016b; New Jersey DWQI, 2018). It has been established that PFOS can induce hepatotoxicity via activation of the nuclear receptor PPAR α . However, PPAR α activation does not explain all of the observed hepatotoxicity. It has been suggested that PFOS may interact with other nuclear receptors, including CAR, PXR, PPAR β/δ , PPAR γ , HNF4 α , and ER α (New Jersey DWQI, 2018). Recently, it was shown that PFOS-induced liver toxicity also appears to act via ER β , as ER β knockout mice did not display the adverse effects (hepatocyte vacuolization, hydropic degeneration, changes in levels of cholesterol and bile acids) observed in wild-type mice (Xu et al., 2017). PFOS also increased expression of ER β in HepG2 cells (Xu et al., 2017). Beggs et al. (2016) demonstrated that PFOS decreased HNF4 α levels in mouse liver and human hepatocytes.

Additional recent studies examining mechanisms of hepatotoxicity are briefly summarized below.

PFOS induced autophagosome formation and lysosome membrane permeabilization in HepG2 cells (Yao et al., 2016). Spinster-1, a sphingolipid transporter involved in cell death, was implicated in toxicity, as knocking out this protein attenuated lysosome membrane permeabilization. PFOS also inhibited activation of protein kinase B in HepG2 cells, which could lead to changes in cell proliferation and apoptosis (Qiu et al., 2016b).

Several studies have examined hepatic transcriptomic/proteomic changes in rats (Dong et al., 2016; Wan et al., 2016; Han et al., 2018a; Han et al., 2018b), mice (Lai et al., 2017; Xu et al., 2017), zebrafish (Cheng et al., 2016; Fai Tse et al., 2016; Cui et al., 2017), mammalian liver cells (Han et al., 2018b; Wan et al., 2016; Beggs et al., 2016; Song et al., 2016), and chicken eggs (Jacobsen et al., 2018) following PFOS administration. In general, PFOS exposure altered the levels of mRNA transcripts and/or proteins involved with apoptosis, lipid metabolism, cell proliferation, necrosis, and carcinogenesis. The transcriptomic/proteomic evidence is indicative of hepatotoxicity and supports the animal toxicity data.

Critical Study Selection

The NOAELs/LOAELs (based on administered dose) determined from these recent PFOS studies showing liver toxicity are orders of magnitude higher than the NOAEL of 0.008 mg/kg-day (administered dose) for immunotoxicity from Dong et al. (2009) (discussed below in the PFOS immunotoxicity section), which was the basis for OEHHA's interim NL recommendation. Therefore, these studies are not considered for POD derivation in support of a final recommendation on the PFOS NL.

Immunotoxicity - PFOA

In a systematic review, NTP (2016) determined that PFOA is “presumed to be an immune hazard to humans” through suppression of antibody response as shown in animal and human studies. Assessments by US EPA (2016a), New Jersey Drinking Water Quality Institute (DWQI) (2017) and the US Agency for Toxic Substances and Disease Registry (ATSDR) (2018) have also described immune toxicity effects in humans and animals. Effects on spleen and thymus have been observed as well as the inability for the immune system to respond to a challenge.

In vivo studies

Since the publication of the above cited assessments, several recent studies reported similar effects on the immune system. These studies are summarized in Table 4.

Table 4. Summary of recent animal toxicity studies of PFOA reporting immune toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Female C57BL/6N PPAR α KO and WT mice (n=6/dose/group)	0, 7.5 or 30 mg/kg-day in drinking water for 15 days	↓ relative spleen and relative thymus weights in WT mice; ↓SRBC-specific IgM antibody responses in KO and WT mice.	LOAEL: 7.5 mg/kg-day for ↓ relative thymus weight in WT mice	Dewitt et al. (2016)
Female C57BL/6N WT mice (n=8/dose)	0, 0.94, 1,88, 3.75, or 7.5 mg/kg-day in drinking water for 15 days	↓ dinitrophenyl-ficoll (DNP)-specific IgM antibody response; ↓ relative spleen and thymus weight (high dose)	NOAEL: 0.94 mg/kg-day for ↓ antibody response	Dewitt et al. (2016)
Female C57BL/6N WT mice (n=4/dose/group)	0, 3.75 or 7.5 mg/kg-day in drinking water for 10, 13 or 15 days	Changes in splenic lymphocyte subpopulations	LOAEL: 3.75 mg/kg-day for changes in splenic lymphocyte subpopulations	Dewitt et al. (2016)
Male ICR mice (n=5/dose)	Treated mice were sensitized with OVA to induce active systemic anaphylaxis on day 0 and 7. OVA + 100 or 150 mg/kg 3 times on days 9 and 13 orally. Control mice had 150 mg/kg PFOA only or OVA only.	↓ rectal temperature; ↑ serum histamine, TNF- α , IgG1 and IgE levels	LOAEL: 100 mg/kg for ↑ TNF- α and IgE levels in sensitized mice	Lee et al. (2017)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
C57BL/6 mice (sex not specified) (n=4/group)	0 or 2 mg/kg via oral gavage for 25 days. Mice infected with <i>Citrobacter</i> at day 7.	↓ weight gain; ↓ in pathogen clearance at late stage infection; induction of IL-22 from ILC3 and Th17 cells; ↓ mucin	NA ^a	Suo et al. (2017)
Male Sprague Dawley rats (n=10/dose)	0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed for 16 weeks	↓ absolute and relative spleen weight; lymphoid follicle atrophy	LOAEL: 14.7 mg/kg-day for ↓ absolute and relative spleen weight	NTP (2018c)
Female Sprague Dawley rats (n=10/dose)	0, 300, or 1,000 ppm (0, 27.7, or 92.7 mg/kg-day) in feed for 16 weeks	Pigment in spleen	LOAEL: 27.6 mg/kg-day for pigment in spleen	NTP (2018c)

^a LOAEL/NOAEL not applicable for single dose studies.

GD, gestation day; IgE, immunoglobulin E; IgM, immunoglobulin M; IL-22, interleukin 22; KO, knockout; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; OVA, ovalbumin; PND, postnatal day; PPAR α , peroxisome proliferator-activated receptor alpha; SRBC, sheep red blood cells; TNF- α , tumor necrosis factor alpha; WT, wild-type

In vitro studies

Lee et al. (2016) investigated the effect of PFOA on mast cells and its association with allergic inflammation. Increased histamine and β -hexoaminidase release was observed in IgE-stimulated mast cells. The increased histamine release was the result of increased intracellular calcium induced by PFOA. Cytokine gene and protein expression were also increased. A decrease in IL-10 was also observed in PFOA-treated multicellular organotypic culture models of human or rat cells (Orbach et al., 2018).

Mechanistic studies

The database of studies on the mechanism for immune toxicity is limited. US EPA (2016a) and New Jersey DWQI (2017) suggested that the effects of PFOA on the immune system may have a mode of action that is both PPAR α -dependent and independent.

Lee et al. (2016) found that the mechanism for cytokine induction observed in PFOA-treated mast cells was the result of activation of nuclear factor kappa B (NF- κ B), a nuclear factor that helps regulate immune response in cells.

Critical Study Selection

The NOAELs/LOAELs (based on administered dose) determined from these recent immunotoxicity studies are substantially higher than the LOAEL of 0.05 mg/kg-day for liver toxicity from the Li et al. (2017) study, which is selected as a critical study for development of a noncancer RL. Therefore, these studies are not considered for POD derivation in support of a final recommendation on the PFOA NL.

Immunotoxicity – PFOS

A systematic review by NTP (2016) determined that PFOS is presumed to be an immune hazard to humans. The database of studies investigating the immune toxicity of PFOS is limited and has been reviewed in recent assessments by US EPA (2016a), New Jersey DWQI (2018) and ATSDR (2018). Effects on immune organs as well as immune suppression have been observed.

In vivo studies

OEHHA conducted a literature search to find additional studies published after the above-cited reviews and these recent studies are summarized in Table 5.

Table 5. Summary of recent animal toxicity studies of PFOS reporting immune toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male and female Sprague Dawley rats (n=10/sex/dose)	0, 0.312, 0.625, 1.25, 2.5 or 5 mg/kg-day for 28 days via oral gavage	Males: ↓white blood cells, ↓neutrophils, ↓ eosinophils, ↓ relative thymus weight Females: ↓ relative thymus weight at 1.25 mg/kg-day (not statistically significant at higher doses)	NOAEL: 2.5 mg/kg-day for all endpoints in males	NTP (2018a)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male ICR mice (n=5/dose)	Treated mice were sensitized with OVA to induce active systemic anaphylaxis on day 0 and 7. OVA + 50, 100 or 150 mg/kg, 3 times on days 9, 11 and 13 orally. Control mice had 150 mg/kg PFOS only or OVA only.	↓ rectal temperature; ↑ histamine, TNF- α , IgG and IgE levels in sensitized mice	LOAEL: 50 mg/kg for ↑TNF- α and IgE levels	Lee et al. (2018)
Male Sprague Dawley rats (n=6/dose)	0, 1, or 10 mg/kg-day orally for 4 weeks	↑ serum TNF- α and IL-6 levels	LOAEL: 1 mg/kg-day for ↑ serum TNF- α and IL-6 levels	Han et al. (2018b)

IgE, immunoglobulin E; IgG, immunoglobulin G; IL-6, interleukin 6; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; OVA, ovalbumin; TNF- α , tumor necrosis factor alpha

There are no new studies that are more sensitive than the Dong et al. (2009) study for derivation of the noncancer RL for PFOS. In this study, 10 adult male C57BL/6 mice per dose group were administered 0, 0.008, 0.083, 0.417, 0.833, or 2.08 mg/kg-day PFOS via oral gavage for 60 days. Significant toxicity endpoints include: decreased body, spleen, thymus, and kidney weights; increased liver weight; decreased splenic and thymic cellularity, and T cell CD4/CD8 subpopulations; altered natural killer cell activity; decreased splenic lymphocyte proliferation; and decreased sheep red blood cell (SRBC)-specific IgM plaque forming cell response. Serum concentrations were reported for each dose. A NOAEL of 0.008 mg/kg-day (serum concentration of 0.674 mg/L) was identified for decreased plaque-forming cell response.

In vitro studies

Han et al. (2018b) compared changes in tumor necrosis factor alpha (TNF- α) and interleukin 6 (IL-6) levels between Kupffer cells and hepatocytes treated with PFOS. Exposure to 100 μ M PFOS for 48 hours caused a transient but significant increase in TNF- α in Kupffer cells while levels remained unchanged in hepatocytes. Interleukin 7 (IL-7) was significantly elevated in Kupffer cells for the entire 48-hour exposure duration

while levels remained unchanged in hepatocytes. Blockage of TNF- α and IL-6 inhibited gadolinium chloride-induced hepatocyte proliferation. The authors suggest that cytokine expression in Kupffer cells is involved in hepatocyte proliferation through a NF- κ B/TNF- α /IL-6 dependent pathway. In a study in primary human decidual stromal cells, PFOS inhibited cortisone induced reduction of the inflammatory cytokines, IL-6 and interleukin 1 beta (IL-1 β) (Yang et al., 2016).

Mechanistic studies

In the risk assessment by New Jersey DWQI (2018), the authors summarized that the immunotoxicity of PFOS may be PPAR α mediated, or it may be due to lipid imbalance or be a stress response, but the specific mechanism remains unclear.

Han et al. (2018b) found that hepatocyte proliferation observed in PFOS treated mice was influenced by PFOS-induced cytokine expression in Kupffer cells, and occurred through the NF- κ B/TNF- α /IL-6 pathway. Blocking TNF- α and IL-6 inhibited hepatocyte proliferation.

Critical Study Selection

The recent immunotoxicity studies of PFOS are much less sensitive than the Dong et al. (2009) study, which was the basis for OEHHA's interim NL recommendation. Thus, these recent immunotoxicity studies are not considered as critical studies for POD derivation.

Thyroid Toxicity – PFOA

Thyroid effects have been reported in animals environmentally exposed to perfluoroalkyl and polyfluoroalkyl substances (PFAS). Levels of the thyroid hormone, T3 (triiodothyronine), were negatively associated with PFAS in polar bears and hooded seals (Bourgeon et al., 2017; Grønnestad et al., 2018).

Several recent mechanistic studies showed that PFOA, PFOS, and other medium-chain PFAS bind to the thyroxine transport protein transthyretin (Ren et al., 2016; Zhang et al., 2016b; Xin et al., 2018). Xin et al. (2018) also showed that PFOS can bind to thyroid hormone receptors.

NTP recently released subacute (28 days) and chronic (16 or 107 weeks) bioassays for PFOA conducted in male and female rats. Animals were given PFOA in feed (concentrations are provided in Table 6). For the chronic studies, an additional cohort of animals was exposed to PFOA during gestation and lactation (perinatal exposure). Although the initial chronic study in male rats with concentrations of 0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed was ended at 21 weeks due to overt toxicity, it appears a subset of animals receiving these doses were examined at 16 weeks, and

the study was repeated with lower doses. Results are summarized in Table 6. Thyroid follicular cell hypertrophy was observed in male and female rats in the 28-day studies, and in female rats in the 107-week study. Thyroid toxicity was not observed in female rats in the 16-week study and male rats in the 107-week study (NTP, 2018c). It should be noted, however, that male rats exposed perinatally in the 107-week study had higher incidences of thyroid follicular cell hypertrophy, although statistical significance was not reached ($p=0.087$, Fisher's exact test, done by NTP). OEHHA identified a LOAEL of 0.625 mg/kg-day (corresponding to a plasma concentrations of 50.7 and 0.49 mg/L in males and females, respectively) for changes in thyroid hormone levels in male and female rats for the 28-day studies, and a NOAEL of 14.7 mg/kg-day (plasma concentration of 193 mg/L) for thyroid follicular cell hypertrophy and changes in thyroid weight in male rats in the chronic studies.

Table 6. Thyroid toxicity from the NTP (2018b,c) subacute and chronic studies of PFOA in Sprague Dawley rats

Sex	Exposure	Endpoints	NOAEL/ LOAEL
Male (n=10/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day via oral gavage for 28 days	Thyroid follicular cell hypertrophy (trend), increased relative thyroid weight, decreased TSH, T3, fT4 and tT4	LOAEL: 0.625 mg/kg-day for changes in thyroid hormones
Female (n=10/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day via oral gavage for 28 days	Thyroid follicular cell hypertrophy, increased TSH, decreased fT4 and tT4	LOAEL: 0.625 mg/kg-day for increased TSH
Male (n=10/dose)	0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed for 16 weeks	Decreased relative and increased absolute thyroid weight, thyroid follicular cell hypertrophy	NOAEL: 14.7 mg/kg-day for all thyroid endpoints
Male (n=10/dose)	0, 20, 40, or 80 ppm (0, 1.8, 3.7, or 7.5 mg/kg-day) in feed for 16 weeks	Decreased absolute thyroid weight (not significant at the highest dose)	NOAEL: 1.8 mg/kg-day for decreased thyroid weight
Female (n=50/dose)	0, 300, or 1,000 ppm (0, 18, or 63 mg/kg-day) in feed for 107 weeks	Thyroid follicular cell hypertrophy	NOAEL: 18 mg/kg-day for thyroid follicular cell hypertrophy

LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; TSH, thyroid stimulating hormone; T3, triiodothyronine; fT4, free thyroxine; tT4, total thyroxine

PFOA was associated with hyperthyroidism in a case control study of 72 cats (Bost et al., 2016).

Critical Study Selection

Thyroid toxicity observed in the NTP (2018b,c) subacute and chronic studies of PFOA is not considered for POD derivation because this endpoint is much less sensitive than the hepatotoxicity endpoints reported by Li et al. (2017).

Thyroid Toxicity – PFOS

NTP (2018a) conducted subacute studies in male and female rats with PFOS. Animals were given 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day PFOS via oral gavage for 28 days. Decreases in T3, fT4 (free thyroxine), and tT4 (total thyroxine) were observed in both sexes, while decreased absolute thyroid weight was reported in males only (NTP, 2018). A LOAEL of 0.312 mg/kg-day (corresponding to plasma concentrations of 23.7 and 30.5 mg/L for males and females, respectively) was identified based on decreases in fT4 and tT4 in both sexes.

A recent study in male and female cynomolgus monkeys given 14 mg/kg PFOS via oral gavage on three separate occasions over an observation period of 422 days showed a slight reduction in serum tT4 in both sexes (Chang et al., 2017b). There were no significant changes in TSH or fT4. The authors did not consider the reduction in tT4 to be toxicologically relevant because a sufficient reservoir of inactive (bound to protein) T4 remained available to maintain thyroid hormone homeostasis.

Critical Study Selection

Thyroid toxicity observed in the subacute NTP (2018a) studies is not considered for POD derivation because this endpoint is much less sensitive than the immunotoxicity reported by Dong et al. (2009).

Reproductive Toxicity – PFOA

In vivo studies

Reproductive effects of PFOA in animals were described in recent assessments by US EPA (2016a), New Jersey DWQI (New Jersey DWQI, 2017) and ATSDR (2018). Additionally, in 2017, PFOA was listed under Proposition 65 as a chemical known to the state of California to cause reproductive toxicity. Subchronic studies in mice showed reproductive toxicity effects such as decreased litter size, increased litter resorptions, and increased fetal death. Male mice exposed to PFOA had decreased testis weight, decreased sperm count and testicular damage. A two-generational study in rats showed no reproductive toxicity.

Studies of PFOA exposure reporting reproductive toxicity effects published after 2016 are summarized in Table 7.

Table 7. Summary of recent animal toxicity studies of PFOA reporting reproductive toxicity

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male Kunming mice (n=6/dose)	0, 2.5, 5, or 10 mg/kg-day for 14 days orally	<p>↓ absolute testis weight (high dose); ↓ sperm count (all doses); morphological changes in seminiferous tubules; ↓ SOD levels, CAT activity in testes (all doses); ↓ MDA and H₂O₂ levels in the testis (mid and high dose)</p>	<p>LOAEL: 2.5 mg/kg-day for ↓ sperm count</p>	<p>Liu et al. (2015)</p>
Male BALB/C mice (n=11/dose)	0, 1.25, 5, or 20 mg/kg-day via oral gavage for 28 days	<p>↓ triglyceride and cholesterol in epididymis (mid and high dose); ↓ in relative epididymis weight (low and high dose); changes in expression of genes and proteins related to triglyceride, cholesterol and fatty acid metabolism in the epididymis; changes in fatty acid composition in epididymis; ↑ MDA levels in epididymis (low and mid dose) ↓ GSH-Px levels in epididymis (mid and high dose)</p>	<p>LOAEL: 1.25 mg/kg-day for ↓ in relative epididymis weight</p>	<p>Lu et al. (2016)</p>

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant Kunming mice (n=10/dose), male offspring evaluated for effects on PND 21 and 70	0,1 ,2.5, or 5 mg/kg-day via oral gavage from GD 1-17	Pups: ↓ number of surviving mice at weaning (high dose); ↑ absolute testis weight (high dose) on PND 21, ↓ absolute testis weight (low dose) on PND 70; ↑ testosterone (low dose) on PND 70, ↓ testosterone in testis (all doses) on PND 21 and (mid and high dose) on PND 70; ↓ Leydig cells (mid and high dose, PND 21 and 70); vacuolization of Sertoli cells and ↓ spermatozoa at high dose	LOAEL: 1 mg/kg-day for ↑ testosterone, ↓ in absolute testis weight	Song et al. (2018)
Pregnant Kunming mice (n=12/dose)	0, 2.5, 5, or 10 mg/kg-day via oral gavage from GD 1 to GD 7 or 13	Dams: ↑ number of resorbed embryos on GD 13 (high dose); ↑ serum estradiol on GD 7 (high dose); ↓ serum progesterone on GD 13 (mid and high dose); ↓ number of corpora lutea on GD 7 (low and mid dose); ↓ number of corpora lutea on GD 13 (mid and high dose); ↓ ratio of corpora lutea to ovarian areas on GD 7 and 13; ↑ CAT and SOD activity, H ₂ O ₂ , and MDA levels in ovary; ↑ apoptosis protein markers (p53 and Bax) in ovary	LOAEL: 2.5 mg/kg-day for oxidative stress, apoptosis markers and ↓ in number of corpora lutea	Chen et al. (2017b)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male BALB/c mice (n=15/dose)	0, 1.25, 5 or 20 mg/kg-day via oral gavage for 28 days	<p>↑ CBG protein levels in testes (low and mid dose);</p> <p>↓ CBG protein levels in testis (high dose);</p> <p>↑ CBG (all doses) and corticosterone levels (mid and high dose)</p> <p>↓ adrenocorticotrophic hormone serum levels (ACTH) (high dose)</p>	<p>LOAEL: 1.25 mg/kg-day for ↑ CBG levels in testis and serum</p>	Sun et al. (2018)
Pregnant C57BL/6J mice (n=6/dose for dams, 9/dose for pups)	Dietary exposure to 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, or 3 mg/kg-day (targeted concentration). Exposure started 2 weeks before mating and continued during mating (1 week), gestation (3 weeks), and lactation (3 weeks). Pups organs evaluated at 26 weeks (males) or 28 weeks (females).	<p>Dams: ↓ litter size at two highest doses</p> <p>Pups (both sexes): ↓ body weight at PND 4; hepatocellular anisokary-osis and karyomegaly</p> <p>Male pups: ↑ absolute and relative liver weight; ↑ eosinophilic liver foci; lipid accumulation in liver</p> <p>Female pups: ↓ triglycerides and cholesterol</p>	<p>Dams: NOAEL: 0.3 mg/kg-day for ↓ litter size</p> <p>Pups: NOAEL: 0.003 mg/kg-day for ↓ body weight in females on PND 4</p>	van Esterik et al. (2016)
Male and female Sprague Dawley rats (n=10/sex/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day for 28 days via oral gavage	<p>Males: ↑ relative testis weight, ↓ absolute epididymis weight, ↓ cauda epididymis weight, ↓ cauda epididymis sperm count</p>	<p>NOAEL: 2.5 mg/kg-day for ↑ relative testis weight and ↓ cauda epididymis weight</p>	NTP (2018b)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male Sprague Dawley rats (n=10/dose)	0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed for 16 weeks	↓ absolute testis weight	NOAEL: 14.7 mg/kg-day for ↓ absolute testis weight	NTP (2018c)
Female Sprague Dawley rats (n=10/dose)	0, 300, or 1,000 ppm (0, 27.7, or 92.7 mg/kg-day) in feed for 16 weeks	Ovarian cysts	NOAEL: 27.7 mg/kg-day for ovarian cysts	NTP (2018c)
Female Sprague Dawley rats (n=50/dose)	0, 300, or 1,000 ppm (0, 18, or 63 mg/kg-day) in feed for 107 weeks	Squamous metaplasia in the endometrium	LOAEL: 18 mg/kg-day for endometrial squamous metaplasia	NTP (2018c)

ACTH, adrenocorticotrophic hormone; CAT, catalase; CBG, corticosteroid binding globulin; GD, gestation day; GSH-Px, glutathione peroxidase; H₂O₂, hydrogen peroxide; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; PND, postnatal day; SOD, superoxide dismutase

As seen in studies cited in previous risk assessments by other agencies, a number of studies in mice reported reproductive toxicity following exposure to PFOA for 1-4 weeks. In male mice, studies reported decreased testis and epididymis weights and sperm count (Lu et al., 2016; Liu et al., 2015; Song et al., 2018). In females, studies reported decreases in litter size, changes in estrous cycle and changes in hormone levels (van Esterik et al, 2016; Chen et al; 2017b). A study by NTP (2018b) was the only study found in rats. Decreased absolute cauda epididymis weight and sperm count and increased relative testis weight were observed after a 28-day gavage study. In a 16-week oral gavage study, decreased absolute testis weight was observed in male rats (NTP, 2018c).

van Esterik et al. (2016) administered 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, or 3 mg/kg-day PFOA in the diet to 6 pregnant C57BL/6J mice per dose group. Exposure started 2 weeks before mating and continued through mating (1 week), gestation (3 weeks), and lactation (3 weeks). Toxicity in the F₁ generation was monitored in 6-9 pups from 2-3 litters in each dose group. Decreased litter sizes were reported at the two highest doses. Additionally, several developmental disorders were reported in pups, including the following: increased liver weight, increased eosinophilic liver foci and lipid

accumulation in liver in males; decreased femur length and femur weight, decreased quadriceps femoris muscle weight, decreased adipocyte cell size, and decreased serum triglycerides and cholesterol in females; and decreased body weight at postnatal day (PND) 4, decreased tibia length, and hepatocellular anisokaryosis and karyomegaly in pups of both sexes. OEHHA determined a NOAEL of 0.003 mg/kg-day based on decreased body weight in female pups on PND 4 ($p < 0.001$; student's T-test determined by OEHHA).

In vitro studies

A number of in vitro studies investigated the effects of PFOA treatment on Leydig cells. Mouse Leydig tumor cell lines showed an increase in gene and protein expression of cortisol binding protein (CBG) (Sun et al., 2018). Decreased mitochondrial membrane potential and increases in reactive oxygen species (ROS) were observed at PFOA concentrations of 50 μM and greater in mouse Leydig tumor cells (Zhao et al., 2017).

PFOA did not induce cell death or ROS production in male human embryonic stem cells; however, cells showed a decrease in spermatid production (Steves et al., 2018). Cytotoxicity was observed at concentrations greater than 250 μM PFOA in the human cell lines HEK293T, MCF-7, LNCaP, and H295R while no cytotoxicity was observed in MDA-kb2 cells at concentrations up to 500 μM (Behr et al., 2018). In the same study, a concentration of 100 μM PFOA co-incubated with estradiol (E2) increased ER β activity in HEK293T cells. An increase in the production of estrone was measured in H295R cells treated with 100 μM PFOA.

Mechanistic studies

An earlier assessment by New Jersey DWQI (2017) reviewed possible mechanisms for reproductive toxicity, specifically in male mice. Possible modes of action described were PPAR α activation and disruption of the blood-testis barrier leading to oxidative stress and estrogenic effects of PFOA. A recent study by Zhao et al. (2017) showed impairment of mitochondrial function and increase in ROS in mouse Leydig tumor cells.

Mice treated with 5 mg/kg-day PFOA for 28 days showed alterations in gene and protein expression related to endocytosis and the blood-testis barrier that are supportive of studies showing toxicity in the male testis (Lu et al., 2017).

A possible mechanism for male reproductive toxicity involves changes in cholesterol and fatty acid metabolism. Lu et al. (2016) showed PFOA activated Akt/AMPK signaling, a pathway that regulates lipid metabolism.

Critical Study Selection

van Esterik et al. (2016) generated a NOAEL of 0.003 mg/kg-day based on decreased body weight in female pups on PND 4. This NOAEL is two orders of magnitude lower than the NOAEL of 0.3 mg/kg-day from Loveless et al. (2006), which is associated with increased relative liver weight in male mice and was the basis for the interim NL. Although the van Esterik et al. (2016) study may provide a lower POD than the Loveless et al. (2006) study, the most appropriate dose metric, serum levels of PFOA, is not available for RL derivation.

Reproductive Toxicity – PFOS

In vivo studies

Reproductive effects of PFOS were described in recent assessments by US EPA (2016b), New Jersey DWQI (2018) and ATSDR (2018). Additionally, in 2017, PFOS was listed under Proposition 65 as a chemical known to the state of California to cause reproductive toxicity. More recent studies identified effects such as decreases in testis and/or epididymis weights, decreases in sperm count, increases in apoptosis and apoptosis markers in the ovary or testis, decreases in litter size, changes in hormone levels, and changes in estrous cycle. These studies are summarized in Table 8.

Table 8. Summary of recent animal toxicity studies of PFOS reporting reproductive toxicity

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Female ICR mice (n=136/dose)	0 or 0.1 mg/kg-day in drinking water for 4 months ^a	Prolongation of estrous cycle; ↓ estrous cycles per month; increase in atretic follicles; ↓ number of corpora luteum; changes in hormone levels at each estrous cycle	NA	Feng et al. (2015)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Pregnant Sprague Dawley rats (n=4/dose)	0, 5, or 20 mg/kg-day via oral gavage from GD 11-19	Dams: ↓ body weight, serum cholesterol levels Pups: ↓ body weight, body length, absolute testis weight, anogenital distance of male pups, testosterone in testis, Leydig cell number, testosterone biosynthetic enzyme levels, HDL levels in liver and testis; apoptosis in fetal Leydig cells	NOAEL: 5 mg/kg-day for all pup endpoints	Zhao et al. (2014)
Male ICR mice (n=20/dose)	0, 0.25, 2.5, 25, or 50 mg/kg-day via oral gavage for 28 days	↓ sperm count, ↑ Sertoli cell vacuolization and derangement of cells layers; damage in blood-testis barrier between Sertoli cells	NOAEL: 0.25 mg/kg-day for all endpoints	Qiu et al. (2013)
Female ICR mice (n=20/dose)	0 or 10 mg/kg-day orally for 30 days	Prolongation of duration of diestrus; ↓ number of corpora lutea; ↓ serum levels of P4, LH and GnRH on day 7, ↓ serum levels of GnRH, E2, T4 and T3 on day 14; ↑ serum levels of CORT on day 14	NA	Wang et al. (2018)
Male C57 mice (n=12/dose)	0, 0.5 or 10 mg/kg-day via oral gavage for 5 weeks	↓ body weight, absolute and relative testis weight, sperm count, serum testosterone levels; vacuolization in spermatogonia, spermatocytes and Leydig cells; ↑ apoptotic cells in testes, apoptosis related proteins, ↑ ERα and ERβ protein expression	LOAEL: 0.5 mg/kg-day for ↑ ERβ protein expression	Qu et al. (2016)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male ICR mice (n=10/dose)	0, 0.5, 5 or 10 mg/kg-day via oral gavage for 4 weeks	↓ sperm count	NOAEL: 0.5 mg/kg-day for ↓ sperm count	Qiu et al. (2016a)
Male and female Sprague Dawley Rats (n=10/sex/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day for 28 days via oral gavage	Females: ↑ testosterone	NOAEL: 0.625 mg/kg-day for ↑ testosterone	NTP (2018a)

^a Animals were exposed up to 6 months. Due to a significant decrease in body weight of PFOS exposed animals at 6 months, only animals treated for 4 months were used for subsequent endpoints. It is unclear how many animals were exposed to PFOS for 4 months or 6 months.
 CORT, corticosterone; ER α , β , estrogen receptor α , β ; E2, estradiol; GnRH, gonadotrophin-releasing hormone; HDL, high density lipoprotein; LH, luteinizing hormone; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; P4, progesterone; T3, triiodothyronine; T4, thyroxine

In vitro studies

Studies in Sertoli cells isolated from mice, rats and humans reported effects such as perturbation of tight junction proteins resulting in inhibition of the tight junction permeability barrier (Li et al., 2016; Qiu et al., 2016a; Chen et al., 2017a; Gao et al., 2017). Decreases in mitochondrial membrane potential and increases in ROS generation were observed at PFOS concentrations of 50 μ M and greater in mouse Leydig tumor cells (Zhao et al., 2017).

Cytotoxicity was observed at concentrations greater than 250 μ M in human HEK293T, MCF-7, LNCaP, H295R and MDA-kb2 cell lines (Behr et al., 2018). In the same study, concentrations greater than 50 μ M PFOS co-incubated with E2 increased ER β activity in HEK293T cells. In MDA-kb2 cells, PFOS co-incubated with dihydrotestosterone (DHT) increased androgen receptor (AR) activity. An increase in production of estrone and progesterone was measured in H295R cells treated with 100 μ M PFOS. In porcine ovarian theca and granulosa cells, PFOS at 1.2 μ M caused a decrease in basal secretion of progesterone, androstenedione and estradiol (Chaparro-Ortega et al., 2018).

Mechanistic studies

An earlier assessment by US EPA (2016b) reviewed possible mechanisms for reproductive toxicity. Male reproductive toxicity may be caused by disruptions in gap junction intercellular communication by PFOS, compromising the blood-testis barrier in Sertoli cells. Recent in vitro studies assessed effects of PFOS on the blood-testis barrier and showed that PFOS perturbs tight junction proteins and function, and causes

microfilament disruption resulting in Sertoli cell injury (Gao et al., 2017; Li et al., 2016; Qiu et al., 2016a; Chen et al., 2017a).

Gene and protein expression were analyzed in the testes of rats exposed to PFOS for 28 days (Lopez-Doval et al., 2016). These investigators showed that PFOS inhibits the expression of follicle stimulating hormone receptor and AR, while inducing the expression of gonadotropin-releasing hormone receptor and luteinizing hormone receptor.

PFOS has been suggested to interact with estrogen receptors. Qu et al. (2016) found that PFOS altered expression of ER α and ER β in mouse testis after exposure to at least 0.5 mg/kg-day for 5 weeks. ER α -induced anteroventral periventricular nucleus (AVPV)-kisspeptin neuron activation was suppressed by PFOS, causing alterations in the estrous cycle in female ICR mice (Wang et al., 2018).

Critical Study Selection

The NOAELs/LOAELs (based on administered dose) determined from these recent studies reporting reproductive effects are orders of magnitude larger than the NOAEL of 0.008 mg/kg-day (administered dose) for immunotoxicity from Dong et al. (2009), which was the basis for OEHHA’s interim NL recommendation. Therefore, these studies are not considered for POD derivation in support of a final recommendation on the PFOS NL.

Cancer – PFOA

Cancer bioassays in laboratory animals conducted prior to 2016 have been thoroughly described previously (US EPA, 2016; New Jersey, 2018; IARC, 2017). These studies are briefly described below, and significant cancer incidences are reported in Table 9.

Table 9. Significant tumor incidences following exposure to PFOA

Sex/Species	Exposure	Tumor type	Dose (mg/kg-day)	Incidence	Reference
Male Sprague Dawley rats (n=50/dose)	Oral in diet for 106 weeks	Leydig Cell Adenoma	0, 1.3, or 14.2	0/33, 2/36, 7/44*	Butenhoff et al. (2012b), data from Sibinsky (1987)
Male Sprague Dawley rats (n=76-79/dose)	Oral in diet for 104 weeks	Hepatocellular adenoma or carcinoma	0 ^a or 13.6	3/79, 10/76*	Biegel et al. (2001)

Sex/Species	Exposure	Tumor type	Dose (mg/kg-day)	Incidence	Reference
Male Sprague Dawley rats (n=76-79/dose)	Oral in diet for 104 weeks	Pancreatic acinar cell adenoma or carcinoma	0 ^a or 13.6	1/79, 8/76*	Biegel et al. (2001)
Male Sprague Dawley rats (n=76-79/dose)	Oral in diet for 104 weeks	Leydig cell adenoma	0 ^a or 13.6	2/78, 8/76*	Biegel et al. (2001)
Pregnant CD-1 mice (n=6-14 dams/dose or 21-37 female pups/dose)	Oral in drinking water from GD 1 to GD 17, pups followed for 18 months	Hepatocellular adenoma	0, 0.01, 0.1, 0.3, 1, or 5	0/29, 1/29, 1/37, 4/26*, 0/31, 1/21	Filgo et al. (2015)

^a Pair-fed control, fed same amount of food as treated group

*p<0.05, pairwise comparison with Fisher's exact test, statistical analysis by OEHHA

GD, gestation day

Sibinsky (1987), as reported by Butenhoff et al. (2012), administered 0, 30, or 300 ppm PFOA to rats (0, 1.3, or 14.2 mg/kg-day for males; 0, 1.6 or 16.1 mg/kg-day for females) in the diet for 105-106 weeks. In male animals, a significant increase in Leydig cell adenomas and preneoplastic pancreatic acinar cell hyperplasia was observed in the high dose group. In females, a significant increase in preneoplastic ovarian tubular hyperplasia was observed (later reclassified as gonadal stromal hyperplasia in a pathology review by Mann and Frame, 2004). An increase in mammary gland fibroadenoma was initially reported, but a follow-up examination of the pathology revealed no significant increase over controls (Hardisty et al., 2010).

Biegel et al. (2001) administered 0 or 300 ppm (0 or 13.6 mg/kg-day) to male rats in the diet for 24 months. A significant increase in several tumor types was reported, including hepatocellular adenomas or carcinomas, pancreatic acinar cell adenomas or carcinomas, and Leydig cell adenomas. Additionally, preneoplastic pancreatic acinar cell hyperplasia and Leydig cell hyperplasia were increased.

Filgo et al. (2015) exposed three different strains of pregnant mice (CD-1, 129/SV wild-type, and 129-SV PPAR α knock-out) to doses of PFOA in drinking water ranging from 0 to 5 mg/kg-day from gestation day (GD) 1 to GD 17. Offspring were observed for 18

months. A significant increase in hepatocellular adenomas, and a significant trend for hepatic hemangiosarcomas, were observed in the CD-1 F₁ generation. Liver tumors were not significantly increased in wild-type or knock-out 129/SV mice. It should be noted that the liver was the only organ evaluated in these studies.

Recently, NTP (2018c) released carcinogenicity data from chronic bioassays of PFOA in male and female rats (see Liver Toxicity section for study details). Significant increases in hepatocellular adenomas/carcinomas and pancreatic acinar cell adenomas/carcinomas were observed in male rats, shown in Table 10. Female rats had an increase in uterine adenomas/carcinomas, shown in Table 11. Animals that died before the first observed tumor incidence were not included in the dose-response analysis. Furthermore, carcinogenicity data from animals exposed perinatally were not considered for derivation of the RL because the tumor incidences in these animals were comparable to animals that were not exposed perinatally, suggesting that exposure during gestation and lactation had minimal impact on tumor development later in life.

Table 10. Hepatocellular and pancreatic tumor incidences in male rats exposed to PFOA in the diet for 107 weeks (NTP, 2018c)

Conc. (ppm)	Dose (mg/kg-day)	Plasma conc. (mg/L)	Human Equivalent Dose (mg/kg-day)	Hepatocellular adenoma or carcinoma	Pancreatic acinar cell adenoma or carcinoma
0	0	BD ^a	0	0/36 ^b	3/43
20	1.0	81.4	0.011	0/42	29/49***
40	2.3	131	0.018	7/35**	26/41***
80	4.8	160	0.022	11/37***	32/40***

^a BD = below the limit of detection. Values were considered 0 for the dose-response analysis

^b Incidence/effective number of animals

p<0.01, *p<0.001, pairwise comparison with Fisher's exact test, statistical analysis by OEHHA

Table 11. Uterine tumor incidences in female rats exposed to PFOA in the diet for 107 weeks (NTP, 2018c)

Concentration (ppm)	Dose (mg/kg-day)	Plasma Concentration (mg/L)	Uterine adenoma or carcinoma
0	0	BD ^a	2/32
300	18	20.4	5/40
100	63	72.3	8/35

^a BD = below the limit of detection. Values were considered 0 for the dose-response analysis

^b Incidence/effective number of animals

#p<0.05 for trend test, statistical analysis by OEHHA

Plasma concentrations in the chronic male rat study were determined at 16 weeks, but because the serum half-life of PFOA is estimated to be 4-6 days in male rats, it is

anticipated that by 16 weeks, a steady-state concentration would have been reached. Therefore, the plasma concentration would remain relatively stable over the 107-week period of continuous dosing. Plasma concentrations are converted to human equivalent doses (HEDs) using the human clearance factor of 1.4×10^{-4} L/kg-day for PFOA, determined by US EPA (2016a). The formula is shown below:

$$\text{Serum concentration (mg/L)} \times \text{clearance factor (L/kg-day)} = \text{HED (mg/kg-day)}$$

The resulting HEDs are presented in Table 10. Hepatic adenomas/carcinomas and pancreatic acinar cell adenomas/carcinomas are critically evaluated for RL development.

Cancer – PFOS

Summaries of the sole report of carcinogenicity bioassays (Butenhoff et al., 2012a) for PFOS have been previously published (US EPA, 2016b; New Jersey DWQI, 2018). The study design and significant results are briefly described below.

Butenhoff et al. (2012a) published a report of carcinogenicity studies from 2002 by 3M, a former PFOS manufacturer (Thomford, 2002). In these studies, male and female Sprague Dawley rats were administered 0, 0.5, 2, 5, or 20 ppm PFOS (0, 0.024, 0.098, 0.242, or 0.984 mg/kg-day for males; 0, 0.029, 0.120, 0.299, or 1.251 mg/kg-day for females) in the diet for two years. An additional group was administered 20 ppm for one year, and then control diet for the next year (data not shown). An increase in hepatocellular adenoma incidence was observed in both male and female animals at the highest dose. Combined hepatocellular adenoma/carcinoma incidence was also increased in female rats. Positive trends for hepatocellular adenomas were reported in both sexes. Tumor incidence data are summarized in Tables 12 and 13. It should be noted that the relatively low effective number of female rats was not due to high levels of premature mortality (mortality in treated groups was comparable to controls), but due to the fact that the first incidence of hepatocellular adenoma/carcinoma appeared quite late in the bioassay (day 653).

Table 12. Hepatocellular tumor incidences in male rats exposed to PFOS in the diet for 2 years (Butenhoff et al., 2012a)

Conc. (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L) ^a	Human equivalent dose (mg/kg-day)	Hepatocellular adenoma
0	0	0.014	1.2×10^{-6}	0/41 ^b
0.5	0.024	2.64	2.1×10^{-4}	3/42
2.5	0.098	12.1	9.8×10^{-4}	3/47
5	0.242	32.3	2.6×10^{-3}	1/44
20	0.984	121	9.8×10^{-3}	7/43*

^a Calculated by OEHHA

^b Incidence/effective number of animals. Animals that died before the first tumor incidence were not considered in the dose-response assessment.

*p<0.05, reported by study authors

Table 13. Hepatocellular tumor incidences in female rats exposed to PFOS in the diet for 2 years (Butenhoff et al., 2012a)

Conc. (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L) ^a	Human equivalent dose (mg/kg-day)	Hepatocellular adenoma or carcinoma
0	0	0.841	6.8×10^{-5}	0/28 ^b
0.5	0.029	5.49	4.5×10^{-4}	1/29
2.5	0.120	23.0	1.9×10^{-3}	1/16
5	0.299	66.4	5.4×10^{-3}	1/31
20	1.251	215	1.7×10^{-2}	6/32*

^a Calculated by OEHHA

^b Incidence/effective number of animals. Animals that died before the first tumor incidence were not considered in the dose-response assessment.

*p<0.05, reported by study authors

Because the biological half-life of PFOS differs greatly between rats (9-10 weeks) and humans (4-5 years), administered dose is not the appropriate dose metric for toxicity assessment. Serum PFOS concentration is a more suitable dose metric for extrapolating toxicity in rodents to toxicity in humans. Serum concentrations at various time points were measured, and the results are reported in Table 14.

Table 14. Mean serum PFOS concentrations (in mg/L) in rats from Butenhoff et al. (2012)

Week	Sex	0 ppm	0.5 ppm	2.5 ppm	5 ppm	20 ppm
4	Male	0	0.907	4.33	7.57	41.8
4	Female	0.026	1.61	6.62	12.6	54
14	Male	0	4.04	17.1	43.9	148

Week	Sex	0 ppm	0.5 ppm	2.5 ppm	5 ppm	20 ppm
14	Female	2.67	6.96	27.3	64.4	223
53	Male	0.025	-	-	-	146
53	Female	0.395	-	-	-	220
103	Male	-	-	-	-	-
103	Female	-	-	20.2	-	-
105	Male	0.012	1.31	7.6	22.5	69.3
105	Female	0.084	4.35	-	75	233

A dash (-) indicates that data were not collected at that time point

In males, the maximum serum concentration was reached at 14 weeks. At 105 weeks, serum concentrations in all dose groups were typically 2- to 3-fold less than at 14 weeks, indicating that PFOS is more rapidly eliminated at later time points. The authors attributed this increased urinary elimination of PFOS to chronic progressive nephritis. In females, serum concentrations measured at 105 weeks were typically comparable to the values measured at 14 weeks. Because serum concentrations in males declined after 14 weeks, the PFOS serum concentration values at terminal sacrifice would underestimate the serum concentrations at earlier time points. Therefore, OEHHA calculated the area under the curve (AUC) for each dose group using a simple linear interpolation. The time-weighted average serum concentration at each dose is determined by dividing the AUC by the duration of the study (103 or 105 weeks). These time-weighted average serum concentrations are presented in Tables 12 and 13.

Serum concentrations in rats are converted to HEDs using the human clearance factor of 8.1×10^{-5} L/kg-day for PFOS, determined by US EPA (US EPA, 2016). These values are presented in Tables 12 and 13, and the conversion formula is shown below:

$$\text{Serum concentration (mg/L)} \times \text{clearance factor (L/kg-day)} = \text{HED (mg/kg-day)}$$

There is sufficient evidence to consider and critically evaluate the liver tumors in male and female rats for RL development. First, the two chronic bioassays reported in Butenhoff et al. (2012) are of sufficient quality (appropriate length, suitable number of animals per dose, adequate reporting, etc.) to warrant consideration as critical studies. Second, recent studies of PFOA by NTP (2018c) provide additional support for considering carcinogenicity as a critical endpoint for PFOS. In their assessment, NTP (2018c) showed that chronic exposure to PFOA led to a significant increase in hepatocellular adenomas and/or carcinomas in male rats (data presented in Table 10), which is similar to the carcinogenic effects of PFOS reported by Butenhoff et al. (2012). The similarity in molecular structure between PFOS and PFOA suggests that these two chemicals may have comparable biological activities, and in fact, the noncancer toxicology profiles of these two chemicals are similar. The International Agency for Research on Cancer (IARC, 2017) designated PFOA possibly carcinogenic to humans (Group 2B). PFOS also produced a positive trend for pancreatic carcinomas in male

rats (data not shown), which is a critical tumor type in PFOA-exposed male rats in the NTP (2018c) bioassay (Table 10). It should be noted that the highest administered dose in the Butenhoff et al. (2012) PFOS bioassay (0.984 mg/kg-day) was essentially the same as the lowest administered dose in the NTP (2018c) PFOA bioassay (1.0 mg/kg-day). This suggests that the Butenhoff et al. (2012) studies are less sensitive than the NTP (2018c) studies, and that the modest, but significant, tumor incidences observed (when compared against the NTP (2018c) PFOA data) are the result of overall lower administered doses. Third, although there is minimal evidence to indicate PFOS is genotoxic or mutagenic (US EPA, 2016b; New Jersey DWQI, 2018), increases in hepatic oxidative stress (Xing et al., 2016; Han et al., 2018a), hepatocellular hypertrophy (Han et al., 2018a; Bagley et al., 2017; Wan et al., 2016; NTP, 2018a), and cell proliferation (Han et al., 2018b) in rodents have been observed in recent short-term studies of PFOS. Additional data regarding the modes of action of PFOS are needed to clarify whether or not these effects are precursors of liver tumors, but at present, there is not enough evidence to rule out the possibility.

DOSE-RESPONSE ASSESSMENT AND REFERENCE LEVEL CALCULATIONS

Noncancer – PFOA

From Table 1, the lowest LOAEL is 0.05 mg/kg-day, which corresponds to a serum concentration of 0.97 mg/L, for hepatic mitochondrial membrane potential changes and increased apoptosis and oxidative DNA damage (Li et al., 2017). These endpoints were also frequently observed in in vitro studies. OEHHA selected the data from this study as the basis of a POD for calculating an RL for noncancer effects. For the purpose of comparison, the recommended interim NL was based on increased relative liver weight in a mouse study, from which a NOAEL of 0.3 mg/kg-day (based on administered dose) was determined (Loveless et al., 2006).

A NOAEL of 0.003 mg/kg-day was identified from the van Esterik et al. (2016) study, based on reduced female pup body weight on PND 4 in animals exposed to PFOA during gestation and lactation. However, serum concentrations were not reported in this study, and due to the complexity of the dosing scheme (PFOA was administered to dams during pregnancy and lactation), kinetic modeling was not conducted to predict serum concentrations. Therefore, this study is not considered for derivation of a noncancer RL. However, OEHHA acknowledges the potential for developmental toxicity at PFOA levels below the selected POD, and an additional uncertainty factor is added to account for this (discussed below).

The most sensitive endpoints from the Li et al. (2017) study (increased oxidative DNA damage, changes in mitochondrial membrane potential, and increased biomarkers of apoptosis in the liver of female mice) were analyzed with benchmark dose (BMD) software (BMDS version 2.6, US EPA). BMD modeling of the endpoints in Table 2 did

not generate any models with an acceptable goodness-of-fit. Therefore, the LOAEL of 0.97 mg/L is selected as the POD for noncancer effects.

Pharmacokinetic (PK) modeling

Administered dose in rodent studies of PFOA is not the optimal dose metric for toxicity evaluation because of the great difference in the chemical's half-life between humans (2-3 years) and rodents (1-3 weeks). The preferred dose metric is PFOA serum concentration. OEHHA evaluated available PK models to predict PFOA serum concentrations from administered doses. However, after critical evaluation, OEHHA found several shortcomings with the available models that lowered overall confidence in these models' ability to adequately predict serum concentrations. Therefore, OEHHA is using reported serum concentrations for RL derivation.

Acceptable Daily Dose Calculation

To calculate the acceptable daily dose (ADD), which is an estimated maximum daily dose of a chemical that can be consumed by humans for an entire lifetime without toxic effects, the POD is divided by the total uncertainty factor (UF). Because the dose metric for the POD is serum concentration, the ADD is first expressed as a target human serum concentration rather than the typical mg/kg-day dose.

A total uncertainty factor (UF) of 300 is applied in calculating the ADD for PFOA: 3 for interspecies extrapolation, 10 for intraspecies variability, 3 for LOAEL to NOAEL extrapolation, and 3 for the potential for developmental toxicity at the point of departure. When developing a health-protective RL of a chemical in drinking water, the adverse effect or an upstream physiological change that leads to an adverse effect occurring at the lowest dose is selected as the critical effect. Because the critical endpoints here are upstream physiological changes that can lead to adverse effects in a known target organ of PFOA toxicity, the liver, OEHHA is applying a LOAEL to NOAEL UF of 3 rather than 10. OEHHA also is applying a subchronic to chronic extrapolation UF of 1, consistent with the New Jersey DWQI (2017) assessment for PFOA, in which the critical endpoint was increased liver weight from a 14-day study, and a subchronic to chronic UF of 1 was used. New Jersey DWQI's rationale was that, based on evaluation of multiple studies, early manifestations of liver toxicity do not appear to increase in magnitude with chronic exposures. This rationale would also apply to the upstream endpoints used as the basis of the POD from the Li et al. (2017) critical study. For animal studies, OEHHA typically uses a UF of 10 for interspecies extrapolation ($\sqrt{10}$ for pharmacokinetics and $\sqrt{10}$ for pharmacodynamics) and a UF of 30 for intraspecies variability (10 for pharmacokinetics and $\sqrt{10}$ for pharmacodynamics). Since PFOA is not known to be metabolized in animals or humans, and because PFOA serum concentration is the dose metric used in the dose-response analysis, the pharmacokinetic components of the interspecies and intraspecies uncertainty factors

are reduced. An intraspecies pharmacokinetics UF of $\sqrt{10}$ (rather than 10) is kept to account for potential PK differences in infants and children. Thus,

$$\text{ADD} = \text{POD} \div \text{UF} = 0.97 \text{ mg/L} \div 300 = 0.0032 \text{ mg/L (target human serum concentration)}.$$

A NOAEL (based on administered dose) of 0.003 mg/kg-day was determined by OEHHA from the van Esterik et al. (2016) study, based on decreased female pup body weight in the F₁ generation of dams administered PFOA throughout gestation and lactation. By comparison, the administered dose LOAEL from the Li et al. (2017) study is 0.05 mg/kg-day. Although it is unknown what the serum concentrations are in the van Esterik et al. (2016) study, it is possible that developmental toxicity occurred at a lower concentration than the hepatotoxicity in the Li et al. (2017) study. Therefore, an additional uncertainty factor of 3 is included to account for this possibility.

The ADD, expressed as a target human serum concentration of 3.2 µg/L, is slightly higher than average PFOA serum levels nationally and in California. Biomonitoring data from California¹ reported a geometric mean of 1.49 µg/L PFOA in the serum of 337 people in 2013 (the 95th percentile was 4.57 ng/mL). This is comparable to the geometric mean of 1.94 µg/L PFOA in the serum of the general US population, as determined from the National Health and Nutrition Examination Survey (NHANES) data (ATSDR, 2018).

As noted above, in order to account for PFOA's long half-life in humans relative to rodents, the ADD is expressed as a target serum concentration. To calculate a noncancer RL, the target serum concentration must be converted to an HED expressed as a dose in mg/kg-day. This is done by multiplying the ADD by a daily clearance factor, which reflects the clearance of PFOA from the body, of 1.4×10^{-4} L/kg-day for PFOA (US EPA, 2016a), as shown below.

$$\text{ADD} = 3.2 \text{ µg/L} \times 1.4 \times 10^{-4} \text{ L/kg-day} = 4.5 \times 10^{-4} \text{ µg/kg-day or } 0.45 \text{ ng/kg-day}$$

The relative source contribution (RSC) is the proportion of exposures to a chemical attributed to tap water (including inhalation and dermal exposures, e.g., during showering), as part of total exposure from all sources (including food and air pollution). The RSC values typically range from 20 to 80 percent (expressed as 0.20 to 0.80), and are determined based on available exposure data. The default RSC of 0.2 is selected because there is not enough data to determine specific exposure patterns for PFOA. In addition to drinking water, there are several other sources of PFOA that may contribute to exposure in the general population, including air, soil, food, and consumer and industrial products. PFOA released to air may adsorb to airborne particles and travel long distances (US EPA, 2016a). Additionally, the use of PFOA in many consumer

¹ From Biomonitoring California, [Results for Perfluorochemicals \(PFCs\) \(last accessed February 1, 2019\)](#)

products and its environmental persistence has led to the presence of PFOA in indoor air and dust. In fact, US EPA (2016a) reports that the most common exposure routes of PFOA are diet and indoor dust. Thus, an RSC of 0.2 is appropriate, and consistent with RSCs used by other agencies, including US EPA and the State of New Jersey.

Oral ingestion is the primary route of exposure for PFOA in drinking water. PFOA is not very volatile in its ionized form (its predominant form in water) (Johansson et al., 2017), so inhalation of PFOA directly from drinking water is not anticipated to be a major route of exposure. Dermal absorption is also not anticipated to be a significant route of exposure from typical household uses of tap water. Ionized PFOA penetrates skin poorly compared to the neutral form, and PFOA should remain ionized in the stratum corneum due to its buffering capacity (Franko et al., 2012).

PFOA can permeate mouse and human skin in vitro, and be absorbed following dermal application in mice in vivo (Franko et al., 2012). However, a time-course of >5 hours is needed for PFOA to penetrate full-thickness human skin, and this exposure scenario is unlikely to occur from typical household uses of tap water. Additionally, solid PFOA and 1% PFOA in acetone were determined to be non-corrosive in an in vitro epidermal cell viability assay following three minutes of exposure. It should be noted, however, that solid PFOA was corrosive following one hour of exposure, whereas 1% PFOA in acetone was not.

Because oral ingestion is considered to be the only significant route of drinking water exposure, a lifetime average drinking water intake rate of 0.053 L/kg-day (OEHHA, 2012) is used to determine the noncancer RL, which is calculated using the following formula:

$RL = ADD \times RSC \div DWI$, where

ADD = acceptable daily dose of 0.45 ng/kg-day,

RSC = relative source contribution of 0.2, and

DWI = daily water intake rate of 0.053 L/kg-day

$RL = (0.45 \text{ ng/kg-day} \times 0.2) \div 0.053 \text{ L/kg-day} = 2 \text{ ng/L or 2 ppt}$

Thus, the reference drinking water level for the noncancer effects of PFOA is 2 ng/L or 2 ppt based on a recent hepatotoxicity study in mice (Li et al., 2017).

Noncancer – PFOS

OEHHA did not identify any new studies to replace the Dong et al. (2009) study as the critical toxicity study for the noncancer effects of PFOS. Decreased plaque forming cell response was the most sensitive endpoint, and a NOAEL of 0.008 mg/kg-day was identified. The data are summarized in Table 15.

Table 15. Plaque forming cell response in male mice exposed to PFOS (Dong et al., 2009)

Dose (mg/kg-day)	Serum Concentration (mg/L)	Human Equivalent Dose (mg/kg-day)	Plaque Forming Response ^a (PFC/10 ⁶ spleen cells)
0	0.048 ± 0.014 ^b	3.9 × 10 ⁻⁶	597 ± 64 ^b
0.008	0.674 ± 0.166	5.5 × 10 ⁻⁵	538 ± 52
0.083	7.132 ± 1.039	5.8 × 10 ⁻⁴	416 ± 43*
0.417	21.638 ± 4.410	1.8 × 10 ⁻³	309 ± 27*
0.833	65.426 ± 11.726	5.3 × 10 ⁻³	253 ± 21*
2.08	120.670 ± 21.759	9.8 × 10 ⁻³	137 ± 16*

^a Data taken from New Jersey DWQI (2018). Authors state they received numerical data via personal communication with GH Dong.

^b Mean ± SEM (n = 10/dose)

* p<0.05, reported by study authors

Using the equations shown above for PFOA, serum concentrations are converted to HEDs using the human clearance factor of 8.1 × 10⁻⁵ L/kg-day for PFOS, determined by US EPA (US EPA, 2016b). The formula is shown below:

$$\text{Serum concentration (mg/L)} \times \text{clearance factor (L/kg-day)} = \text{HED (mg/kg-day)}$$

The resulting HEDs are presented in Table 15.

BMD modeling was performed using both serum concentrations and HEDs as the dose metric. However, an adequate model fit was not attained in either case. Therefore, the NOAEL of 0.008 mg/kg-day (corresponding to a serum concentration of 0.674 mg/L, Table 15) is selected as the POD.

A total UF of 30 is applied in calculating the ADD for PFOS: 3 for interspecies extrapolation and 10 for intraspecies variability. For animal studies, OEHHA typically uses a UF of 10 for interspecies extrapolation ($\sqrt{10}$ for pharmacokinetics and $\sqrt{10}$ for pharmacodynamics) and a UF of 30 for intraspecies variability (10 for pharmacokinetics and $\sqrt{10}$ for pharmacodynamics). However, because PFOS is not known to be metabolized in animals or humans, and because PFOS serum concentration is the dose metric used in the dose-response analysis, the pharmacokinetic components of the interspecies and intraspecies uncertainty factors are reduced. A subchronic to chronic UF of 3 is typically applied when the study duration is 8-12% of the animal's lifetime (OEHHA, 2008), in order to account for the potential exacerbation of toxicity following chronic exposure. However, New Jersey DWQI (2018) argues that the subchronic to chronic uncertainty factor is not necessary because the maximum decrease in plaque forming cell response remained relatively constant (~70-85%) across studies with different exposure durations (ranging from 7 to 60 days), thus increased exposure

duration does not lead to increased toxicity. OEHHA agrees and is applying a subchronic to chronic UF of 1 rather than 3.

To determine the ADD, expressed as a target human serum concentration, the POD is divided by the total UF, as shown below.

$$\text{ADD} = 0.674 \text{ mg/L} \div 30 = 0.022 \text{ mg/L or } 22 \text{ } \mu\text{g/L (target human serum concentration)}$$

The target serum concentration of 22 $\mu\text{g/L}$ is higher than average PFOS serum levels nationally and in California. Biomonitoring data from California² report a geometric mean of 5.21 $\mu\text{g/L}$ PFOS in the serum of 337 people in 2013 (the 95th percentile was 17.6 ng/mL). This is comparable to the geometric mean of 4.99 $\mu\text{g/L}$ PFOS in serum of the general US population, as determined from NHANES data (ATSDR, 2018).

The ADD is converted to an HED by multiplying the target human serum concentration by a daily clearance factor of 8.1×10^{-5} L/kg-day (US EPA, 2016b), as shown below.

$$\text{ADD} = 22 \text{ } \mu\text{g/L} \times 8.1 \times 10^{-5} \text{ L/kg-day} = 1.8 \times 10^{-3} \text{ } \mu\text{g/kg-day or } 1.8 \text{ ng/kg-day}$$

The default RSC of 0.2 is selected because there is not enough specific data to determine specific exposure patterns to PFOS. In addition to drinking water, there are several other sources of PFOS that may contribute to exposure in the general population, including air, soil, food, and consumer and industrial products. PFOS released to air may adsorb to airborne particles and travel long distances (US EPA, 2016b). Additionally, the use of PFOS in many consumer products and its environmental persistence has led to the presence of PFOS in indoor air and dust. As with PFOA, US EPA (2016b) reports that the most common exposure routes of PFOS are diet and indoor dust. Thus, an RSC of 0.2 is appropriate, and consistent with RSCs used by other agencies, including US EPA and the State of New Jersey.

Oral ingestion is the primary route of exposure for PFOS in drinking water. Volatilization of the predominant anionic form in water ($\text{pK}_\text{a} < 1.0$) is not expected to occur (HSDB, 2018).

Dermal absorption is also not anticipated to be a significant route of exposure from typical household uses of tap water, based on its physicochemical similarities to PFOA. However, no specific studies could be identified that addressed absorption of PFOS following dermal exposure. ATSDR (2018) reports the results of an unpublished single-dose dermal absorption study in rabbits, where potassium PFOS or its diethanolamine salt (at doses up to 20 $\mu\text{g/kg}$) was applied to clipped, intact skin (Johnson et al., 1995a,b, as reported by ATSDR, 2018). Compared to controls, no increase in organic fluoride in the liver was detected, suggesting that PFOS was not absorbed.

² From Biomonitoring California, [Results for Perfluorochemicals \(PFCs\) \(last accessed February 1, 2019\)](#)

Because oral ingestion is considered to be the only significant route of drinking water exposure, a lifetime average drinking rate of 0.053 L/kg-day (OEHHA, 2012) is used to determine the noncancer RL, which is calculated using the following formula:

$RL = ADD \times RSC \div DWI$, where

ADD = acceptable daily dose of 1.8 ng/kg-day,

RSC = relative source contribution of 0.2, and

DWI = daily water intake of 0.053 L/kg-day

$RL = (1.8 \text{ ng/kg-day} \times 0.2) \div 0.053 \text{ L/kg-day} = 7 \text{ ng/L or 7 ppt}$

Thus, the reference drinking water level for the noncancer effects of PFOS is 7 ng/L or 7 ppt based on immunotoxicity in mice.

Cancer – PFOA

Hepatocellular adenoma/carcinoma and pancreatic acinar cell adenoma/carcinoma in male rats were evaluated for RL derivation. For individual tumor sites, OEHHA uses the linear multistage cancer model from US EPA's BMD software (BMDS version 2.6, US EPA) to determine the dose associated with a benchmark response (BMR) of 5% increased risk of developing a tumor and the lower 95% confidence limit of that dose, the BMDL₀₅. For carcinogens that induce tumors at multiple sites and/or in different cell types at the same site in a particular species and sex, BMDS can be used to derive maximum likelihood estimates (MLEs) for the parameters of the multisite carcinogenicity model by summing the MLEs for the individual multistage models from the different sites and/or cell types. This multisite model is then used to provide a basis for estimating the cancer potency of a chemical that causes tumors at multiple sites. Using the HEDs as the dose metric, multisite benchmark dose modeling was performed to determine the cancer slope factor (CSF) for the hepatic and pancreatic tumors in male rats.

A multisite BMDL₀₅ of 0.000648 mg/kg-day was determined from the animal bioassay data (Table 10). To estimate from animal data an HED that would result in an equal lifetime risk of cancer, OEHHA uses body weight (BW) scaling to the ³/₄ power (OEHHA, 2009). This adjustment accounts for interspecies differences in pharmacokinetics and pharmacodynamics. Because pharmacokinetic differences have already been accounted for by using serum concentration as the dose metric instead of administered dose, the BMDL₀₅ only needs modification for pharmacodynamic differences (BW^{1/8} adjustment). The equation is provided below.

$$BMDL_{05}(\text{human}) = BMDL_{05}(\text{animal}) \times (BW_{\text{animal}}/BW_{\text{human}})^{1/8}$$

where BW_{animal} is 0.509 kg, the time-weighted average body weight of control male rats from the NTP (2018c) 2-year bioassay, and BW_{human} is the default value of 70 kg. Thus,

the BMDL_{05(human)} is 3.5×10^{-4} mg/kg-day. The human CSF is determined using the following equation:

$$\text{CSF} = \text{BMR} \div \text{BMDL}_{05} = 0.05 \div 3.5 \times 10^{-4} \text{ mg/kg-day} = 143 \text{ (mg/kg-day)}^{-1}$$

As described in the noncancer reference level derivation, oral ingestion is the primary route of exposure to PFOA in drinking water, and inhalation and dermal exposures are considered negligible.

When determining cancer risk, OEHHA typically applies age sensitivity factors (ASFs, unitless) to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2 years of age, and a factor of 3 is applied for exposures that occur from 2 through 15 years of age. These factors are typically applied unless chemical-specific data exist to better guide the risk assessment.

NTP (2018c) administered 300 ppm PFOA from GD 6 through PND 21 to a concurrent cohort of animals. There were no significant differences in tumor incidences between animals with and without perinatal exposure in the 20, 40 and 80 ppm dose groups (Table 16). This suggests that early-life exposures to PFOA do not substantially increase the likelihood of tumor formation later in life. Therefore, OEHHA is not applying ASFs for derivation of the cancer RL.

Table 16. Comparison of tumors in perinatally and non-perinatally exposed male rats (NTP, 2018c)

Tumor type	Exposure	0 ppm	20 ppm	40 ppm	80 ppm
Hepatocellular adenoma or carcinoma	No perinatal exposure	0/36 ^b	0/42	7/35	11/37
Hepatocellular adenoma or carcinoma	300 ppm perinatal exposure	0/35	1/38	5/38	12/39
Pancreatic acinar cell adenoma or carcinoma	No perinatal exposure	3/43	29/49	26/41	32/40
Pancreatic acinar cell adenoma or carcinoma	300 ppm perinatal exposure	7/41	20/44	30/44	30/43

Perinatal exposure – GD 6 through PND 21

^b Incidence/effective number of animals

Because oral ingestion is considered to be the only significant route of drinking water exposure to the compound, a lifetime average drinking rate of 0.053 L/kg-day (OEHHA, 2012) is used to determine the RL. The RL for carcinogenic effects can be calculated using the following equation:

$$RL = R \div (CSF \times DWI), \text{ where}$$

R = default risk level of one in one million, or 10^{-6}

CSF = cancer slope factor in $(\text{mg}/\text{kg}\text{-day})^{-1}$

DWI = daily water intake rate of 0.053 L/kg-day

Using the total lifetime drinking water exposure estimate of 0.053 L/kg-day, a RL for a one in one million cancer risk from PFOA in tap water is:

$$RL = 10^{-6} \div (143 (\text{mg}/\text{kg}\text{-day})^{-1} \times 0.053 \text{ L}/\text{kg}\text{-day}) = 1.3 \times 10^{-7} \text{ mg}/\text{L}$$

$$RL = 0.1 \text{ ng}/\text{L} \text{ or } 0.1 \text{ ppt (rounded)}$$

The cancer RL of 0.1 ppt should protect against the noncancer effects of PFOA since it is lower than the 2 ppt level for noncancer effects.

Cancer – PFOS

Hepatocellular adenomas in male rats, and hepatocellular adenomas/carcinomas in female rats were evaluated for RL derivation. As noted above, PFOS is being evaluated as a carcinogen because of the positive animal carcinogenicity bioassay data from Butenhoff et al. (2012), and because of the similarities in chemical structure and biologic activity between PFOS and PFOA. Calculation of the PFOS RL for cancer uses the same methods as used above for PFOA. Using the HEDs as the dose metric, BMD modeling produces a BMDL_{05} of 0.0020 mg/kg-day for male rats and a BMDL_{05} of 0.0027 mg/kg-day for female rats.

Applying the $\text{BW}^{1/8}$ adjustment for pharmacodynamics differences between animals, where the time-weighted average male body weight is 0.690 kg (from Thomford 2002), the time-weighted average female body weight is 0.414 kg (from Thomford 2002), and the body weight of humans is the default of 70 kg, the human BMDL_{05} is 0.0011 mg/kg-day for males and 0.0014 mg/kg-day for females. These BMDLs result in human CSFs of $45.5 (\text{mg}/\text{kg}\text{-day})^{-1}$ for males and $35.7 (\text{mg}/\text{kg}\text{-day})^{-1}$ for females. The higher CSF from male animals is used to derive the RL for PFOS. As described in the noncancer RL derivation, oral ingestion is the primary route of exposure to PFOS in drinking water, and inhalation and dermal exposures are considered negligible.

When determining cancer risk, OEHHA typically applies ASFs to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2

years of age, and a factor of 3 is applied for exposures that occur from 2 through 15 years of age. These factors are typically applied unless chemical-specific data exist to better guide the risk assessment.

However, ASFs are not included when deriving the cancer RL for PFOA because the NTP (2018c) study provided evidence that early life exposure did not increase tumor incidences later in life (Table 16). Because it is anticipated that PFOS behaves in a similar manner as PFOA, OEHHA is excluding ASFs in the RL derivation for cancer.

Since oral ingestion is considered to be the only significant route of drinking water exposure to the compound, a lifetime average drinking rate of 0.053 L/kg-day (OEHHA, 2012) is used to calculate the RL for carcinogenic effects:

$$RL = 10^{-6} \div (45.5 \text{ (mg/kg-day)}^{-1} \times 0.053 \text{ L/kg-day}) = 4.2 \times 10^{-7} \text{ mg/L}$$

$$RL = 0.4 \text{ ng/L or } 0.4 \text{ ppt (rounded)}$$

The cancer RL of 0.4 ppt should protect against the noncancer effects of PFOS since it is lower than the 7 ppt level for noncancer effects.

RECOMMENDED NOTIFICATION LEVELS

The cancer RLs for PFOA and PFOS should protect against both cancer and noncancer effects of these chemicals. However, these levels are below concentrations of PFOA and PFOS that can be reliably detected in drinking water, which limits the utility of setting the NLs at these levels.

OEHHA recommends that SWRCB establish the NLs at the lowest levels that can be reliably detected in drinking water using currently available and appropriate technologies.

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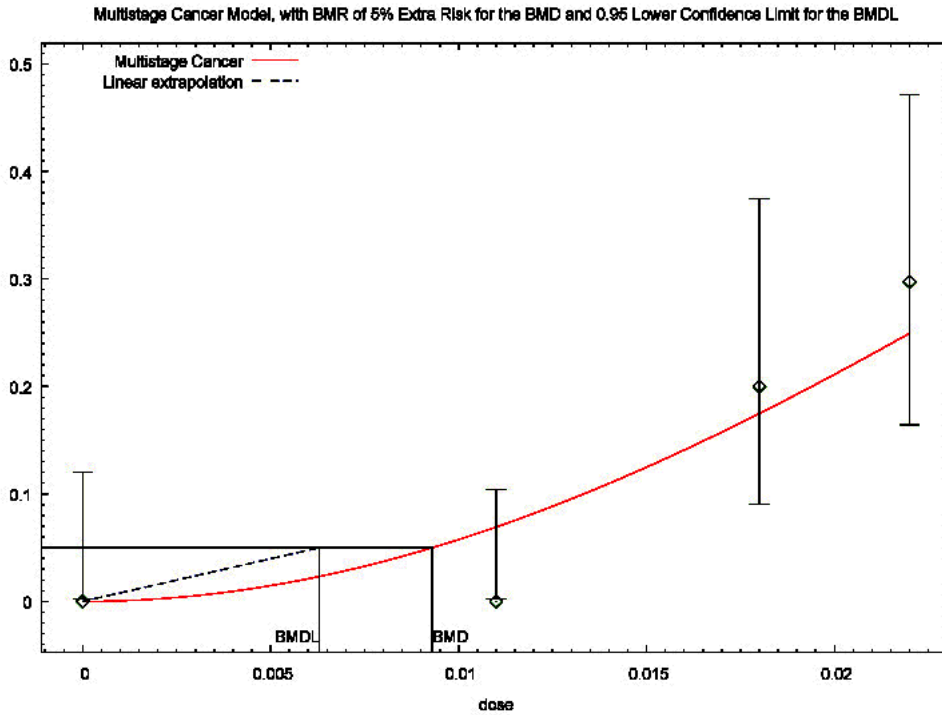
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APPENDIX I. BENCHMARK DOSE MODELING RESULTS

Figure A1. Linear multistage cancer model output for liver adenoma/carcinoma in male rats exposed to PFOA (NTP, 2018c)



```

=====
MS_COMBO. (Version: 1.9; Date: 05/20/2014)
Input Data File: K:\BMD saved files\Chemicals\PFOA\ntp2018 pfoa multi.(d)
Gnuplot Plotting File: K:\BMD saved files\Chemicals\PFOA\ntp2018 pfoa multi.plt
Fri Jan 11 15:24:06 2019
=====

```

```

BMSD_Model_Run
~~~~~

```

The form of the probability function is:
 $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2)]$

The parameter betas are restricted to be positive

Dependent variable = Effect
 Independent variable = Dose
 Data file name = NTP2018livercancermaleeff.dax

Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

Background = 0
 Beta(1) = 0
 Beta(2) = 791.355

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1) have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	592.678	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-40.0307	4			
Fitted model	-43.3357	1	6.61006	3	0.08542
Reduced model	-55.0387	1	30.0161	3	<.0001

AIC: 88.6714

Log-likelihood Constant 36.287873953351991

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0	0	0.000	36.000	0
0.011	0.0692	2.907	0.000	42.000	-1.767
0.018	0.1747	6.115	7.000	35.000	0.394
0.022	0.2494	9.227	11.000	37.000	0.674

Chi² = 3.73 d.f. = 3 P-value = 0.2919

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.00930296

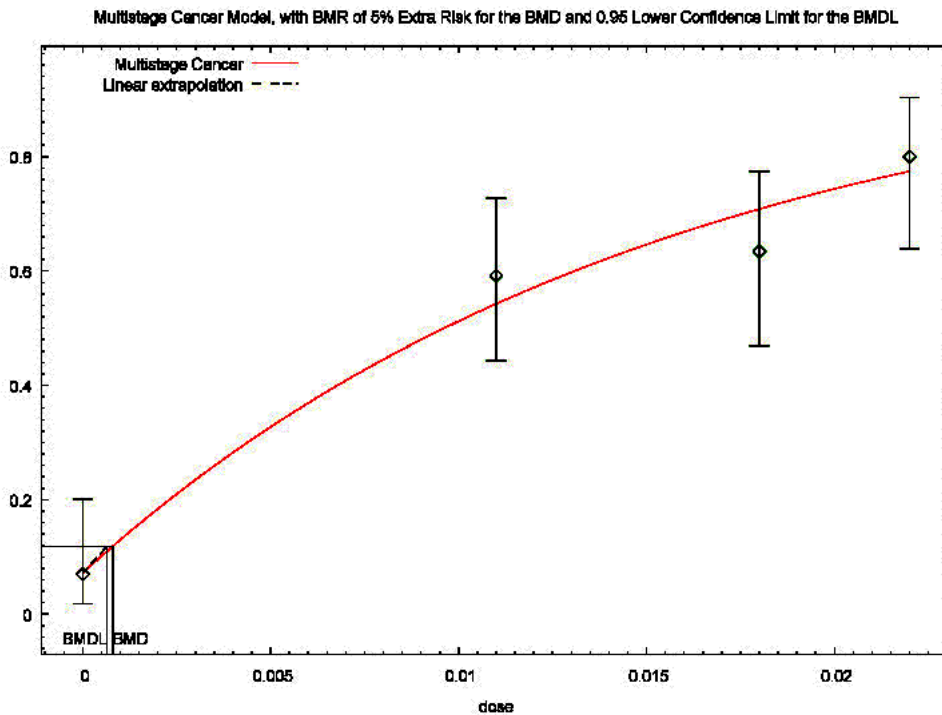
BMDL = 0.00629827

BMDU = 0.0114503

Taken together, (0.00629827, 0.0114503) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 7.93869

Figure A2. Linear multistage cancer model output for pancreatic acinar cell adenoma/carcinoma in male rats exposed to PFOA (NTP, 2018c)



```

=====
MS_COMBO. (Version: 1.9; Date: 05/20/2014)
Input Data File: K:\BMD saved files\Chemicals\PFOA\ntp2018 pfoa multi.(d)
Gnuplot Plotting File: K:\BMD saved files\Chemicals\PFOA\ntp2018 pfoa multi.plt
Fri Jan 11 15:24:06 2019
=====

```

BMDS_Model_Run

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

The parameter betas are restricted to be positive

Dependent variable = Effect

Independent variable = Dose

Data file name = NTP2018panccancermaleeff.dax

Total number of observations = 4

Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1

Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
 Background = 0.0836357
 Beta(1) = 63.4118

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.7
Beta(1)	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0721712	*	*	*
Beta(1)	64.3322	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-90.9548	4			
Fitted model	-91.7978	2	1.68581	2	0.4305
Reduced model	-119.773	1	57.6359	3	<.0001

AIC: 187.596

Log-likelihood Constant 83.425086842392645

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0.0722	3.103	3.000	43.000	-0.061
0.011	0.5428	26.596	29.000	49.000	0.69
0.018	0.7086	29.051	26.000	41.000	-1.048
0.022	0.7747	30.987	32.000	40.000	0.383
Chi^2 = 1.73		d.f. = 2	P-value = 0.4221		

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.00079732

BMDL = 0.000651028

BMDU = 0.00100245

Taken together, (0.000651028, 0.00100245) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 76.8016

Multisite liver adenoma/carcinoma and pancreatic acinar cell adenoma/carcinoma in male rats (NTP, 2018c)

**** Start of combined BMD and BMDL Calculations. ****

Combined Log-Likelihood -135.13347144919277

Combined Log-likelihood Constant 119.71296079574464

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

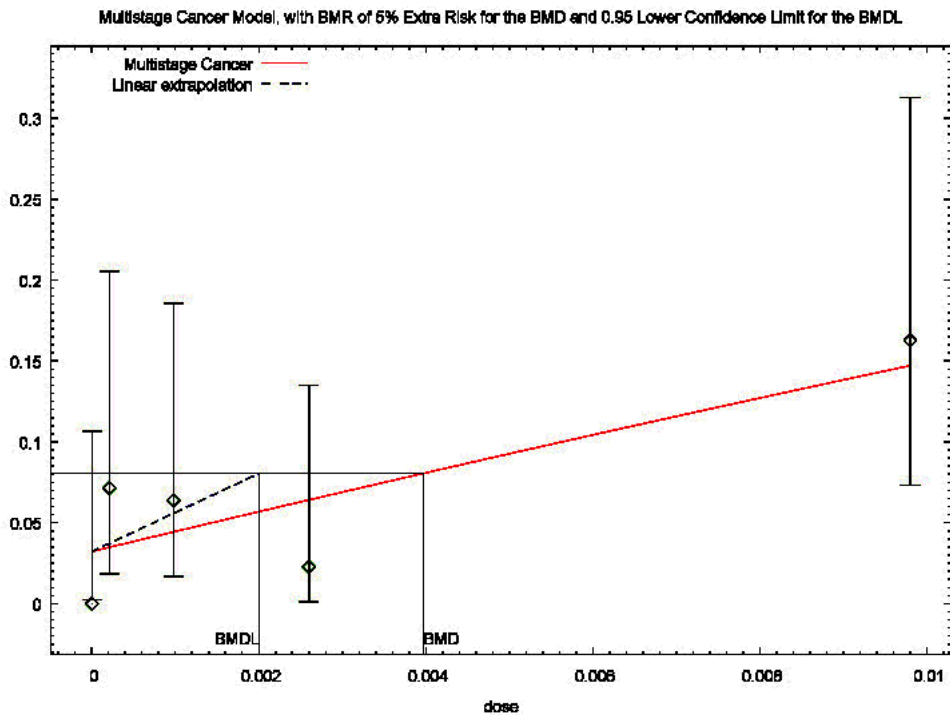
Confidence level = 0.95

BMD = 0.000791547

BMDL = 0.000647865

Multistage Cancer Slope Factor = 77.1766

Figure A3. Linear multistage cancer model output for hepatocellular adenomas in male rats exposed to PFOS (Butenhoff et al., 2012)



10:36 03/18 2019

=====
 Multistage Model. (Version: 3.4; Date: 05/02/2014)
 Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff 2012 male hep ad_Opt.(d)
 Gnuplot Plotting File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff 2012 male hep ad_Opt.plt
 Mon Mar 18 10:36:34 2019
 =====

BMDS_Model_Run

~~~~~  
 The form of the probability function is:

$$P[\text{response}] = \text{background} + (1-\text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

The parameter betas are restricted to be positive

Dependent variable = Effect  
 Independent variable = Dose

Total number of observations = 5  
 Total number of records with missing values = 0  
 Total number of parameters in model = 2  
 Total number of specified parameters = 0  
 Degree of polynomial = 1

Maximum number of iterations = 500  
 Relative Function Convergence has been set to: 1e-008  
 Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values  
 Background = 0.0288938  
 Beta(1) = 14.2842

Asymptotic Correlation Matrix of Parameter Estimates

|            | Background | Beta(1) |
|------------|------------|---------|
| Background | 1          | -0.46   |
| Beta(1)    | -0.46      | 1       |

Parameter Estimates

| Variable   | Estimate  | Std. Err. | 95.0% Wald Confidence Interval |                   |
|------------|-----------|-----------|--------------------------------|-------------------|
|            |           |           | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0.0321681 | 0.016529  | -0.000228122                   | 0.0645644         |
| Beta(1)    | 12.9208   | 6.5633    | 0.0569607                      | 25.7846           |

Analysis of Deviance Table

| Model         | Log(likelihood) | # Param's | Deviance | Test d.f. | P-value |
|---------------|-----------------|-----------|----------|-----------|---------|
| Full model    | -45.8404        | 5         |          |           |         |
| Fitted model  | -48.8812        | 2         | 6.08161  | 3         | 0.1077  |
| Reduced model | -51.9101        | 1         | 2.1394   | 4         | 0.01634 |

AIC: 101.762

Goodness of Fit

| Dose   | Est._Prob. | Expected | Observed | Size   | Scaled Residual |
|--------|------------|----------|----------|--------|-----------------|
| 0      | 0.0322     | 1.32     | 0.000    | 41.000 | -1.168          |
| 0.0002 | 0.0348     | 1.461    | 3.000    | 42.000 | 1.296           |
| 0.001  | 0.0443     | 2.084    | 3.000    | 47.000 | 0.649           |
| 0.0026 | 0.0641     | 2.822    | 1.000    | 44.000 | -1.121          |
| 0.0098 | 0.1473     | 6.333    | 7.000    | 43.000 | 0.287           |

Chi<sup>2</sup> = 4.80      d.f. = 3      P-value = 0.1868

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.00396983

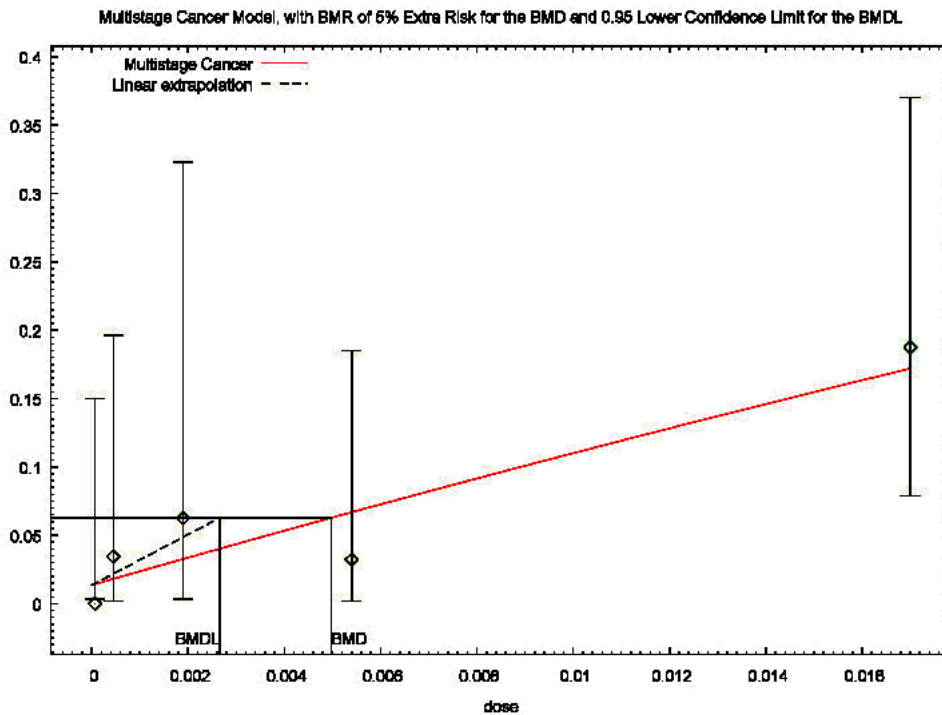
BMDL = 0.00200609

BMDU = 0.0138674

Taken together, (0.00200609, 0.0138674) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 24.9241

**Figure A4. Linear multistage cancer model output for hepatocellular adenoma/carcinoma in female rats exposed to PFOS (Butenhoff et al., 2012)**



```

=====
Multistage Model. (Version: 3.4; Date: 05/02/2014)
Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff 2012 female hep
ad car_Opt.(d)
Gnuplot Plotting File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff 2012
female hep ad car_Opt.plt
Thu Mar 14 12:08:12 2019
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```

BMDS\_Model\_Run

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^{\wedge}1)]$$

The parameter betas are restricted to be positive

Dependent variable = Effect

Independent variable = Dose

Total number of observations = 5

Total number of records with missing values = 0

Total number of parameters in model = 2

Total number of specified parameters = 0  
 Degree of polynomial = 1

Maximum number of iterations = 500  
 Relative Function Convergence has been set to: 1e-008  
 Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values  
 Background = 0.0140434  
 Beta(1) = 10.8528

Asymptotic Correlation Matrix of Parameter Estimates

|            |            |         |
|------------|------------|---------|
|            | Background | Beta(1) |
| Background | 1          | -0.45   |
| Beta(1)    | -0.45      | 1       |

Parameter Estimates

| Variable   | Estimate  | Std. Err. | 95.0% Wald Confidence Interval |                   |
|------------|-----------|-----------|--------------------------------|-------------------|
|            |           |           | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0.0134513 | 0.0167796 | -0.0194362                     | 0.0463387         |
| Beta(1)    | 10.3116   | 4.61895   | 1.25862                        | 19.3646           |

Analysis of Deviance Table

| Model         | Log(likelihood) | # Param's | Deviance | Test d.f. | P-value |
|---------------|-----------------|-----------|----------|-----------|---------|
| Full model    | -27.951         | 5         |          |           |         |
| Fitted model  | -29.095         | 2         | 2.28895  | 3         | 0.5146  |
| Reduced model | -33.134         | 1         | 10.3672  | 4         | 0.03468 |

AIC: 62.1903

Goodness of Fit

| Dose   | Est._Prob. | Expected | Observed | Size   | Scaled Residual |
|--------|------------|----------|----------|--------|-----------------|
| 0.0001 | 0.0141     | 0.396    | 0.000    | 28.000 | -0.634          |
| 0.0004 | 0.018      | 0.523    | 1.000    | 29.000 | 0.667           |
| 0.0019 | 0.0326     | 0.521    | 1.000    | 16.000 | 0.674           |
| 0.0054 | 0.0669     | 2.073    | 1.000    | 31.000 | -0.772          |
| 0.017  | 0.1721     | 5.507    | 6.000    | 32.000 | 0.231           |

Chi^2 = 1.95      d.f. = 3      P-value = 0.5831

## Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.00497433

BMDL = 0.00265944

BMDU = 0.0133689

Taken together, (0.00265944, 0.0133689) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 18.801