

**INSTRUCTIONS FOR STAFF WORKSHEET**

Contains a summary of available data and ongoing staff analysis  
 Data and analysis are subject to change

Last Revised: 5/3/17

**\*\*\* 1. Chemical Identification \*\*\*****Perfluorooctane Sulfonate**

Supplement to Original Review (completed on 12/07/2007)  
 Re-Evaluation Focused on Key Studies Identified in US EPA  
 Health Effects Support Documents Released May 2016

Refer to original review worksheet (\\Data3fb\eh\HRA\COMMON\Guidance - Water\Tox reviews-completed\Final\PFOS\PFOS 2007Review\PFOS Final Nov 07.pdf) developed in 2007 for additional information

**CAS #s 1763-23-1 (acid)**

29081-56-9 (ammonium salt)

70225-14-8 (diethanolamine salt)

2795-39-3 (potassium salt)

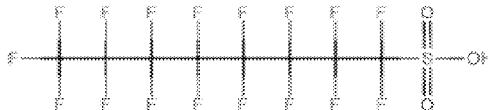
29457-72-5 (lithium salt)

[Note: perfluorooctanoate anion does not have a specific CAS number.]

Synonyms: *PFOS, Perfluorooctane sulfonic acid*

Chemical Formula: C<sub>8</sub>H-F<sub>17</sub>-O<sub>3</sub>-S

Structure:



	Initials	Date Started	Date Completed
Initial Primary Re-review	HMG	5/27/2016	7/1/2016 (2ndary review discussion); 8/5, 9/12 & 12/2016 (2ndary review discussion)
(Partial)Final Primary Re-review (Final) Final Primary Re-review		9/6/2016	
Initial Secondary Re-review	JAJ	7/29/2016	8/10/2016
(Partial) Final Secondary Re-review (Final) Final Secondary Re-review		9/15/2016	9/23/2016
Interim Team Re-review		8/15/2016	8/30/2016
(Partial) Final Team Re-review (Final) Final Team Re-review		11/14/2016 3/31/2017	11/23/2016 4/20/2017

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**Exhibit  
 2476**

State of Minnesota v. 3M Co.,  
 Court File No. 27-CV-10-28862

STATE\_07438096

2476.0001

## \*\*\* 2. MDH Health-based Criteria History \*\*\*

### Current MDH Criteria:

Acute nHRL (2009)\* = Not Derived (Insufficient Data)\*\*

Short-term nHRL (2009)\* = Not Derived (Insufficient Data)\*\*

Subchronic nHRL (2009)\* = Not Derived (Insufficient Data)\*\*

Chronic nHRL (2009)\* = 0.3 ug/L (Development, Hepatic system, Thyroid (E))

\* Values officially became HRLs (i.e., promulgated into rule) in May 2009, however, the full review and values (as nHBVs) were finalized in Dec 2007.

\*\*Serum concentrations are the best dose-metric for extrapolating to humans. At the present time the information necessary to estimate less than chronic doses (i.e., acute, short-term or subchronic) that would result in a given serum concentration is not available. Additional uncertainty exists regarding toxicokinetics in early life. Therefore, acute, short-term and subchronic HRLs were not derived.

### MDH 2017 Health-Based Guidance Evaluation

PFOS is a manmade chemical in a large family of chemicals called perfluoroalkyl substances (PFASs). PFOS has been used in a variety of consumer products, and continues to be used as a fire repellent in firefighting foams, and generated as a degradation product of other perfluorinated compounds. PFOS is very persistent in the environment and the human body; it has been detected in water, wildlife, and humans worldwide.

PFOS was selected for re-evaluation under the Contaminants of Emerging Concern (CEC) program because the US EPA recently published a new final Health Advisory (HA) (USEPA 2016d) along with a Health Effects Support Document (HESD) (USEPA 2016c) for PFOS which contain new information and more in-depth assessments (e.g., pharmacokinetic modeling) of pre-existing studies. MDH initiated a re-evaluation of the 2009 HRL value to determine whether changes to this value are warranted. US EPA's published documents include a comprehensive review of the toxicological literature. This comprehensive review will not be duplicated in the re-evaluation. Rather, the re-evaluation will focus on the key studies identified in US EPA's risk response assessment.

PFOS is a highly bioaccumulative chemical. High, short-term exposures result in an internal body burden that can take years to be eliminated from the body. Therefore, a single Health-based Value has been derived that is protective of short-term exposures such as formula-fed and breast-fed infants as well as long-term exposures.

Noncancer HBV = 0.027 ug/L (Development, Immune system, Liver system, Thyroid (E))  
RfD (MDH 2017)

Cancer cHBV = Not Applicable

**\*\*\* 3. Other Relevant Water Criteria \*\*\***

*Note: Table below is only a partial list and focuses on more recently available guidance values.*

Value	Type/Description	Source	Date Obtained
0.07 ug/L	Lifetime drinking water health advisory (HA)	(USEPA 2016d) Based on: RfD derived from a developmental tox study in rats (decreased pup BW), RSC of 0.2, and lactating women intake rate (0.054 L/kg-d). HA is protective of short as well as lifetime exposure. [previous provisional HA was 0.2 ug/L (2009)]	5/19/2016
0.6 ug/L	Draft Groundwater value	Alaska (August 22, 2015) personal communication from Ted Wu to Jimmy Seow. Based on US EPA 2014 draft toxicity values.	8/22/2015
0.2 ug/L	Drinking water guideline value	Delaware Dept of Resources and Environmental Control aci (USEPA 2016d)	
0.2 ug/L	Provisional groundwater remediation objective	Illinois EPA aci (ASTSWMO 2015)	6/8/2016
0.1 ug/L	Groundwater remediation action guideline	Maine DEP aci (ASTSWMO 2015)	6/8/2016
0.11 ug/L	Drinking water guideline value	Michigan Dept of Environmental Quality 2013 aci (USEPA 2016d) Michigan Dept of Environmental Quality 2013 aci (ASTSWMO 2015)	
0.012 ug/L	Ambient water quality standard		
0.56 ug/l	Drinking water	(TCEQ 2016) Based on RfD 0.000023 mg/kg-d	6/28/2016
0.5 ug/L 5 ug/L	Interim Drinking water Quality Guideline Recreational Water Guideline	(Australian Health Protection Principal Committee 2016) <a href="http://www.health.nsw.gov.au/environment/factsheets/Documents/pfas-interim-health-values-ahppc.pdf">http://www.health.nsw.gov.au/environment/factsheets/Documents/pfas-interim-health-values-ahppc.pdf</a> Based on TDI of 0.00015 mg/kg-d.	6/29/2016
0.6 ug/L	Drinking water screening value (2016a) & proposed Drinking Water Guideline (2016b)	(Health Canada 2016a) Screening Value and draft proposed drinking water guideline (Health Canada 2016b). Draft document included calculation of a cancer based value of 10 ug/L. Noncancer value based on $POD_{HEQ}$ of 0.0015 mg/kg-d (Butenhoff et al 2012 rat study) and composite UF of 25 resulting in a TDI of 0.00006 mg/kg-d. The TDI was combined with a 0.2 RSC and 1.5L/70 kg - d to calculate proposed guideline. Documents are expected to be finalized in 2017. [previous (2010) Drinking Water Guidance Value for PFOS was 0.3 ug/L]	5/27/2016
0.1 ug/L	Drinking Water (and ground water used for drinking water)	(Environment. 2015) Based on TDI of 0.00003 mg/kg-d, 'RSC' of 0.1, and intake rate of 0.03 L/kg-d. Value is also recommended for PFOSA. Since tox profiles of PFOS, PFOA and PFOSA are similar compliance with a composite drinking water quality criteria, i.e., addition of the concentration/limit value ratios should be kept <1. The water guidance for PFOA is 0.3 ug/L	6/2/2016

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0.53 ug/L	MPC <sub>dw, water</sub>	Dutch National Institute for Public Health and the Environment (RIVM 2010)	6/2/2016
0.00065 ug/L	MPC <sub>hh food, water</sub>	MPC <sub>dw, water</sub> - based on EFSA TDI of 0.00015 mg/kg-d, 'RSC' of 0.1, and intake rate of 2L/70 kg-d. MPC <sub>hh food, water</sub> – based on protection for humans upon consumption of fishery products, based on same TDI, 'RSC' 0.1, and fish intake of 115 g/day per 70 kg	
0.3 ug/L	Lifelong precautionary value	(Health. 2006) Drinking Water value - lifelong health tolerable guidance value for all populations groups (from 2003)	1/5/2007
>0.1-0.6 ug/L	Precautionary Action Values (PAV)	PAVs tolerable for a maximum of 10 yrs, 3 yrs, 1 yrs, or immediate action. PVA is for composite of PFOA and PFOS. In addition, in accordance of the Drinking Water Ordinance, efforts are to be made, as expeditiously as possible and insofar as financial resources and the local circumstances allow, to reduce composite perfluorocarbon levels to less than the HPV (health-based precautionary value) of 0.1 µg/L.	
>0.6-1.5 ug/L	PAV <sub>10</sub>		
>1.5-5.0 ug/L	PAV <sub>3</sub>		
5.0 ug/L	PAV <sub>1</sub>		
0.5 ug/L	PAV <sub>0</sub>		
	PAV for infants & pregnant women		
0.09 ug/L		(Sweden) Livsmedelsverket (2014), aci (Environment. 2015). A maximal tolerable level of 0.09 µg/L for PFOS was derived for drinking water based on the TDI of 0.15 µg/kg bw/d derived by EFSA (2008) and considering an exposure scenario where 10% of this value was allocated to the consumption of infant formula based on drinking water. As a precautionary measure, the limit value of 0.09 ug/L was further applied for the sum of seven PFAS substances found in contaminated drinking water: Perfluorooctane sulfonate (PFOS); Perfluorhexane sulfonate (PFHxS); Perfluorobutane sulfonate (PFBS); Perfluorooctanoic acid (PFOA); Perfluoroheptanoic acid (PFHpA); Perfluorohexanoic acid (PFHxA); and Perfluoropentanoic acid (PFPeA).	
0.3 ug/L	Health Value	(United Kingdom. Drinking Water Inspectorate 2007) Level 1 = 0.3 ug/L (consult local health professionals & monitor DW) Level 2 = 1.0 ug/L (Level 1 + put measures in place to reduce to below 10 ug/L) Level 3 = 9 ug/L (Level 1 + 2 + take action to reduce exposure w/i 7 days)	

**\*\*\* 4. Existing Toxicological Criteria or Reviews \*\*\***

*Note: Table below is only a partial list and focuses on more recently released reviews.*

<b>Value and/or Type of Review</b>	<b>Type/Description (Year of Publication)</b>	<b>Source (Refer to the General HRL EndNote Library)</b>	<b>Date Obtained</b>
0.00002 mg/kg-d	RfD (2016)	(USEPA 2016c) Health Effects Support Document for Perfluorooctane Sulfonate (PFOS)	5/19/2016
0.00003 mg/kg-d	Draft Intermediate MRL  Draft Toxicological Review (2015)	(ATSDR 2015) <a href="http://www.atsdr.cdc.gov/toxprofiles/tp200.pdf">http://www.atsdr.cdc.gov/toxprofiles/tp200.pdf</a> Draft Toxicological Profile for Perfluoroalkyls. MRLs were derived based on non-human primate study (it was felt that extrapolating from the rodent studies incurred too much uncertainty). A BMDL10 for liver weight from Seacat et al 2002 was used to generate an HED POD of 0.00252 mg/kg-d. Using a total UF of 90 (3A, 10H, 3 DB) an intermediate MRL of 0.00003 mg/kg-d was calculated.	9/15/2015
0.00015 mg/kg-d	Interim TDI	(Australian Health Protection Principal Committee 2016) <a href="http://www.health.nsw.gov.au/environment/factsheets/Documents/pfas-interim-health-values-ahppc.pdf">http://www.health.nsw.gov.au/environment/factsheets/Documents/pfas-interim-health-values-ahppc.pdf</a>	6/29/2016
0.00006 mg/kg-d (proposed)		(Health Canada 2016a) Screening Value and draft proposed drinking water guideline (Health Canada 2016b). Draft document included calculation of a cancer based TDI of 0.0011 mg/kg-d, which was less conservative than the noncancer TDI. Noncancer value based on $POD_{HEQ}$ of 0.0015 mg/kg-d (Butenhoff et al 2012 rat study) and composite UF of 25 resulting in a TDI of 0.00006 mg/kg-d. Candidate TDI calculations also included use of a $POD_{HEQ}$ of 0.0075 mg/kg-d based on thyroid hormone changes (Seacat et al 2002) – candidate TDI = 0.0075/75 (composite UF) = 0.0001 mg/kg-d. Documents are expected to be finalized in 2017. <i>[The previous Drinking Water Guidance Value of 0.3 ug/l (Health Canada 2010) was based on HED of 0.00003 mg/kg-d (based on monkey study by Seacat et al and serum level of 14.5 ug/mL @LOEL)].</i>	6/30/2016
0.00003 mg/kg-d	TDI	(Environment. 2015) Based on $BMDL_{10}$ of 0.033 mg/kg-d from Thomford et al 2002 rat study. Adjusted for TK difference (factor of 41, based on serum half-life of 48 days in rats), 3 for $UF_A$ and 10 $UF_{II}$ . This TDI was also recommended for PFOSA.	6/2/2016
0.00015 mg/kg-d	TDI	(EFSA 2008) Administered dose NOAEL of 0.03 mg/kg-d (subchronic study in Cynomolgus monkeys) was selected and an overall UF of 200 (10A, 10H, & 2 to compensate for uncertainties related to internal dose kinetics) resulted in a TDI of 0.00015 mg/kg-d.	1/14/2009

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## \*\*\* 5. Toxicokinetic and Toxicodynamic Information \*\*\*

### Toxicokinetics:

**Source:** (USEPA 2016d) and (USEPA 2016c) (See Chapter 2 for additional information) as well as previous MDH 2007 review worksheet.

- Absorption:** Uptake and egress of PFOS from cells is largely regulated by transporters in cell membranes based on data collected for PFOA, a structurally similar PFAS. PFOS is absorbed from the gastrointestinal tract as indicated by the serum measurements in treated animals and distributed to the tissues based on the tissue concentrations found in the pharmacokinetic studies.
- Distribution:** The highest tissue concentrations are usually those in the liver. Postmortem tissues samples collected from 20 adults in Spain found PFOS in liver, kidney, and lung (Pérez et al. 2013). The levels in brain and bone were low. In serum, it is electrostatically bound to albumin, occupying up to 11 sites and sometimes displacing other substances that normally would occupy a site (Weiss et al. 2009). Linear PFOS chains display stronger binding than branched chains (Beesoon and Martin 2015). Binding causes a change in the conformation of serum albumin, thereby changing its affinity for the endogenous compounds it normally transports. PFOS binds to other serum proteins, including immunoglobulins and transferrin.

During pregnancy, PFOS is transferred to the fetus (Chang et al. 2009; Luebker et al. 2005b). Lactational transfer was not measured, but was inferred based on the postnatal declines in maternal serum during lactation (Chang et al. 2009). Mondal et al. (2014) collected serum samples from 633 breast-feeding women and 49 of their infants in West Virginia and Ohio. They found that each month of breast feeding lowered the maternal PFOS levels in serum by 3% (95% CI [-2%, 3%]) and increased the infant serum levels by 4% (95% CI [1%, 7%]).

*MDH Notes: Publications by (Cariou 2015), (Kim 2011), (Liu 2011), (Fromme 2010), and (Karrman 2007) indicate that levels in human cord blood/serum are typically ~40% of maternal serum concentrations and levels in breast milk are ~1.3% of maternal serum concentrations. One study (Fromme 2010) also measured serum concentrations in mothers and breastfed infants at 6 months after delivery and reported similar serum concentrations in infants and their mothers.*

**Metabolism:** PFOS is not metabolized.

**Elimination:** Electrostatic interactions with proteins are an important toxicokinetic feature of PFOS. Studies demonstrate binding or interactions with receptors (e.g., peroxisome proliferator activated receptor-alpha [PPAR $\alpha$ ]), transport proteins (e.g., transthyretin [TTR]), fatty acid binding proteins, and enzymes (Luebker et al. 2002; Ren et al. 2015; S. Wang et al. 2014; Weiss et al. 2009; Wolf et al. 2008, 2012; L. Zhang et al. 2013, 2014). Saturable renal resorption of PFOS from the glomerular filtrate via transporters in the kidney tubules is believed to be a major contributor to the long half-life of this compound. No studies were identified on specific tubular transporters for PFOS but many are available for PFOA. All toxicokinetic models for PFOS and PFOA are built on the concept of saturable renal resorption first proposed by Andersen et al. (2006). Some PFOS is removed from the body with bile (Chang et al. 2012; Harada et al. 2007), a process that also is transporter-dependent. Accordingly, the levels in fecal matter represent both unabsorbed material and that discharged with bile.

An upward trend of increased urinary excretion was observed in the rats administered  $\geq 5$  mg/kg/day PFOS.

The arithmetic mean half-life in humans for occupationally exposed workers (Olsen et al. 2007) was 5.4 years (95% confidence interval [CI] [3.9, 6.9]). Half-lives from animals include 120.8 days for monkeys, 33 to 35 days for male and female Sprague-Dawley rats, and 36.9 days for male and female CD-1 mice (Chang et al. 2012). The half-life differences between male and female rats observed for PFOA were not observed with PFOS. This indicates a lack of gender related differences in renal excretion for rats, and implies that the renal excretion and/resorption transporters for PFOS differ from those for PFOA. No comprehensive studies of PFOS transporters in humans or laboratory animals were identified during EPA's assessment. A study by Zhao et al. (2015) evaluated whether transporters involved in the enterohepatic circulation of bile acids are involved in the disposition of specific PFASs, including PFOS. Uptake of PFOS was measured using hepatocytes from both humans and rats with and without sodium. The results showed sodium-dependent uptake for PFOS. Transport of PFOS was also evaluated using stable CHO Flp-In cells. PFOS was transported by human apical sodium-dependent bile salt transporter (ASBT), but not rat ASBT. Human organic solute transporter (OST)  $\alpha/\beta$  was also able to transport PFOS. The study authors concluded that the long half-life and the hepatic accumulation of PFOS in humans can possibly be attributed, at least in part, to transport by sodium taurocholate co-transporting polypeptide (NTCP) and ASBT.

Comments: MDH's East Metro PFC biomonitoring project sampled a subset of people living in the East Metro region who were connected to a contaminated public water supply. Treatment to remove PFCs was added to the PWS and volunteer participants had blood levels measured at three time points: 2008, 2010 and 2014 (Nelson 2016):  
2008 – 35.7 geo mean ug/L (CI 31.4 – 40.5); 95<sup>th</sup> percentile 100 ug/L (range 3.2 – 448)  
2010 – 24.9 geo mean ug/L (CI 22.1 – 28); 95<sup>th</sup> percentile 69.5 ug/L (range 1.6 – 234)  
2014 – 18.5 geo mean ug/L (CI 16.1 – 21.3); 95<sup>th</sup> percentile 70 ug/L (range 1 – 180)

New Oakdale residents (N=156) were also sampled in 2014. Since these individuals did not have historical exposure to the contaminated water their serum samples may be representative of non-water exposures: 2014 – 7.2 geo mean ug/L (CI 6.5-8.0); 95<sup>th</sup> percentile 21 ug/L (range 0.34-30).

Personal communication (Scher D 2016) re: FDL study indicated lower levels in this population compared to East Metro 2014 levels (and the 2011-2012 NHANES levels).

NHANES biomonitoring data - The CDC's Fourth National Report on Human Exposure to Environmental Chemicals (CDC 2009) included exposure data for PFOS from 2003 to 2004 collected by NHANES. PFOS was detected in 99.9% of the general U.S. population. Since that time, the CDC has issued several updates to the tables. The most recent update was released in 2015 (CDC 2015) (CDC 2017):

Geometric mean ug/L (95th% CI) and 95th Percentile ug/L (95th% CI) from 1999 through 2010 were:

1999 – 2000: 30.4 (27.1-33.9) and 75.7 (58.1-97.5) ug/L  
2003-2004: 20.7 (19.2-22.3) and 54.6 (44.0-66.5)  
2005-2006: 17.1 (16.0-18.2) and 47.5 (42.7-56.8)  
2007-2008: 13.2 (12.2-14.2) and 40.5 (35.4-47.4)  
2009-2010: 9.32 (8.13-10.7) and 32.0 (22.6-48.5)  
2011-2012: 6.31 (5.84-6.82) and 21.7 (19.3-23.9)  
2013-2014: 4.99 (4.50-5.52) and 18.5 (15.4-22.0)

Taken together, the data suggest that PFOS concentrations in human serum in the U.S. declined between 1999 and 2014. Over the course of the study, the geometric mean concentration of PFOS

in human serum decreased from 30.4 µg/L to 4.99 µg/L and the 95th percentile concentration decreased from 75.7 µg/L to 18.5 µg/L. During this time, there has been a major reduction in environmental emissions by the manufacturers as well as a phase-out of production of C-8 compounds in the United States. Analysis of the NHANES 2003–2004 subsample demonstrated higher levels of PFOS and PFOA in males and a slight increase in levels of PFOS with age (Calafat et al. 2007).

**Toxicodynamics:**

*Source: (USEPA 2016d). Also see previous full review worksheet.*

**Mode/Mechanism of Action Information:** Noncancer Effects –  
Oral animal studies of short-term and subchronic duration are available in multiple species including monkeys, rats and mice. These studies report developmental effects (decreased body weight, survival, and increased serum glucose levels and insulin resistance in adult offspring), reproductive (mating behavior), liver toxicity (liver weight co-occurring with decreased cholesterol, hepatic steatosis), developmental neurotoxicity (altered spatial learning and memory), immune effects, and cancer (thyroid and liver). Overall, the toxicity studies available for PFOS demonstrate that the developing fetus is particularly sensitive to PFOS induced toxicity. Human epidemiology data report associations between PFOS exposure and high cholesterol, thyroid disease, immune suppression, and some reproductive and developmental parameters, including reduced fertility and fecundity.

No published cohesive MOA exists that accounts for the varied toxicological properties of PFOS; however, a number of the unique properties of the compound contribute to its toxicity:

- Metabolic stability accompanied by persistence in tissues as an apparent consequence of saturable renal resorption.
- Electrostatic binding to biopolymers, especially proteins, with resultant alterations in conformation and activity.
- Actual or potential displacement of endogenous/exogenous substances normally bound to serum albumin such as fatty acids, bile acids, pharmaceuticals, minerals, and T3.
- Renal resorption (Andersen et al. 2006) and biliary excretion that are dependent on unidentified transporters genetically encoded for management of natural substances (endogenous and exogenous) that prolong systemic retention of absorbed PFOS and explain its long half-life.
- Binding to and activating receptors such as PPAR, thereby initiating activation or suppression of gene transcription.
- Interference with intercellular communication.

Cancer Effects –

A single chronic cancer bioassay in animals is available for PFOS. Increased incidence of hepatocellular adenomas in the male and females at the high dose and combined adenomas/carcinomas in the females at the high dose were observed.

Some human studies suggest an association with bladder, colon, and prostate cancer, however, the literature is inconsistent and some studies are confounded by failure to control for risk factors such as smoking.



**Table 6-A1. Study summary of Key Studies Considered for RfD Derivation**

<b>Relevant Epidemiology Studies or Human Information:</b>
<p>(USEPA 2016d) [See Section 4.1.2 for more details]                      and (USEPA 2016c) [See Section 3.1 for more details] <i>[reviewed by MDH epi staff no suggested edits]</i></p> <p>Numerous epidemiology studies have been conducted evaluating occupational PFOS exposure and environmental PFOS exposure including a large community highly-exposed to PFOA (the C8 Health Project) and background exposures in the general population in several countries. Occupational and general populations have evaluated the association of PFOS exposure to a variety of health endpoints. Health outcomes assessed include blood lipid and clinical chemistry profiles, thyroid effects, immune function, reproductive effects, pregnancy related outcomes, fetal growth and developmental outcomes, and cancer.</p> <p><i>Serum lipids –</i></p> <p>Multiple epidemiologic studies have evaluated serum lipid status in association with PFOS concentration. These studies provide support for an association between PFOS and small increases in total cholesterol in the general population at mean serum levels of 0.0224 to 0.0361 µg/mL. Hypercholesterolemia, which is clinically defined as cholesterol greater than 240 mg/dL, was associated with PFOS exposure in a Canadian cohort and in the C8 Health Project cohort, PFOS levels in these studies were 0.0084 µg/mL &amp; 0.022 µg/mL, respectively. Cross-sectional occupational studies demonstrated an association between PFOS and total cholesterol with much higher serum levels of up to 1.40 µg/mL. Evidence for associations between other serum lipids and PFOS is mixed including HDL cholesterol, low density lipoprotein (LDL), VLDL, and non-HDL cholesterol, as well as triglycerides.</p> <p>The studies on serum lipids in association with PFOS serum concentrations are largely cross sectional in nature and were largely conducted in adults, but some studies exist on children and pregnant women. Limitations to these studies include the frequently high correlation between PFOA and PFOS exposure; not all studies control for other PFASs, such as PFOA, in study design. Also studied were populations with known elevated exposure to other environmental chemicals including PFOA, polybrominated diphenyl ethers (PBDEs), and other persistent chemicals. Overall, the epidemiologic evidence supports an association between PFOS and increased total cholesterol.</p> <p><i>Thyroid –</i></p> <p>Numerous epidemiologic studies evaluated thyroid hormone levels and/or thyroid disease in association with serum PFOS concentrations. These epidemiologic studies provide support for an association between PFOS exposure and incidence or prevalence of thyroid disease, and include large studies of representative samples of the general U.S. adult population. In studies of pregnant women, PFOS was associated with increased TSH levels. Pregnant women testing positive for the anti-thyroid peroxidase (TPO) biomarker for autoimmune thyroid disease showed a positive association with PFOS and TSH. In a second study, an association with PFOS and THS and T3 was found in a subset of the NHANES population with both low-iodide status and positive anti-TPO antibodies. These studies used anti-TPO antibody levels as an indication of stress to the thyroid system, not a disease state. Thus, the association between PFOS and altered thyroid hormone levels is stronger in people at risk for thyroid</p>

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insufficiency or disease. In people without diagnosed thyroid disease or without biomarkers of thyroid disease, thyroid hormones (i.e., TSH, T3 or T4) show mixed effects across cohorts.

Studies of thyroid disease and thyroid hormone concentrations in children and pregnant women found mixed effects.

*Fertility, Pregnancy, and Birth Outcomes -*

Data also suggest a correlation between higher PFOS levels ( $> 0.033 \mu\text{g/mL}$ ) and decreases in female fecundity and fertility, as well as decreased body weights in offspring and other measures of postnatal growth.

Fetal growth retardation was examined through measures including mean birth weight, low birth weight, and small for gestational (SGA) age. Mean birth weight examined as a continuous outcome was the most commonly examined endpoint for epidemiology studies of serum/cord PFOS exposures. Although three studies were null, birth weight deficits ranging from 29 to 149 grams were detected in five studies. Larger reductions (from 69 to 149 grams) were noted in three of the five studies based on per unit increases in serum/cord PFOS exposures. Although a few of these studies showed some suggestion of dose-response relationships across different fetal growth measures, study limitations, including the potential for exposure misclassification, likely precluded the ability to adequately examine exposure-response patterns.

A small set of studies observed an association with gestational diabetes, pre-eclampsia and pregnancy-induced hypertension in populations with serum PFOS concentrations of 0.012 to 0.017  $\mu\text{g/mL}$ .

Although some suggested association between PFOS exposures and semen quality parameters exists in a few studies most studies were largely negative.

Small increased odds of infertility was found for PFOS exposures in a limited number of studies. One study was null for PFOS exposures associated with decreased fecundability ratios (FRs), however, several did find longer time to pregnancy. Reverse causality has been suggested as an explanation for these observations. Although some concern remains about the possibility of reverse causation explaining some previous study results, these collective findings indicate a consistent association with fertility and fecundity measures and PFOS exposures.

*Immune Function -*

A few studies have evaluated associations with measures indicating immunosuppression. Two studies reported decreases in response to one or more vaccines in children aged 3, 5, and 7 years (e.g., measured by antibody titer) in relation to increasing maternal serum PFOS levels (ranging 0.0056–0.027  $\mu\text{g/mL}$ ) during pregnancy, or at 5 years of age (Grandjean et al. 2012; Granum et al. 2013). Decreased rubella antibody concentrations in relation to serum PFOS concentration were found among 12- to 19-year-old children in the NHANES, particularly among seropositive children (Stein et al. 2015). A third study of adults found no associations with antibody response to influenza vaccine (Looker et al. 2014). In the three studies examining exposures in the background range among children (i.e., general population exposures, geometric means  $< 0.02 \mu\text{g/mL}$ ), the associations with PFOS were also seen with other correlated PFASs, complicating the conclusions drawn specifically for PFOS.

No clear associations were reported between prenatal PFOS exposure and incidence of infectious disease among children (Fei et al. 2010; Okada et al. 2012), although an elevated risk of hospitalization for infectious disease was found among girls, suggesting an effect at the higher maternal serum

levels measured in the Danish population (mean maternal plasma levels were 0.0353 µg/mL). With regard to other immune dysfunction, serum PFOS levels were not associated with risk of ever having had asthma among children in the NHANES with median levels of 0.017 µg/mL (Humblet et al. 2014). A study among children in Taiwan with higher serum PFOS concentrations (median with and without asthma: 0.0339 and 0.0289 µg/mL, respectively) found higher odds ratios for physician-diagnosed asthma with increasing serum PFOS quartile (Dong et al. 2013). Associations also were found for other PFASs. Among asthmatics, serum PFOS was also associated with higher severity scores, serum total immunoglobulin E, absolute eosinophil counts, and eosinophilic cationic protein levels.

*[Note: NTP recently completed a draft monograph (NTP 2016a) regarding the immunotoxicity associated with exposure to PFOA and PFOS. A peer review meeting was held July 19, 2016. (see Figures D1 – D-37) The panel agreed that:*

- *The scientific evidence for suppression of the antibody response from experimental animal studies and human studies of PFOS support a high and moderate level of evidence, respectively.*
- *Moderate level of evidence in experimental animal studies for reduction of disease resistance and suppression of natural killer cell activity, and only low or inadequate (no studies) evidence in humans.*
- *Low or inadequate (no studies) of hypersensitivity-related outcomes or autoimmunity-related effects in animal studies, and very low or inadequate evidence in humans.*

*The NTP monograph has been finalized (NTP September 2016)*

#### *Cancer -*

Several human epidemiology studies evaluated the association between PFOS and cancers including bladder, colon, and prostate. A large increase in mortality risk from bladder cancer was demonstrated, and a subsequent study of bladder cancer incidence in the same cohort found rate ratios of 1.5 to 1.9 in the two highest cumulative exposure categories compared to an internal referent population (Alexander et al. 2003; Alexander and Olsen 2007). The risk estimates lacked precision because the number of cases were small. Smoking prevalence was higher in the bladder cancer cases, but the analysis did not control for smoking because data were missing for deceased workers; therefore, positive confounding by smoking is a possibility in this analysis. No elevated bladder cancer risk was observed in a nested case-control study in a Danish cohort with plasma PFOS concentrations at enrollment between 0.001 and 0.0131 µg/mL (Eriksen et al. 2009). Other studies that evaluated cancer risk for specific sites (e.g., prostate, breast) in the general population were inconsistent (Bonfeld-Jørgensen et al. 2011, 2014; Hardell et al. 2014; Innes et al. 2014) (see section 4.1.2).

The associations for most epidemiology endpoints are mixed. Although mean serum values are presented in the human studies, actual estimates of PFOS exposure (i.e., doses/duration) are not currently available. Thus, the serum level at which the effects were first manifest and whether the serum had achieved steady state at the point the effect occurred cannot be determined. It is likely that some of the human exposures that contribute to serum PFOS values come from PFOS derivatives or precursors that break down metabolically to PFOS. These compounds might originate from PFOS in diet and materials used in the home, which creates potential for confounding. Additionally, most of the subjects of the epidemiology studies have many PFASs and/or other contaminants in their blood. Although the study designs adjust for other potential toxicants as confounding factors, their presence constitutes a level of uncertainty that is usually absent in the animal studies.

Taken together, the weight of evidence for human studies supports the conclusion that PFOS exposure is a human health hazard. At this time, EPA concludes that the human studies are adequate for use qualitatively in the identification hazard and are supportive of the findings in laboratory animals.

**Supplement to Original Review Completed on 12/07/2007.**

PFOS is bioaccumulative compound and the most appropriate dose-metric regardless of duration is average serum concentration\*. Therefore a single study summary table is provided below rather than one table for each duration. The contents of the table below focuses on the key endpoints and studies largely identified in the US EPA Health Advisory (HA) and Health Effects Support Document (HESD) Released May 2016. For additional information regarding MDH's previous assessment refer to [review worksheet](#) from 2007. The studies included in EPA's HESD and HE were determined by EPA to provide the most current and comprehensive description of the toxicological properties of PFOS and the risk it poses to humans through drinking water. From these studies, those that presented serum data amenable for modeling (i.e., determination of HEDs) were selected for dose-response analysis. The resulting subset of studies is limited because of the need to have dose and species-specific serum values for model input, as well as exposure durations of sufficient length to achieve values near to steady-state projections or applicable to developmental endpoints with lifetime consequences following short-term exposures. The pharmacokinetically modeled average serum values from the animal studies are restricted to the animal species selected for their low-dose response to oral PFOS intake. Additional studies have been included by MDH if they provided information on additional endpoints of interest.

*\* EPA used a peer-reviewed pharmacokinetic model developed by (Wambaugh 2013) to calculate the average serum concentrations associated with the candidate NOAELs and LOAELs from the toxicological database. Average serum levels of PFOS from the model were used to determine the HED associated with the study NOAEL and LOAEL. The Wambaugh et al. (2013) model is based on the Andersen et al. (2006) concept that saturable renal resorption is responsible for the long serum half-lives seen in humans and animals. A unique feature of the pharmacokinetic approach is the use of a single model for the three species and reliance on the serum PFOS level as the measure of exposure. For each species, the model accommodated the appropriate toxicokinetic variables for the species/strain. The pharmacokinetic analysis facilitated examination for consistency in the average serum values associated with effect and no-effect doses from the animal PFOS studies. A nonhierarchical model for parameter values was assumed wherein a single numeric value represented all individuals of the same species, gender, and strain. Body weight, the number of doses, and magnitude of the doses were the only parameters that varied.*

Study Description – duration, route/ vehicle, species/ strain, age @ dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
<b>Animal Studies:</b> Note - EPA used a peer-reviewed pharmacokinetic model developed by (Wambaugh 2013) to calculate the average serum concentrations associated with the candidate NOAELs and LOAELs from the toxicological database. Average serum levels of PFOS from the model are shown in blue, and were used to determine the HED associated with the study NOAEL and LOAEL. The HED is calculated using animal serum concentration multiplied by estimated human Clearance rate (0.000081 L/kg-d).						
<i>Reproductive/Developmental Effects</i>						
Developmental Gavage Study – Sprague-Dawley Rats Dosed beginning GD2 until term (~GD21) ½ mothers killed on GD18 other ½ allowed to deliver  Study Duration = 19 days	0, 1, 2, 3, 5, or 10 <i>Measured final serum concentrations: 19.69, 44.33, 70.62, 79.39, &amp; 189.4 ug/mL.</i>  Predicted AUC ug/mL*h 8,020, 16,000, 24,000, 40,100, 79,800 (Table 4-3)  Average serum concentration = Predicted AUC/(19 d x 24 hr-d) = 17.6 ug/mL 35.1 52.6 87.9 175	<i>Maternal –</i> ≥ 17.6 ug/mL – ↓ TT4 & FT4 by GD7 (≥40% estimated from Fig 4), ↓ T3 but to lesser extent (no feedback response of TSH was observed based on circulating serum levels); ≥ 35.1 ug/mL - ↓ BW; ↓ food & water consump profound at two highest doses; 175 ug/mL - ↑ rel liver wt (20%); ↓ serum triglycerides & cholesterol (34% triglycerides & 14% cholesterol)  <i>Author BMD/BMDL<sub>5</sub> for ↓ maternal BW 0.224/0.150 admin dose &amp; for ↓ T4 (GD7) 0.234/0.046 admin dose. [MDH estimated serum concentrations ~ 4.1/2.8 ug/mL BW &amp; 4.3/1.0 ug/mL T4. Note: MDH typically uses BMR of 10% for maternal BW &amp; 20% for change in T4]</i>  <u>MDH BMD Modeling:</u> <i>Maternal BW on GD15 (BMR10%) (modeling with all dose grps not successful. Removed highest dose grp, which was severely affects) – 23.0/20.6 ug/mL</i>  <i>Maternal tT4 GD7 (BMR20%) (highest dose grp removed) – 10.1/5.57 ug/mL</i>	Maternal & Develop  17.6 ug/mL EPA NOAEL  35.1 ug/mL EPA LOAEL based on ↓ pup & maternal BW; ↓ pup survival; delayed eye opening  <i>[NOAEL/LOAEL<sub>HED</sub> ~ 0.0014/0.0028 mg/kg-d]</i>  <i>Maternal</i> 5.57 ug/mL MDH BMDL  10.1 ug/mL MDH BMD <i>Based on maternal tT4</i> <i>[BMDL/BMD<sub>HED</sub> ~ 0.00045/0.00082 mg/kg-d]</i>	30 (3A, 10H - EPA)  100 (3A, 10H, 3 DB)	0.00005 (EPA)  0.0000045 (MDH)	(Lau 2003) and (Thibodeaux 2003) and aci (US EPA 2016a)  <i>MDH BMD modeling: requested &amp; received from authors data reported in figures, etc.</i>  <i>File at: \\Data3fb\eh\HR A\COMMON\G uidence - Water\Tox reviews-completed\Final \PFOS\BMD Modeling\Thibo deaux-Lau_2003\Copy of Data\orMDH_ PFOS_Thibodea</i>

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> )  <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
		<p>Developmental –            ≥ 17.6 ug/mL - ↑sternal defects (# per fetus) (1.7, 2.1*, 2.6, 2.1, &amp; 3.4* vs 1.2 in controls); ↑pup rel liver wt @PND2 &amp; 9 only (10-14% vs control, however, no clear dose response); hypothyroxinemia (low T4)            35.1 ug/mL - ↓ pup survival; ↓BW (PND0 - 8, 10, &amp; 15%; PND3 – 17, 21, &amp; 30% (BW @ highest dose not reported as all pups died)) ; delayed eye opening; ↑pup rel liver wt @PND5, 9, 15, &amp; 21 (10-17% vs control, however, no clear dose response)            52.6 ug/mL - ~50% pup survival; ↑pup rel liver wt, statis signif @PND2 &amp; 5 only (10-14% vs control)            87.9 ug/mL - &lt;5% pup survival            175 ug/mL - ↓fetal BW (13%), ↑ incidence cleft palate (60% vs 0 in controls), &amp; anasarca (edema); 0% pup survival  <i>Authors BMD/BMDL<sub>5</sub> for fetal sternal defects 0.313/0.122 (adm dose) &amp; for pup survival (PND8) 1.07/0.58 (adm dose)</i>  <i>[MDH estimated corresponding serum concentrations ~ 5.6/2.3 ug/mL &amp; 18.9/10.3 ug/mL]</i></p> <p><u>MDH BMD Modeling:</u>  <i>For 'comparison purposes only' since optimal BMD modeling could not be conducted because we do not have full nested dataset, which would require individual animal data.</i></p>	<p><i>Developmental</i>            8.56 ug/mL            MDH BMDL</p> <p>10.8 ug/mL            MDH BMD</p> <p><i>Based on pup tT4</i>  <i>[BMDL/BMD<sub>HED</sub> ~ 0.00069/0.00087 mg/kg-d]</i></p>	<p>MDH )  100 (3A, 10H, 3 DB – MDH )</p>	<p>0.0000069 (MDH)</p>	<p><i>ux&amp;Lau2003.xls</i>  <i>x</i></p>

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
		<p><i>Fetal sternal defects BMR<sub>05</sub> – no models fit data.</i></p> <p><i>Pup survival BMR<sub>05</sub> (highest dose grp removed – no pups survived) – no models fit data (variance not well modeled and other problems even when included all dose groups or taking out top two dose groups)</i></p> <p><i>Pup BW BMR<sub>05</sub> (highest two dose grps removed – no or too few pups) – 12.1/9.71 ug/mL</i></p> <p><i>Pup eye opening – no models adequately fit data</i></p> <p><i>Pup tT4 BMR<sub>20</sub> on PND9 (highest two dose grps removed – no or too few pups) 10.8/8.56 ug/mL</i></p>				
<p>Developmental Gavage Study – CD-1 Female Mice Dosed GD1-17 ½ mothers killed on GD18 other ½ allowed to deliver</p> <p>Study Duration = 17 days</p>	<p>0, 1, 5, 10, 15 or 20</p> <p><i>Serum concentrations were not reported.</i></p> <p>Predicted AUC ug/mL*h 13,500, 57,700, 88,900, 106,000, 118,000 (Table 4-3)</p> <p>Average serum concentration =</p>	<p><i>Maternal –</i></p> <p><i>≥ 33.1 ug/mL – ↓ triglycerides (stat sign at ≥ 141 ug/mL); rapid ↓ T4 but similar to controls at last week of pregnancy</i></p> <p><i>≥141 ug/mL - dose-dependent ↑ rel liver weight (22%/65%/95%/109%)</i></p> <p><i>289 ug/mL - ↓ BW gain</i></p> <p><i>Author BMD/BMDI<sub>5</sub> for ↓ maternal BW 15.15/3.14 admin dose; ↑ liver wt 2.61/1.31 admin dose; &amp; ↓ T4 0.513/0.352 admin dose. [MDH estimated corresponding serum levels for T4 33/30 ug/mL. Note: we have typically used BMR of 20%]</i></p> <p><i>Developmental –</i></p>	<p>33.1 ug/mL EPA NOAEL</p> <p>141 ug/mL EPA LOAEL based on ↑liv wt, delayed eye opening</p> <p><i>[NOAEL/LOAEL<sub>HED</sub> ~ 0.0027/0.011 mg/kg-d]</i></p>			<p>(Lau 2003) and (Thibodeaux 2003) and aci (US EPA 2016a)</p> <p><i>MDH BMD modeling limitations:</i></p> <p><i>Maternal T4 data only presented in figures – have requested data from authors</i></p> <p><i>Pup survival only reported in figure.</i></p>

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
	Predicted AUC/ (17 dx 24 hr-d) = 33.1 ug/mL 141 218 260 289	<p>≥ 33.1 ug/mL - ↑ pup rel liver weight, stat sign @PND14 &amp; 21 only (6-9% vs control); delayed eye opening</p> <p>≥ 141 ug/mL – ↑ anomalies (defective sternebrae); ↑ pup rel liver weight, stat sign @PND0, 3, 7, 14, &amp; 21 (9-23% vs control)</p> <p>≥ 218 ug/mL - ↓ fetal BW; ↑ mortality (≥ 50%); ↑ growth lags; ↑ pup rel liver weight, stat sign @PND0, 3, 7, 14, &amp; 21 (20-28% vs control)</p> <p>289 ug/mL - ↑ post-implantation loss</p> <p><i>Author's BMD/BMDL<sub>ss</sub> for fetal sternal defects, 0.055/0.016 admin dose; cleft palate 7.03/3.53 admin dose; &amp; survival 7.02/3.88 admin dose. [MDH estimated corresponding serum levels of 23.5/22.8 ug/mL (fetal sternal defects), 165/94 ug/mL (cleft palate), &amp; 165/101 ug/mL. (survival).]</i></p> <p><u>MDH BMD modeling:</u>  <i>For 'comparison purposes only' since optimal BMD modeling could not be conducted because we do not have full nested dataset, which would require individual animal data.</i></p> <p><i>Pup liver wt (Table 3 of Lau et al 2003) states data reported represents mean ±SE from 20-40 mice derived from 21-22 litters. Modeled assuming 22 litters per dose - all models unusable.</i></p>				<p><i>Pup BW (table 1 of Lau et al) provides mean &amp; SE of 8-12 pups obtained from 17-28 litters (specific # of pups or litters per data point are not provided)</i></p> <p><i>Delayed eye opening data reported in narrative (mean ±SE) but no info on number of pups or litters per dose grp.</i></p>



Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
<p>Developmental Gavage Study – Crl:CD(SD)IGS VAF Rats</p> <p>6 wks prior to mating through LD4 ~20/dose</p> <p>Additional 8/grp in control, 1.6 &amp; 2.0 mg/kg-d only were sac'd on GD20 for assessment</p> <p>Study Duration = 63 days</p>	<p>0, 0.4, 0.8, 1.0, 1.2, 1.6, &amp; 2.0</p> <p><i>Serum concentration were not measured.</i></p> <p>Predicted AUC ug/mL*h 30,100, 60,100, 75,000, 90,000, 120,000, 150,000 (Table 4-3)</p> <p>Average serum concentration = Predicted AUC/ (63 d x 24 hr-d) = 19.9 ug/mL 39.7 49.6 59.5 79.4 99.2</p>	<p>Maternal:</p> <p>≥ 19.9 ug/mL - ↓ serum cholesterol (16**, 24**, 25**, 23**, 27**, &amp; 23%**; **=p≤0.01); ↓ 19-84% tT4 when using analog RIA kits (Note: when measured using equilibrium dialysis appeared to be normal)</p> <p>≥ 39.7 ug/mL - ↓ BWG during gestation (75*, 77*, 57, 94**, &amp; 120%*, *p≤0.05, **p≤0.01) &amp; food consumption; ↓ gestation duration (1.7*, 2.2**, 2.6**, 3.9**, &amp; 3%**; p≤0.01); stat signif ↑ rel. liver weight (10, 17 &amp; 12%)</p> <p>≥ 59.5 ug/mL - ↓ serum triglycerides (37, 39*, &amp; 44%**); ↓ liver triglycerides (26, 74**, &amp; 108%**); ↓ 30-38% T3 when using analog RIA kits but suspect negative bias (see above comment for tT4)</p> <p>≥ 79.4 ug/mL - ↑ # dams w/all pups dying PND1-5 (23.5 &amp; 73.7**, p≤0.01); ↓ viability index (49** &amp; 82%**; p≤0.01); ↓ serum glucose (8 &amp; 14%**)</p> <p><i>Authors BMD/BMDLs: 0.45/0.31 mg/kg-d (↓ gestation duration). Note: authors state that ↓ gestation length may have played a role in ↓ survival of neonates. [MDH estimated corresponding serum levels of 22/15 ug/mL]</i></p> <p>Developmental: ≥ 19.9 ug/mL – stat signif ↓ pup BW &amp; BWG (% not given as data presented graphically); ↓ &gt;96%** tT4 but no dose</p>	<p>Maternal:</p> <p>19.9 ug/mL EPA NOAEL</p> <p>39.7 ug/mL EPA LOAEL based on ↓ BWG</p> <p>Offspring: NA EPA NOAEL</p>			<p>(Luebker 2005a) and aci (US EPA 2016a)</p> <p><u>MDH modeling limitations:</u> Gestation duration data only presented in figure</p> <p>Developmental - Pup BW/BWG data only reported in figures</p>

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
		<p>response &amp; not when measured by RIA analog kits but not when measured by chemiluminometric methods</p> <p>≥ 46.9 ug/mL - ↓ liver triglycerides (M/F 29*/36**, 34**/37**, 37*/36*, &amp; 40/57%*, *p&lt;0.05, ** p&lt;0.01)</p> <p>≥ 59.5 ug/mL - ↓ survival (81.7, 49.3**, &amp; 17.1**%)</p> <p>≥ 79.4 ug/mL - ↑ serum cholesterol on GD21 (21* &amp; 21%*) on LD5 (17 &amp; 13%) but not stat signif but large SD; ↑ serum LDL on GD21 (64** &amp; 66%***) on LD5 20 &amp; 42% but not stat signif but large SD;</p> <p><i>Authors BMD/BMDL<sub>5</sub> – 0.39/0.27 (↓ pup BW @PND5); 0.41/0.28 (↓ pup BW gain); 0.63/0.39 (birth BW); 1.06/0.89 (↓ pup survival). [MDH estimated corresponding serum levels of 19/13 ug/mL (BW), 20/14 ug/mL (pup BWG), 31/19 ug/L (birth wt) &amp; 53/44 ug/mL (survival).]</i></p> <p><u>MDH BMD modeling:</u> <i>Viability index reported in Table 4 BUT number of dams is not reported. Assumed 20/dose group for modeling. Modelling results -- all models unusable</i></p>	<p>19.9 ug/mL EPA LOAEL based on ↓ pup BW</p> <p><i>NOAEL/LOAEL<sub>HED</sub> NA/0.0016 mg/kg-d]</i></p>	10H, 3L – EPA)		
2 Generation Reproductive Gavage Study – Crl:CD(SD)IGS VAF Rats Dosed 6 weeks prior to mating	F0 – 0, 0.1, 0.4, 1.6, & 3.2  F1 – 0, 0.1 & 0.4	<p><i>Maternal/Paternal –</i></p> <p>F0: ≥ 99.7 ug/mL - ↓ BW/BW gain &amp; food consumption;</p> <p>F1: 197 ug/mL - ↓ seminal vesicle &amp; prostate weights, ↑ stillborn pups, ↓ duration of</p>	<p><i>F1 offspring:</i> 25.0 ug/mL MDH/EPA NOAEL</p> <p>99.7 ug/mL</p>			(Luebker 2005b)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
<p>(N=35/scx/dose) Female groups consisted of 2 subgroups: 1 dosed until GD10 &amp; killed at end of gestation; 2nd allowed to deliver naturally &amp; killed on lactation day 21. F1 offspring (25/sex/dose) dosed beginning LD22 F2 killed LD21</p> <p>Study Duration = 84 days</p>	<p><i>Measured final serum concentrations: 4.52, 26.2, 136, &amp; 155 ug/mL.</i></p> <p>Predicted AUC ug/mL*h 12,600, 50,400, 201,000, 398,000 (Table 4-3)</p> <p>Average serum concentration = Predicted AUC/ (84 d x 24 hr-d) = 6.26 ug/mL 25.0 99.7 197</p>	<p>gestation &amp; number of implantation sites</p> <p><i>Developmental – [Note no pups @197 ug/mL survived past PND4 &amp; effects below are not reported for this dose grp]</i></p> <p>F1: 25.0 ug/mL – transient delay in righting reflex; ↓ BW (PND1 – 21 ~3-5% &amp; ~14-26% vs control) &amp; BWG (PND1 – 21 ~2-7% &amp; ~15-38% vs control) – statis sign @99.7 ug/mL; slight delayed eye opening ≥99.7 ug/mL - ↓ pup viability (66 &amp; 0%); ↓lactation index; delayed startle reflex, surface righting; delayed physical development (e.g., eye opening, pinna unfolding)</p> <p><i>No BMD modeling results reported in publication.</i></p> <p><i>MDH BMD modeling (using ave serum concen): For ‘comparison purposes only’ since optimal BMD modeling could not be conducted because we do not have full nested dataset, which would require individual animal data. BMD/BMDL<sub>05</sub> 19.9/16.8 ug/mL (F1 pup BW PND7)(HED equivalent – 0.0016/0.0014 mg/kg-d); BMD/BMDL for PND4 viability – model results - unusable</i></p> <p>F2:</p>	<p>MDH/EPA LOAEL based on ↓pup viability &amp; BW</p> <p><i>NOAEL/LOAEL<sub>HED</sub> 0.0020/0.0081 mg/kg-d]</i></p> <p><i>F2 offspring 6.26 ug/mL MDH/EPA NOAEL 25.0 ug/mL MDH/EPA LOAEL, based on ↓pup BW</i></p> <p><i>NOAEL/LOAEL<sub>HED</sub> 0.00051/0.002 mg/kg-d]</i></p>	<p>30 (3A, 10H - EPA)</p> <p>100 (3A, 10H, 3 DB - MDH )</p>	<p>0.00002 (EPA)</p> <p>0.0000051 MDH</p>	

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
		<p>6.26 ug/mL - ↓pup BW (PND1 – 1 ~6% &amp; ~2-13% vs control – statis sign @25.0 ug/mL on PND7-14 w/13 &amp; 10% ↓) &amp; BWG (PND1 – 21 ~2-4% &amp; ~3-25% vs control – statis sign @25.0 ug/mL on PND4 &amp; 7-14 w/~19 &amp; 7%↓)</p> <p><i>MDH BMD modeling (using ave serum concen):</i>  <i>For 'comparison purposes only' since optimal BMD modeling could not be conducted because we do not have full nested dataset, which would require individual animal data.</i>  <i>BMD/BMDL<sub>05</sub>: 9.64/5.91 ug/mL (F2 pup BW PND7) )(HED equivalent – 0.00078/0.00048 mg/kg-d) *NOTE: Only one control and two dosed groups in model, therefore results are used to support selection of NOAEL as POD.</i></p>				
<p>Cross-fostering oral gavage study – CrI:CD(SD)IGS VAF Rats 42 days prior to mating throughout gestation and lactation 25 females/dose</p>	<p>0 or 1.6 mg/kg-d Four grps (<i>in utero/lactation</i>): Control/control Expo/control Control/expo Expo/expo</p> <p><i>Measured pup serum concentrations:</i></p>	<p>↓maternal BW (gestation only); ↓gestation length, number of implantation sites, total litter size, &amp; live litter size</p> <p>Viability – 19% Exp/expo pups found dead by PND2-4 compared to 9% in Expo/control and 1.1% in Control/expo (similar to Control/control)</p> <p>↓Pup BW PND1 in Expo/expo &amp; Expo/control grps ↓Pup BW PND4-21 in all exposed grps, greatest effect in Expo/expo grp.</p>				(Luebker 2005b)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
	<i>Expo/control: 47.6-59.2 ug/mL Control/expo: &lt;DL – 35.7 Expo/expo: 79.5-96.9</i>					
Oral gavage DNT study – Female Sprague-Dawley Rats GD 0 to PND 20 25 females/dose Offspring monitored through PND72  Study duration (gestation) 22 days  (gestation + PND) 41 days	0, 0.1, 0.3, or 1.0 mg/kg/day  <i>Measured final serum levels during Gestation: 1.72, 6.245, or 26.63 ug/mL</i>  <i>Gestation + Postnatal: 3.16, 8.98 &amp; 30.48 ug/mL</i>  Predicted AUC ug/mL*h Gestation 1,060, 3,180, 10,600 Gest+Postnat 3,410, 10,300, 34,100 (Table 4-3)  Average serum concentration =	<i>Maternal</i> 34.7 ug/mL - slight but not statis ↓BWG  <i>Developmental</i> 34.7 ug/mL - ↑motor activity (M) on PND17 (but not observed on PND13, 21 or 61); lack of habituation (M) on PND17	Developmental: 10.5 ug/mL EPA NOAEL  34.7 ug/mL EPA LOAEL based on ↑ motor activity & ↓ habituation  <i>[NOEL/LOAEL]<sub>HED</sub> 0.00085/0.0028 mg/kg-d]</i>	30 (3A, 10H - EPA)  100 (3A, 10H, 3 DB - MDH )	0.00003 (EPA)  0.0000085 (MDH)	Butenhoff et al 2009

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
	Predicted AUC/ (study duration days x 24 hr-d) = Gestation 2.0 ug/mL 6.0 20  Gestation+PND 3.5 ug/mL 10.5 34.7					
Drinking water ‘DNT’ study – Pregnant Wistar Rats GD1 – PND21	0, 5 or 15 mg PFOS/L water <i>Doses to animals were not calculated and BW &amp; water consumption were not reported. EPA estimated doses: 0, 0.8 or 2.4 mg/kg-d</i>	$\geq 0.8$ (adm dose) – water maze escape latency $\uparrow$ (swimming speed & time to reach visible platform similar across all groups) 2.4 (adm dose) - $\downarrow$ pup survival before cross-fostering	0.8 (adm dose) EPA NOAEL  2.4 (adm dose) EPA LOAEL based on $\uparrow$ water maze escape distance & escape latency			Wang et al 2015 ari (US EPA 2016a)
Oral Neurodevelopmental Gavage study – Pregnant Sprague-Dawley Rats GD2-21	0, 0.1, 0.6, or 2.0 mg/kg-d	$\geq 0.1$ (adm dose) - $\uparrow$ number GFAP positive cells in hippocampus & cortex of pups; $\uparrow$ mRNA expression of two inflammatory cytokines (interleukin 1 beta & tumor necrosis factor- $\alpha$ ) in hippocampus PND0; $\uparrow$ mRNA levels of pro-inflammatory transcription factor activation protein-1 @PND0				Zeng et al 2011 ari (US EPA 2016a)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
		≥ 0.6 (adm dose) – ↑S100 calcium binding protein B in pup hippocampus & cortex on PND21; ↑mRNA levels of pro-inflammatory transcription factors nuclear factor-kB & cAMP response element-binding protein @PND0				
28 day gavage study in ICR male mice (20/dose)  corn oil vehicle	0, 0.25, 2.5, 25 or 50 mg/kg-d  Serum levels reported in Figure 7 – unable to est level at 0.25 mg/kg-d. <b>Est level @2.5, 25, &amp; 50 mg/kg-d adm dose = ~44, 233, &amp; 320 ug/mL</b>	≥ 2.5 mg/kg-d [adm dose] - ↑Sertoli cell vacuolization & derangement of cell layers; disruption of blood-testicular barrier (BTB); ↓epididymal sperm count (reported in Figure 4, <b>estimated</b> ↓vs control – 28*, 60**, & 68**%, p<0.05* or 0.01**) 50 mg/kg-d [adm dose] – dislocated immature germ cells found in lumen of seminiferous tubules  <i>In vitro</i> (primary Sertoli cell assay) demonstrated a dose dependent ↓in cell permeability barrier (‘threshold’ between 20 & 30 uM) & transepithelial electrical resistance (‘threshold’ between 10 & 20 uM)  Figure S2 shows PFOS did not significantly change morphology of the caput, corpus, or cauda of epididymis	0.25 (adm dose) NOAEL  2.5 (adm dose) LOAEL			(Qiu 2013)
Hypothalamic-pituitary-testicular (HPT) axis 28 day oral gavage study – Adult Sprague-Dawley Male Rats 19/grp	0, 0.5, 1, 3, or 6 mg/kg/day	≥ 0.5 (adm dose) - ↓serum LH & testosterone (flat dose response), ↑FSH, ↓gene expression for GnRH (but inverse dose response shown) ≥ 1 (adm dose) – histological changes in testes (edema around seminiferous	NA Authors NOAEL  0.5 (adm dose) Authors LOAEL, based on ↓ LH & testosterone & ↑ FSH			Lopez-Duval et al 2014 aci (US EPA 2016a)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
Small number of animals (2-5) examined per endpoint		<p>tubules &amp; malformed spermatids); changes in norepinephrine concen <math>\geq 3</math> (adm dose) - most active pituitary gonadotrophic cells classified as inactive based on the lack of homogeneous endoplasmic reticulum &amp; well developed Golgi complex, many cells in process of degeneration were observed. Histological changes in hypothalamus (basophilia, vacuolation, and irregular nuclear borders). <math>\uparrow</math>gene expression for LH &amp; FSH (but <math>\downarrow</math> @highest dose)</p> <p><i>Authors state results are consistent w/inhibition of the reproductive hypothalamus-pituitary-testicular axis at <math>\geq 0.5</math> mg/kg/day.</i></p>				
<p>HPT axis 28 day oral gavage study – Adult Sprague-Dawley Male Rats</p> <p>N=6/dose</p> <p>Tween 20 vehicle</p>	0, 1, 3, or 6 mg/kg/day	<p><math>\geq 1</math> mg/kg-d [adm dose] – <math>\downarrow</math>Gonadotropin-releasing hormone receptor (GnRHr) relative protein expression in pituitary (largest effect @lowest dose); <math>\downarrow</math>GnRHr gene (flat dose response) &amp; <math>\uparrow</math>GnRHr protein (inverse dose response) relative expression in testis, <math>\uparrow</math>LHr relative gene expression &amp; <math>\downarrow</math>LHr relative protein expression in testes; <math>\downarrow</math>Follicle-stimulating hormone receptor (FSHr) relative gene (low dose only) &amp; relative protein expression (all doses but fairly flat dose-response) in hypothalamus; <math>\downarrow</math>FSHr relative gene &amp; relative protein expression in testes; <math>\downarrow</math>Androgen receptor (Ar) relative protein expression</p>				<p>(Lopez-Doval 2016) <i>Does not appear that histological assessments were conducted</i></p> <p><i>Small group size</i></p>

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
		<p>in hypothalamus &amp; relative gene expression in testes; ↑Ar relative gene expression in pituitary (low dose only)</p> <p>&gt; 3 mg/kg-d [adm dose] - ↓Luteinizing hormone receptor (LHr) relative protein expression in hypothalamus; ↑Ar relative gene expression in hypothalamus; ↓Ar) relative protein expression in pituitary &amp; testes</p> <p>6 mg/kg-d [adm dose] - ↑LHr relative gene expression in hypothalamus</p>				
<p>Lung Developmental oral gavage study – Sprague-Dawley Rats GD1-21</p> <p>n=6/group (no mention regarding sex)</p> <p>Study duration 21 days</p>	<p>0, 0.1, or 2 mg/kg/day</p> <p><i>Measured final serum concentrations</i> 1.7 ug/mL 47.5</p> <p>Predicted AUC ug/mL*h 968, 19,400 (Wambaugh et al 2013)</p> <p>Average serum concentration = Predicted AUC ug/mL-hr/(21 d x 24 hr/d) = 1.9 ug/mL 38.5</p>	<p>38.5 ug/mL - ↓ Pup BW (~20% @ PND21); ↑pup mortality; histopath changes in pup lungs (alveolar hemorrhage, thickened interalveolar septum &amp; inflammatory cell infiltration); ↑biomarkers for oxidative stress</p>	<p><i>Offspring</i> 1.9 ug/mL NOAEL</p> <p>38.5 ug/mL LOAEL based on histopathological changes in lungs, ↓ BW &amp; ↑ mortality <i>[NOAEL/LOAEL]<sub>HED</sub></i> 0.00015/0.0031 <i>mg/kg-d]</i></p>			<p>(Chen 2012) and aci (US EPA 2016a)</p>

<b>Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.</b>	<b>Admin Dose (mg/kg/d)</b> <i>[average serum concen]<sup>#</sup></i>	<b>Effect(s) Observed at each Serum Concentration (or Admin Dose)</b>	<b>Study POD<sub>HED</sub> (mg/kg/d)</b> (e.g. NOAEL <sub>HED</sub> )  <i>[serum concen]<sup>#</sup></i>	<b>UF<sup>1,2</sup></b>	<b>Candidate RfD mg/kg-d</b>	<b>Reference (note limitations in comment filed)</b>
Developmental immune oral gavage study – C57BL/6N Female Mouse GD1-17 10-12/dose Immunotox evaluations on pups performed at 4 & 8 wks (1M & 1F per litter were tested)	0, 0.1, 1, or 5 mg/kg	≥ 1 (adm dose) – suppressed NK activity @8wks (Ms – 42.5 & 32.1%) 5 (adm dose) - suppressed NK activity @8wks (Fs 35.1%); ↓ plaque-forming cell response for SRBC IgM production by B cells (M 35%); ↓ CD3+ & CD4+ in thymocytes. Functional responses (nitrite production) to LPS & interferon-gamma by peritoneal macrophages were not affected with treatment	0.1/1 (M/F) admin dose EPA NOAEL  1/5 admin dose EPA LOAEL based on ↓NK cell activity			Kcil et al 2008 aci (US EPA 2016a)
Glucose & lipid homeostasis oral gavage study – Wistar Rats GD0-PND20 6/grp; Blood samples collected at 10 and 15 weeks (fasted) for lipids and glucose	0, 0.5 or 1.5 mg/kg-d	≥ 0.5 (adm dose) - ↓BW (5-15%); dose-related ↑glucose intolerance; ↓serum adiponectin; ↑epigonadal fat pad wt & fat accumulation	NA EPA NOAEL  0.5 (adm dose) EPA LOAEL, based on ↓ offspring body wt, impaired glucose tolerance			(Lv 2013) and aci (US EPA 2016a)
Glucose & lipid homeostasis oral gavage study – CD-1 Mice Exposure GD3-PND21. Pups assessed @PND21 & PND63. Offspring fed std or hi-fat diet	0, 0.3 or 3 mg/kg-d	≥ 0.3 (adm dose) - ↑fasting serum glucose (std diet); ↑HOMA-IR index @PND63 (hi-fat diet) 3 (adm dose) - ↑ liver wts; ↑expression of CYP4A14, lipoprotein lipase, fatty acids translocase, hepatic insulin receptor, and insulin-like growth factor-1; ↓genes for prolactin receptor & insulin-like growth factor-1; ↑HOMA-IR index @PND63 (std diet); changed glucose tolerance test (hi-fat diet); ↑fasting serum insulin (both diets)	0.3 (adm dose) EPA NOAEL  3 (adm dose) EPA LOAEL, based on ↑ liver wt in dams & M offspring, ↑ fasting serum insulin Ms			Wang et al 2014b aci (US EPA 2016a)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>LHED</sub> )  <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
<i>Nervous System Effects (also see DNT studies under Developmental section above)</i>						
3 month oral gavage study – C57BL6 Mice 15/dose (sex not specified)	0, 0.43, 2.15, or 10.75 mg/kg/day	<p>≥ 2.15 (adm dose) – signif ↑latency to escape &amp; less time in target quadrant in water maze test; signif ↑% apoptotic cells in hippocampus</p> <p>10.75 (adm dose) - ↓dopamine &amp; DOPAC levels; ↑ glutamate levels. (HVA &amp; GABA levels were unchanged). Changes in differential protein expression: Down-regulation of Mib1 protein (an E3 ubiquitin-protein ligase), Herc5 (hect domain &amp; RLD 5 isoform 2), &amp; Tyro3 (TYRO3 protein tyrosine kinase 3). Up-regulation of succinate dehydrogenase flavoprotein subunit (SDHA), Gzma (Isoform HF1 of Granzyme A precursor), Plau (Urokinase-type plasminogen activator precursor), &amp; Lig4 (DNA ligase 4).</p>	<p>0.43 (adm dose) EPA NOAEL</p> <p>2.15 (adm dose) EPA LOAEL based on water maze performance</p>			Long et al 2013
<i>Liver Disease/Function and Serum Lipid Effects</i>						
3 – 21 day Oral gavage study – CD-1 Mice 4 males/dose Study examined mechanistic aspects related to role of PFOS leading to hepatic steatosis	0, 1, 5, or 10 mg/kg/day	<p>≥ 1 (adm dose) – signif ↓mitochondrial β oxidation</p> <p>≥ 5 (adm dose) - ↑ liv wt (only @ day 7 in low dose grp), ↑liver triglycerides, &amp; yellowish coloration; signif ↑transcripts for mRNA for peroxisomal acyl-CoA oxidase, Cyp 4a14, &amp; acyl-CoA dehydrogenase.</p> <p>@10 (adm dose) – microvesicular steatosis at day 14, ↑mRNA &amp; protein expression for fatty acid translocase &amp; lipoprotein lipase, slight but sign ↑total &amp; peroxisomal β oxidation</p>	<p>1 (adm dose) EPA NOAEL</p> <p>5 (adm dose) EPA LOAEL, based on ↑ liver wt, changes in oxidation biochemical parameters</p>			Wan et al 2012

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> )  <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
14 day Oral gavage study – Male BALB/c Mice With regular or high fat diet	0, 5, or 20 mg/kg/day 16 males/dose/diet	<p>≥ 5 (adm dose) - ↑liv wt (Reg/Hi – 81/56 % 99/73%), ↑ liver fat content, ↓liver glycogen, pathological changes in hepatocytes (more severe @ next dose &amp; HiFat grp more susceptible), ↑serum albumin, ↓HDL &amp; cholesterol (HiFat); nonsig ↓ serum testosterone (lrg variability); ↓ BWG (HiFat)</p> <p>@ 20 (adm dose) – ↓BWG &amp; food consump; ↓ fat wt; ↓serum glucose, triglycerides, cholesterol (RegFat) &amp; LDL (RegFat)</p> <p>Regular diet - ~2-fold ↑ (stat sig) liver fat content High fat diet - slight &amp; nonsign ↑liver fat content along with ↓serum glucose and lipid levels</p> <p>PPAR<math>\alpha</math> expression – w/PFOS trt no change in RegFat group, but ↓ HiFat groups (signif @ hi dose). Expression of several genes involved with lipid metabolism (CPT1A &amp; CYP7A1) were examined. CPT1A - role in transport of fatty acid into the mitochondria for beta oxidation, &amp; CYP7A1 - involved w/transformation of cholesterol into bile acids. W/PFOS trt CPT1A expression ↑ w/RegFat but ↓ w/HiFat diet. W/PFOS trt CYP7A1 – no sign change with RegFat but ↓w/HiFat diet. The data support a possible role for PFOS in inhibiting pathways for cholesterol metabolism &amp; export of liver lipids &amp; identify some PFOS associated liver responses that are independent of PPAR<math>\alpha</math> activation.</p>	NA EPA NOAEL  5 (adm dose) EPA LOAEL, based on wt loss on high fat diet			(Wang 2014) and aci (US EPA 2016a)  <i>EPA (2016a) states that Wang et al 2014 demonstrates a clear influence of diet alone on the liver &amp; lipid profile that combined with some dose-related differences in the responses to PFOS exposure. The data support a possible role for PFOS in inhibiting pathways for metabolism &amp; export of liver lipids &amp; identify some PFOS assoc liver responses that are independent of PPAR<math>\alpha</math> activation.</i>

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
<p>28 day oral dietary study – Sprague Dawley Rats 15/sex/dose (0, 2, 20, 50, or 100 mg/kg diet)</p> <p>Study duration 28 days</p>	<p>0.14/0.15, 1.33/1.43, 3.21/3.73, 6.34/7.58 (M/F) mg/kg/day</p> <p><i>Measured serum concentrations</i> 0.95/1.5 ug/mL 13.45/15.4 20.93/31.93 29.88/43.2</p> <p>Predicted AUC ug/mL*h 1,840/2,500 17,400/23,800 42,100/62,100 83,100/126,000 (Wambaugh et al 2013)</p> <p>Average serum concentration = Predicted AUC ug/mL-hr/(28 d x 24 hr/d) = 2.7/3.7 ug/mL 25.9/35.4 62.6/92.4 123.7/187.5</p>	<p>≥ 3.7 ug/mL - stat sign ↑rel liver wt (F – 12, 22, 41, &amp; 71%) ≥ 25.9/35.4 ug/mL – stat sign ↑abs &amp; rel liver wt (M – 12, 35, &amp; 57% rel wt), ↑CYP4A22 (M), ↓T4 (M/F 82/48, 84/60, &amp; 83/57%) ≥ 62.6/92.4 ug/mL - stat sign ↓BW (M/F – 12/12 &amp; 21/20%) &amp; food consump; liver histopath changes (hepatocyte hypertrophy &amp; ↑ cytoplasmic homogeneity), signif ↑ expression of gene for peroxisomal acyl-coenzyme, A oxidase, ↑CYP4A22 (F), ↑ conjug bilirubin (F – 63 &amp; 400%), ↓cholesterol (M/F 36/33 &amp; 88/75%), ↓triglycerides (M/F 43/34 &amp; 89/63%); ↓T3 (F 23 &amp; 31%) @123.7/187.5ug/mL – ↑total &amp; conjug bilirubin, ↓T3 (M24%)</p> <p>67 different fatty acid profiles were examined. Authors state that liver fatty acid profiles showed ↑ total monounsaturated fatty acid levels &amp; ↓ total polyunsaturated fatty acids, which were similar to changes induced by weak peroxisome proliferators.</p>	<p>2.7/NA ug/mL (M/F) EPA NOAEL 25.9/3.7 ug/mL (M/F) EPA LOAEL based on ↑ liver wt <i>[NOAEL/LOAEL<sub>HED</sub> NA/0.00030 mg/kg-d (F)]</i> <i>[MDH NOAEL/LOAEL<sub>HED</sub> 2.7/25.9 ug/mL ~ 0.00022/0.0021 mg/kg-d, based on liver wt change w/histological changes at next dose level up &amp; T4)</i></p>	<p>30 (3A, 10H)</p>	<p>0.0000073 <i>[for comparison purposes]</i></p>	<p>Curran et al 2008 aci (US EPA 2016a)</p>

Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> )  <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
<p>14 week time point from 2 yr dietary cancer bioassay (below) – Sprague-Dawley CrI:CD(SD)IGS BR Rats 5 rats/sex/dose 0, 0.5, 2.0, 5.0, or 20 ppm  Study duration 98 days</p>	<p>0, 0.03/0.04, 0.13/0.15, 0.34/0.40 or 1.33/1.56 (M/F) mg/kg/day</p> <p><i>Measured serum concentrations:</i> 4.04/6.96, 17.1/27.3, 43.9/64.4, &amp; 148/223 ug/mL.</p> <p>Predicted AUC ug/mL*h 3,430/6,620 14,900/24,800 38,900/65,800 152,000/256,000 (EPA Table 4-3)</p> <p>Average serum concentration = Predicted AUC ug/mL-hr/(98 d x 24 hr/d) = 1.5/2.8 ug/mL 6.3/10.5 16.5/28.0 64.6/108.8</p>	<p>No effects were observed on BW, food efficiency, urinalysis evaluation, or peroxisome proliferation (hepatic PCoAO was unchanged).</p> <p>@64.6/108.8 ug/mL - ↓food consump; ↑ abs &amp; rel (M/F 34*/30%*) liver wt &amp; histopath changes; ↓cholesterol (M 72%*) &amp; ↑ ALT (M 80%*) &amp; urea nitrogen (M/F 23*/42%*)</p>	<p>16.5/28.0 ug/mL EPA NOAEL</p> <p>64.6/108.8 ug/mL EPA LOAEL based on ↑liv wt, ALT &amp; BUN</p> <p><i>[NOAEL/LOAEL<sub>HED</sub></i> 0.0013- 0.0023/0.0052- 0.0088 mg/kg-d]</p>	<p>30 (3A, 10H - EPA)</p>	<p>0.00004 (EPA)</p>	<p>(Seacat 2003) and aci (US EPA 2016a)</p>

Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
2-yr dietary study – Sprague-Dawley Crl:CD (SD)IGS BR Rats 0, 0.5, 2, 5, or 20 ppm in diet. Observations were made at 4, 14 and 53 weeks of treatment	0/0, 0.024/0.029, 0.098/0.120, 0.24/0.299, or 0.984/1.251 (M/F) mg/kg-d	<p>≥ 0.024/0.029 (adm dose) – ↑hepatic cystic degeneration (but no clear dose response)</p> <p>≥ 0.098/0.12 (adm dose) - ↓serum glucose (F @ 53 wks), ↑ histomorphological changes in liver (M) (↑ centrilobular hypertrophy)</p> <p>≥ 0.24/0.299 (adm dose) - ↑macroscopic findings in liver (incl enlarged, mottled, diffusely darkened or focally lightened livers), hepatotoxicity characterized by histomorphological changes (M &amp; F)</p> <p>0.984/1.251 (adm dose)- ↓ BW (F); ↑ rel. liver weight; ↑ALT (Ms) (83 ± 84 vs 54 ± 66 in controls; ↓cholesterol; ↓serum glucose; ↑serum urea nitrogen (no microscopic renal findings or changes in serum creatinine); ↑severity of hepatic microscopic changes</p> <p><i>Neoplastic effects:</i> Males - hepatocellular adenoma (Ms 0, 6, 6, 2, &amp; 12% - positive trend); thyroid follicular cell adenoma &amp; carcinoma (10, 12, 10, 10, &amp; 8.5%)</p> <p>Females – hepatocellular adenoma (0, 2, 2, 2, &amp; 8*% - positive trend), 1 carcinoma in highest dose grp; thyroid follicular adenoma &amp; carcinomas (0, 0, 0, 6* &amp; 2%) &amp; C-cell adenoma &amp; carcinoma (20, 14, 12, 16 &amp; 8*%); mammary fibroadenoma/adenoma (38, 60*, 46, 52, &amp; 25*%) and carcinomas (18, 24, 31, 22, &amp; 23%) [high backgrd in controls]</p>	0.024/0.12 admin dose EPA NOAEL  0.098/0.299 admin dose EPA LOAEL based on liver histopathology  <i>Measured serum concentration @NOAEL was 4.04/6.96 ug/mL at week 14 and 1.31/4.35 at week 105</i>			Thomford 2002, (Butenhoff 2012) and aci (US EPA 2016a)  <i>No average serum concentration predicted by PK model in EPA, 2016 HESD</i>

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
<p>26 Week Study – Cynomolgus monkeys PFOS administered in a capsule by gastric intubation. (6/sex/dose – 0, 0.15 or 0.75 mg/kg-d &amp; 4/sex/dose – 0.03 mg/kg-d. Two animals from 0, 0.15 &amp; 0.75 mg/kg/day groups were assigned to a recovery group &amp; were not treated for at least 52 weeks following the last administration of PFOS.</p> <p>Study duration 182 days</p>	<p>0, 0.03, 0.15, or 0.75 mg/kg/day</p> <p><i>Measured serum concentrations: (M/F)</i> 15.8/13.2, 82.6/66.8, 173/171 ug/mL.</p> <p>Predicted AUC ug/mL*<sub>h</sub> 33,800, 166,000, 684,000 (no M/F difference) (EPA Table 4-3)</p> <p>Average serum concentration = Predicted AUC ug/mL-hr (Table 4-3)/(182 d x 24 hr/d) = 7.7 ug/mL 38.0 156.6</p>	<p>7.7 ug/mL – ↓ cholesterol (M/F 28**/24, 3/19, &amp; 68**/49%**<sup>**</sup>, **p&lt;0.01), ↓ HDL cholesterol (M/F 33**/25, 24/36**<sup>**</sup>, &amp; 79**/63%**<sup>**</sup>, **p&lt;0.01))</p> <p>≥ 38.0 ug/mL - ↑ abs (M/F 4/12 &amp; 55/47%*<sup>*</sup>, *p&lt;0.05) &amp; rel (M/F 12.5/17 &amp; 69*/61%*<sup>*</sup>, *p&lt;0.05) liver wt; ↓ T3 (M/F 12/22 &amp; 48**/33%**<sup>**</sup>, **p&lt;0.01); ↑TSH (M/F 151/30 &amp; 160*/82%<sup>*</sup>, *p&lt;0.05); ↓ estradiol (M/F &lt;1/52 &amp; 97**/73%<sup>**</sup>, **p&lt;0.01);</p> <p>156.6 ug/mL - 2 of 6 males did not survive until scheduled sacrifice date; ↓ BWG; liver histological changes (mottled livers, centrilobular or diffuse hepatocellular hypertrophy or vacuolation); hepatic peroxisome proliferation (measured by PCoAO) signif ↑ (but &lt;2-fold)</p> <p><i>Draft ATSDR (2015) BMDL<sub>10</sub> for absolute liver wt of 23.28 ug/mL (HED 0.0016 mg/kg-d) but selected the NOAEL as the basis of the intermediate MRL.</i></p> <p><i>MDH BMD modeling using ave serum concentration: BMD/BMDL<sub>10</sub> 29.0/24.7 ug/mL (~0.0023/0.0020 mg/kg-d) rel liver wt (M); cholesterol and HDL did not model well.</i></p>	<p>38.0 ug/mL EPA NOAEL</p> <p>156.6 ug/mL EPA LOAEL based on ↓BWG, ↓cholesterol &amp; ↑liver wt &amp; histology but no clear evidence of peroxisomal or cell proliferation</p> <p><i>[NOAEL/LOAEL<sub>HED</sub> 0.0031/0.013 mg/kg-d]</i></p> <p>24.7 ug/mL MDH BMDL<sub>10</sub></p> <p>29.0 ug/mL MDH BMD<sub>10</sub>, based on rel liver wt <i>[BMDL/BMD<sub>HED</sub> 0.0021/0.024 mg/kg-d]</i></p>			(Scacat 2002) and aci (US EPA 2016a)
4 week western-type diet study – APOE*3-Leiden. CETP Mice (a strain that exhibits	0 or 3 mg/kg-d	Signif ↓ triglycerides (50%), total cholesterol (60%), HDL (74%), non-HDL (60%), & VLDL (only presented				(Bijland 2011) and aci (US EPA 2016a)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
human-like lipoprotein metabolism)		graphically). Plasma VLDL reduced by 55% & liver production ↓ 87%; ↑liv wt.  PFOS was found to ↓ hepatic VLDL production leading to ↑ retention of triglycerides (steatosis) & hepatomegaly. Gene expression was evaluated: overall, genes upregulated (1- to 2-fold) were those involved w/fatty acid uptake & transport & catabolism; triglyceride synthesis; cholesterol storage; & VLDL synthesis. Genes downregulated were involved w/HDL synthesis, maturation, clearance, & bile acid formation & secretion (1-fold for most genes to ~4-fold for genes involved in secretion). Many of the activated genes are assoc w/nuclear pregnane X receptor (PXR) to a greater extent than PPARα.				
Single or 3 repeat dose Oral gavage study – Young Adult Cynomolgus Monkeys 6/sex/dose  Focus of study was on changes in serum clinical chemistry. Blood samples were drawn prior to (baseline), during, and up to 1 yr after PFOS administration	SD = study day  0, 9 mg/kg (single dose on SD106) or 11-17.2 mg/kg (three separate doses on SD43, 288, & 358) Serum concen. measured on SD 8, 113, & 420 in single dose grp & SD 8, 50, 295, 365,	Single dose grp (mean serum concen SD 113 & 420 - 67.7 & 14.1 ug/mL) – Three dose grp (mean serum concen SD 50, 295, 365, & 420 - 104.8, 141.0, 160.8, & 130.5 ug/mL) – ↓HDL (4 & 12% @1 & 3 wk post-dose)  No trt related changes in liver enzymes or kidney parameters were noted. Decreases in HDL & tT4 (while remaining w/i normal range) appeared to be associated with trt. Data was only reported in figure form. Authors conducted BMD modeling of HDL. tT4 was not considered clinically-relevant for interpreting thyroid function by the				(Chang 2016)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
	& 420 in three dosing grp	authors since they considered it reflective of biologically-inactive thyroxine.  <i>Authors conducted BMD modeling using BMR of LSD (Note: Bayesian analysis of background values was performed for HDL, followed by BMD modeling): BMC/BMC<sub>LSD</sub> – 104.409/74.259 (M) &amp; 106.148/76.373 (F) ug/mL</i>  <i>*Authors noted that half-life estimations (102 to 124 days) were consistent w/those reported in single dose TK studies (110-132 days) &amp; that no sex difference in uptake or elimination were observed (again consistent with previous work).</i>				
<i>Immune Function</i>						
7 day gavage study – C57BL/6 Mice 6 M/grp	0, 5, 20, or 40 mg/kg-d	≥ 5 (adm dose) - ↑ liv wt (34, 79 & 117%); ↓ plaque-forming cell response (63, 77 & 86%) ≥ 20 (adm dose) - ↓ BW, splenic & thymic wts; ↑ serum corticosterone; ↓ splenic & thymic cellularity; ↓ CD4+ & CD8+ cells (markers of functional cell types of spleen & thymic lymphocytes); ↓ NK activity (note no data presented for low dose grp)	NA EPA NOAEL  5 (adm dose) EPA LOAEL based on liver wt & suppression of plaque-forming cell response			Zheng et al 2009 aci (US EPA 2016a)
21 day immune challenge study – B6C3F1 Mice 30 female mice/grp. Exposed 21 days then exposed intranasally to	0, 0.005, or 0.025 mg/kg	0.025 (adm dose) - ↓ survival (17% vs 46% in controls) when challenged with Influenza A				Guruge et al 2009 aci (US EPA 2016a)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
100 plaque forming units influenza A virus suspension. Observed for add's 20 days						
28 day oral gavage study – B6C3F1 Mice N=5/grp  Study duration 28 days	0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, & 0.166 mg/kg/day  <i>Measured final serum concentrations (M/F) as reported in Wambaugh 2013</i> 0.0121/ND, 0.0178/0.0881, 0.0915/0.666, ND/ND, rND/ND, or ND/ND  Predicted AUC ug/mL*h 5.5, 55.7, 111, 556, 1110, & 5540 (Wambaugh et al 2013)  Average serum concentration	Survival, behavior, body weight, spleen, thymus, kidney, gonad and liver weights, and lymphocytic proliferation were not affected by treatment.  Lysozyme activity – ↑ @ 1.83 & 9.14 ug/mL (F) (response was not dose-related)  NK cell activity - ↑ (2-2.5 fold) 0.827, 1.65 & 8.24 (M)  Splenic T-cell immunophenotypes – altered ≥ 0.165 ug/mL (M) Thymic T-cell - ↑ (F) 1.83 & 9.14 ug/mL  SRBC plaque-forming response – dose related suppressed (52-78% in M ≥ 0.083 ug/mL & 50-74% in F ≥ 0.915 ug/mL)	0.0082/0.183 ug/mL (M/F) EPA NOAEL  0.083/0.915 ug/mL EPA LOAEL based on ↓ plaque forming cell response			Peden-Adams et al 2008 aci (US EPA 2016a)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
	=based on Predicted AUC ug/mL-hr/(28 d x 24 hr/d) = 0.0082/0.0092 ug/mL 0.083/0.092, 0.165/0.183, 0.827/0.915, 1.65/1.83, 8.24/9.14					
60 day gavage study – C57BL/6 Adult Male Mice 10/grp  Study duration 60 days	Total dose: 0, 0.5, 5, 25, 50 & 125 mg/kg  0, 0.008, 0.083, 0.417, 0.833, and 2.083 mg/kg/day  <i>Measured final serum concentrations (M) as reported in Wambaugh 2013</i> 0.674, 7.132, 21.638, 65.426, or 120.67 ug/mL  Predicted AUC ug/mL*h	≥ 0.75 ug/mL - ↑ rel liv wt (8, 12*, 29*, 58*, & 122*%, *p≤0.05) ≥ 7.4 ug/mL – 38% ↑ splenic NK cell activity w/↓ @≥ 73.6 ug/mL (~35* & 50*%, data provided in figure 5); ↓ SRBC-specific IgM (~30% to 75% at HDT, data provided in figure 7), ↓ BWG (9, 65*, 179*, & 254*%); ↓ rel spleen (8, 35*, 39*, 53*%) & thymus (14, 38*, 52*, & 59*%) wts; ↓ splenic CD4/CD8 cell subpopulations (CD4 - 10, 27*, 52*, & 73*%; DP - 16, 31*, 49*, & 62*%; DN - 7, 22*, 32*, & 46*%; CD8 <sup>+</sup> - 5, 12, 34*, & 66*%; B220 <sup>+</sup> - 5, 9, 23*, & 33*%); ↓ thymic CD4/CD8 cell subpopulations (CD4 - 21, 29, 41*, & 56*%; DP - 23, 39*, 47*, & 61*%; DN - 13, 23, 34, & 45*%; CD8 <sup>+</sup> - 8, 23*, 36*, & 44*%) ≥ 36.9 ug/mL statis signif ↓ splenic & thymic cellularity (data provided in figure 4); & ↓ rel kidney 10, 18*, & 16*%) wt;	0.75 ug/mL EPA NOAEL  7.4 ug/mL EPA LOAEL, based on SRBC & NK cell response  <i>[NOAEL/LOAEL<sub>HED</sub></i> 0.000061/0.00060 mg/kg-d]  <i>BMDL<sub>1SD-HED</sub></i> 0.00146 mg/kg-d	<i>minimal LOAEL<sub>LHED</sub> w/total 100 (3A, 10H, 3L) for comparison purposes</i>  <i>BMDL-based w/total UF 30 (3A, 10H)</i>	<b>For comparison purposes only:</b>  0.000006 (minimal LOAEL based)  0.000049 (based on BMDL <sub>1SD</sub> for ↓ IgM PFC)	(Dong 2009) and aci (US EPA 2016a) <i>Note: (Dong 2011) in essence replicated these effects. The study included an intermediate dose level (1 mg/kg total dose), confirming 5 mg/kg as the total dose LOAEL. Total dose 1 mg/kg was a NOAEL &amp; supports a candidate RfD of ~0.000006 mg/kg-d</i>

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> )  <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
	1080, 10700, 53200, 106000, & 260000 (Wambaugh et al 2013)  Average serum concentration = Predicted AUC ug/mL-hr/(60 d x 24 hr/d) = 0.75, 7.4, 36.9, 73.6, & 180.6 ug/mL	≥ 73.6 ug/mL - ↓ food consump; signif ↓serum corticosterone (data provided in figure 3); ↓ splenic lymphocyte proliferation index  EPA states that the SRBC-specific IgM plaque forming cell (PFC) response showed a dose-related decrease with statistical significance at ≥ 0.083 mg/kg/day (7.4 ug/mL). <i>MDH conducted BMD<sub>1SD</sub> modeling of functional parameter of ↓PFC was also modeled, based on NTP data link – BMD/BMDL<sub>1SD</sub> of 27.6/18.0 ug/mL.</i>				
<i>Thyroid (also see other studies above that also reported thyroid effects)</i>						
1, 3, or 5 day Oral gavage – Male Sprague-Dawley Rats N=5	10 mg/kg-d	↓tT4 (~47-80%) & fT4 (~60-82%) @ all time points ↓(~23%) tT3 @ day 5 ↓Cholesterol @ day 3 & 5  Hepatomegaly, hepatocellular hypertrophy, & macrovesicular steatosis. Genes associated w/thyroid hormone release & synthesis pathway included type 3 deiodinase DIO3, which catalyzes the inactivation of T3 & type 1 deiodinase DIO1, which bioactivates prohormone T4 to T3. DIO1 repression & Dio3 induction @ day 5.  <i>The authors suggested a link between PFOS, PPAR, &amp; thyroid hormone homeostasis based on work by Miller et al. (2001) who observed ↓ serum T4 and T3 levels &amp; ↑ hepatic proliferation following</i>				Martin et al 2007 aci (US EPA 2016a)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
		<i>exposure to peroxisome proliferators. They also noted that PFOS exhibited similarities to compounds that induce xenobiotic metabolizing enzymes through PPAR<math>\gamma</math> &amp; constitutive androstane receptor (CAR).</i>				
3 Day Oral study Evaluating carrier protein binding interference Female Sprague-Dawley Rats	5 mg/kg-d x 3	No changes to serum TSH and FT4 when checked by & ED-RIA. However, FT4 was signif ↓ when measured with two analog methods, chemiluminescence immunoassay & simple RIA. <i>Authors suggest reference method ED-RIA be used for serum FT4</i>				Chang et al 2007 aci (US EPA 2016a)
Single Oral Dose Study Evaluating serum binding protein – Female Sprague-Dawley Rats 5-15  Study duration 1 day	15 mg/kg  <i>Measured final serum concentration 61.58 ug/mL @24 hr</i> Predicted AUC ug/mL*h 666 (Wambaugh et al 2013)  Average serum concentration = Predicted AUC ug/mL-hr/(1 d x 24 hr/d) = 27.75 ug/mL	↓tT4 (24% @2hr; 38% @ 6hr & 53% @ 24hr) ↑fT4 (68% @2hr & 90% @6 hr) ↓tT3 & rT3 @24 hr only ↓serum & liver <sup>125</sup> I but ↑ in urine & feces ↑ME & UGT1A mRNA @24 hr; ↑ME activity @24hr				Chang et al 2008 aci (US EPA 2016a)
Thyroid cross-foster dietary study – Pregnant Wistar Rats	Doses not calculated and BW & food	CT, TC, & TT - ↓tT4 in offspring @PND21 & 35 (response in CT (71-75% of controls) & TT (63-64% of controls))				Yu et al 2009a aci (US EPA 2016a)

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Study Description – duration, route/ vehicle, species/ strain, age @dosing, N/sex/group, etc.	Admin Dose (mg/kg/d) <i>[average serum concen]<sup>#</sup></i>	Effect(s) Observed at each Serum Concentration (or Admin Dose)	Study POD <sub>HED</sub> (mg/kg/d) (e.g. NOAEL <sub>HED</sub> ) <i>[serum concen]<sup>#</sup></i>	UF <sup>1,2</sup>	Candidate RfD mg/kg-d	Reference (note limitations in comment filed)
Control diet or diet containing 3.2 mg PFOS/kg food Pups cross fostered	consump data not presented	grps larger than TC (80-81% of controls) grp				
Thyroid hormone transport protein transthyretin (TTR) binding study Tested PFASs chemicals capability for competing with T4 & displacing hormone from human TTR		PFOS ranked 2 <sup>nd</sup> highest in binding potency among PFASs tested. Binding potency ~1/15 of T4.  <i>EPA notes that ↓T3 &amp; T4 observed in adult monkeys &amp; rodents @ serum concen ~70-90 ug/mL. But pregnant &amp; neonatal rats appear more sensitive &amp; t14 ↓ appear @20-40 ug/mL. But TBG (rather than TTR) is the major thyroid hormone transporter in rats.</i>				Weiss et al 2009 aci (US EPA 2016a)
Comments:						

<sup>#</sup> Serum concentrations – Serum concentration value are superior to external dose as a POD. Several studies measured serum concentrations at specific time points. EPA performed PK modeling to calculate AUCs to determine an average serum concentration for each data set. Average serum concentration has the advantage of normalizing across the different exposure durations to generate a uniform metric for internal dose in situations where the dosing durations varied and serum measurements were taken immediately prior to sacrifice. Serum concentration data listed are from publication or as reported in EPA Tables 4-3 through 4-8 (US EPA 2016a).

<sup>1</sup> HED (Human Equivalent Dose) is calculated by multiplying the average serum concentration (ug/L) by the clearance rate. Clearance can be calculated from the rate of elimination (derived from half-life) and the volume of distribution:  $Vd \times (\ln 2 \div t_{1/2}) = 0.23 \text{ L/kg bw} \times (0.693 \div 1,971 \text{ days}) = 0.000081 \text{ L/kg bw/day}$ .

<sup>2</sup> Interspecies (animal to human) extrapolation denoted as A  
 Intraspecies variability (variability within human subpopulations – including life stages) denoted as H  
 Database uncertainty factor denoted as DB  
 LOAEL to NOAEL extrapolation denoted as L-to-N  
 Subchronic-to-chronic extrapolation denoted as S-to-C

**Table 6-A2. RfD Derivation**

**Identify the critical acute effects study selected by MDH:**

Critical study (source, date, rationale for the selection)

In this expedited review MDH has focused on key studies identified by EPA in the Dose Response Assessment of the Health Effects document (EPA 2016a) and has utilized the predicted average serum concentration as the preferred dose metric. The 2 generation study by Luebker et al 2005b was selected as the key study. This study includes exposure over all life stages.

BMD/BMDL values have been generated by authors for several of the key studies. However, substantial improvements have been made to the BMD software BMD modeling and therefore BMD modeling was also conducted by MDH when possible. *Note: BMD modeling results were not reported (or utilized) in EPA's 2016 final document. Rationale for not using BMD modeling (the preferred approach) was not provided. Developmental endpoints were difficult to model as suggested by EPA guidelines due to a lack of individual animal/litter data for using the required nested models. The non-nested results are shown for comparison purposes only and do not represent true quantitative candidate points of departure.*

A summary of key studies (MDH has also included the 2002 study in monkeys) along with estimated average serum concentrations @the NOAEL/LOAEL or BMD/BMDL<sup>a</sup> are presented below

Study (duration)	Effects	Average Serum Concentration (ug/mL)	
		NOAEL/LOAEL	BMDL/BMD <sup>a,b</sup>
Luebker et al 2005b 2 gen study	↓pup BW (F1 & F2)	25/99.7 (F1) 6.26/25 (F2)	<i>MDH BMDL/BMD:</i> <i>[for comparison purposes only]</i> 16.8/19.9 (F1 BW PND7) 5.9/9.6 (F2 BW PND7) (but only 2 dose grps)
Luebker et al 2005a 1 gen study	↓gestation length  ↓pup BW	19.9/39.7  NA/19.9	<i>Authors (BMDL/BMDs):</i> 15/22 <sup>a</sup> (gestation length) 13/19 <sup>a</sup> (pup BW PND 5) <i>[numerical data needed was unavailable – MDH unable to model]</i>
Butenhoff et al 2009 DNT study	↑motor activity & ↓habituation	10.4/34.6	NA



Thibodeaux et al & Lau et al 2003 Developmental study in rats	↓maternal & pup BW ↓pup survival	17.6/35.1 <i>[Note: ↓T4 was observed @NOAEL]</i>	<i>Authors BMDL/BMD<sub>5</sub>:</i> 2.8/4.1 (maternal BW) <sup>a</sup> 1.0/4.3 (↓T4) <sup>a</sup> 2.3/5.6 (fetal sternal defects) <sup>a</sup> 10.3/18.9 (pup survival) <sup>a</sup>  <i>MDH BMDL/BMD</i> 20.6/23.0 (maternal BW, BMR 10%) 5.57/10.1 (maternal tT4, BMR 20%) <i>[for comparison purposes only]</i> 9.71/12.1 (pup BW, BMR 5%) 8.56/10.8 (pup tT4, BMR 20%) <i>[MDH unable to successfully model fetal sternal defects or pup survival]</i>
Dong et al 2009	Altered immune parameters	0.75/7.4 (minimal LOAEL)	<i>[MDH modeling for comparison purposes only generated BMDL<sub>10</sub> of ~18.0 ug/L for PFC response. NOAEL of ~2 ug/L supported by Dong et al 2011]</i>
Seacat et al 2003 14 wk time-point within 2 yr rat study	Liver effects (Ms) ↑ALT & BUN	16.5/64.6	NA
Seacat et al 2002 6 month Monkey study	↑liver wt & histopath; ↓BW; ↓T3 & TSH	38/157	<i>MDH BMDL/BMD<sub>10</sub>:</i> 24.7/29.0 (rel liver wt)

<sup>a</sup> **NOTE:** BMD/BMDL values reported by authors were for administered dose and used older BMD software. MDH estimated average serum concentration that corresponded to the BMD/BMDL administered doses by using the relationship between the average serum concentration and the NOAEL or LOAEL administered dose which was consistent within a given study. See [Admin dose to Serum Extrapol spreadsheet](#). MDH conducted BMD modeling using most recent software and used predicted average serum concentration as the dose metric.

<sup>b</sup> MDH BMD/BMDL modeling reports can be found at [\\Data3fb\ch\HRA\COMMON\Guidance - Water\Tox reviews-completed\Final\PFOS\BMD Modeling](#).

Critical effect(s) and dose:  
(LOAEL<sub>HED</sub>/BMD<sub>HED</sub>)

Luebker et al 2005a & 2005b – reported decreased pup BW @ an administered dose of 0.4 mg/kg-d (LOAEL) and decreased pup survival @ an administered dose of 0.8 mg/kg-d (only a 2-fold difference). The average serum concentration at the LOAEL for the 2 generation study (Luebker et al 2005b) was calculated by EPA to be 25 ug/mL (or 25 mg/L). MDH conducted BMD modeling using EPA’s predicted average serum concentrations for the dose metric. Resulting BMD<sub>05</sub> values were 19.9 ug/ml (F1) and 9.64 ug/mL (F2) for decreased pup BW on PND 7 (time-point of largest effect).

Point of Departure The NOAEL for the 2 generation study was an administered dose of 0.1 mg/kg-d and the average serum concentration at this dose was calculated by EPA to be 6.26 ug/mL (or 6.26 mg/L). MDH conducted

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(NOAEL<sub>HED</sub>, LOAEL<sub>HED</sub>, BMDL<sub>HED</sub>) BMD modeling using EPA's predicted average serum concentrations for the dose metric. Resulting BMDL<sub>05</sub> values were 16.8 ug/ml (F1) and 5.91 ug/mL (F2) for decreased pup BW on PND 7. However, due to low survival in the two highest dose groups in the F1 generation only two treatment groups were carried through to F2 which compromises the utility of BMD modeling. The modeling results support a NOAEL of 6.26 ug/mL.

Human Equivalent Dose Adjustment: The following equation is used to calculate an HED from the POD serum concentration<sup>2</sup>:  

$$\text{HED (mg/kg-d)} = \text{POD}_{\text{ave serum concn}} \times \text{Clearance.}$$
*Where*  

$$\text{Clearance} = \text{Vol of Distribution (L/kg)} \times (\text{Ln}2/\text{human half-life}) = 0.23 \text{ L/kg} \times (0.693/1971 \text{ d}) = 0.000081 \text{ L/kg-d}$$

$$\text{HED} = 6.26 \text{ mg/L} \times 0.000081 \text{ L/kg-d} = 0.00051 \text{ mg/kg-d}$$

Uncertainty/Variability Factors:	Interspecies Extrapolation:	3	LOAEL-to-NOAEL:	NA	Database:	3
	Intraspecies Variability:	10	Subchronic-to-chronic:	NA	Other:	
	Total <sup>1</sup> : 100					

UF/VF Comments: Interspecies UF of 3 applied to address TD differences and in the absence of chemical information to the contrary the default value of 10 for Intraspecies Variability. Additional research regarding immunotoxicity is warranted. Dong et al 2009 reported a NOAEL/LOAEL serum level of 0.75/7.4 ug/mL. The LOAEL was associated with decreases in select immune markers (e.g., SRBC IgM plaque forming cells) with significant decreases in multiple markers at the next dose level up (36.9 ug/mL). The LOAEL serum concentration of 7.4 ug/mL is similar in magnitude to the critical study NOAEL serum level (6.26 ug/L) and raises concerns regarding potential immune suppression. In addition, effects reported in Dong et al 2011, which included an intermediate dose, support a LOAEL of 7.4 ug/L and a NOAEL of ~2 ug/L. Some epi studies have suggested an association between serum concentration and immune parameters such as decreased antigen levels, however, the associations have not been consistent across studies and specific studies examining pathogenic health outcome (e.g., increase in infections) have failed to find significant correlations. The recent NTP monograph and peer review concluded that the evidence of suppressed antibody response from animal studies was high and that the human studies provide a moderate level of evidence that PFOS is associated with suppression of the vaccination response. Epidemiological evidence to date do not indicate a functional decline in disease resistance. A DB UF of 3 has been applied to address concerns regarding immune suppression<sup>3</sup>.

**MDH RFD:** 0.00051/100 = 0.0000051 mg/kg-d [corresponding serum concentration 6.26/100=0.063 ug/mL]

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<sup>1</sup> Total UF for derivation of a HRL or HBV RfD is  $\leq 3000$  (RAA could be  $\leq 3000$  or  $> 3000$ )

<sup>2</sup> US EPA 2016 Lifetime Health Advisory Evaluation (USEPA 2016d):

Predicted serum concentrations are converted into an oral equivalent dose by recognizing that, at steady state, clearance from the body equals the dose to the body. Clearance (CL) can be calculated if the rate of elimination (derived from half-life) and the volume of distribution are both known. EPA used Olsen et al. (2007) calculated human half-life of 5.4 years and the Thompson et al. (2010) volume of distribution (Vd) of 0.23 L/kg body weight (bw) to determine a clearance of 0.000081 L/kg bw/day using the following equation:

$$CL = Vd \times (\ln 2 \div t_{1/2}) = 0.23 \text{ L/kg bw} \times (0.693 \div 1971 \text{ days}) = 0.000081 \text{ L/kg bw/day}$$

Where:

Vd = 0.23 L/kg

ln 2 = 0.693

$t_{1/2}$  = 1971 days (5.4 years  $\times$  365 days/year = 1971 days)

Multiplying the derived average serum concentrations (in  $\mu\text{g/mL}$ ) for the NOAELs and LOAELs by the clearance value predicts oral HEDs in mg/kg/day for each corresponding serum measurement. The HED values are the predicted human oral exposures necessary to achieve serum concentrations equivalent to the NOAEL or LOAEL in the animal toxicity studies using linear human kinetic information. *[MDH Note: this is the same equation used in the MDH 2007 evaluation to estimate HED values. Parameter values were identical, with the exception of Vd which was 0.2 L/kg instead of 0.23 L/kg.]*

<sup>3</sup> Immunotoxicity is an identified hazard of PFOA and PFOS exposure (as determined by NTP, 2016 and in MDH's identification as immune changes as a co-critical effect), however, the lack of dose response and indications of immune system deficits in functional responses to pathogenic challenges in even highly exposed cohorts, hampers quantitative inclusion of the effects observed in epi studies in deriving a reference dose (RfD). The presence of multiple perfluorinated compounds in human serum worldwide hampers the ability to determine the toxicity from a single chemical. However, the epidemiological literature provides a clear indication that the additivity of PFAS is strongly associated with immunosuppression. Coupled with these observations in human studies, alterations in animal immune function in response to sheep red blood cells has been shown to occur in replicated studies (e.g., Dong, 2009, Dong, 2011) at serum concentrations near the point of departure for the critical study. Therefore, MDH will include a 3-fold database uncertainty factor. MDH's current practice of comparing drinking water values to a composite hazard index of PFOA, PFOS, PFHxS, PFBA, and PFBS is well-justified and confers additional health protection benefits in the context of risk management, and underlies the choice for a 3-fold DBUF versus using a higher uncertainty factor.

## CRITICAL/KEY STUDY SUMMARY

### Critical Study(s): Luebker et al 2005b (from EPA, 2016)

#### 2 Generation Reproductive Study – Rats

Doses: A two-generation reproductive study was conducted in CrI:CD(SD)IGS VAF rats with five groups of 35 rats/sex/group administered 0, 0.1, 0.4, 1.6, or 3.2 mg/kg/day of PFOS by gavage for 6 weeks prior to and during mating (Luebker et al. 2005b). Treatment in males continued through the cohabitation interval, and females were treated throughout gestation, parturition, and lactation.

Effects: *F0*: In the *F0* generation male rats, mortality, clinical signs, and mating/fertility parameters were unaffected. During pre-mating, decreases in terminal body weight, body weight gain, and food consumption occurred at 1.6 and 3.2 mg/kg/day in males. The only effect

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on weight of the organs evaluated was a significant reduction in the absolute weight of the seminal vesicles (with fluid) and prostate in males administered 3.2 mg/kg/day. In the F0 generation female rats, there were no deaths and no effects on the reproductive parameters measured in both dams sacrificed on GD 10 and those allowed to deliver naturally. The F0 dams administered  $\geq 0.4$  mg/kg/day had localized alopecia during pre-mating, gestation, and lactation, and a decrease in body weight and food consumption.

*F1 Generation:* The F1 generation pup viability was significantly reduced at 1.6 and 3.2 mg/kg/day, therefore only the 0.1 and 0.4 mg/kg/day dose groups were carried into the second generation. Twenty-five F1 rats/sex/dose were administered 0, 0.1, or 0.4 mg/kg/day of PFOS by oral gavage beginning at weaning on post-natal day (PND) 22 and continuing until sacrifice. One rat/sex/litter was tested in a passive avoidance paradigm at 24 days of age and one rat/sex/litter was evaluated in a water-filled M-maze on PND 70. On PND 28, females were evaluated for vaginal patency and on PND 34 males were examined for preputial separation. On PND 90, rats were assigned within each dose group to cohabitation, and once confirmed pregnant, the females were housed individually. The F1 generation male rats were sacrificed after mating, necropsied, and evaluated as described in the F0 generation. All F1 generation females were allowed to deliver and were sacrificed and necropsied on LD 21.

Mortality occurred in the F1 offspring of dams administered 1.6 or 3.2 mg/kg/day. At 1.6 mg/kg/day, over 26% of the pups were found dead between LDs 2 and 4. At 3.2 mg/kg/day, 45% of the pups were found dead on LD 1, with 100% dead by LD 2. The dams dosed with 3.2 mg/kg/day also had a significant increase in stillborn pups and the viability index was 0% at 3.2 mg/kg/day and 66% at 1.6 mg/kg/day. The lactation index was 94.6% at 1.6 mg/kg/day. At 3.2 mg/kg/day, there were significant decreases in gestation length and number of implantation sites, and reductions in litter size. Statistically-significant decreases in pup body weight were also observed at the two highest doses. Additional adverse effects in pups at 3.2 mg/kg/day included impacts on lactation (i.e., high number [ $\sim 75\%$ ] of pups not nursing and not having milk present in the stomach), an increased incidence of stillborn pups, and a high incidence of maternal cannibalization of the pups.

In the F1 generation offspring, pups administered 3.2 mg/kg/day could only be evaluated on LD 1 due to the high mortality. All viable pups from the 1.6 mg/kg/day group had significantly ( $p < 0.05$  or  $0.01$ ) delayed eye opening, pinna unfolding, surface righting, and air righting during lactation. No delays were observed in rats administered doses  $\leq 0.4$  mg/kg/day. Sexual maturation was not affected in the 0.1 and 0.4 mg/kg/day groups after weaning. The results from the passive avoidance (beginning at 24 days of age) and water maze tests (beginning at 70 days of age) for neurobehavioral effects showed no dose-related effects on learning and memory.

Since F1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups, only the 0.1 and 0.4 mg/kg/day dose groups were carried into the second generation.

*F2 Generation* – F1 parental animals displayed no clinical signs or mortality. Food consumption was transiently decreased in F1 males, but it was not affected in F1 females. Reproductive performance was unaffected in the F1 dams. All F2 generation pups were sacrificed, necropsied, and examined on LD 21 as previously described for the F1 generation pups. In the F2 generation pups, decreases in mean pup body weights were observed at 0.1 mg/kg/day on LDs 4 and 7, but mean pup body weights were similar to controls by LD 14. The pups in the 0.4 mg/kg/day group displayed significant decreases in body weight on LDs 7–14; after LD 21, body weights remained lower than controls, but were not statistically-significant. No other treatment-related effects were observed.

- F0 NOAEL = 0.1 mg/kg-d  
LOAEL = 0.4 mg/kg-d, based on decreases in body weight gain
- F1 NOAEL = 0.4 mg/kg-d  
LOAEL = 1.6 mg/kg/day based on the significant decrease in the pup viability, pup weight, and survival
- F2 NOAEL = 0.1 mg/kg-d  
LOAEL = 0.4 mg/kg/day, based on the significant decreases in mean pup body weight

#### Co-critical Study(s):

##### Developmental study in Pregnant Sprague-Dawley Rats (Thibodeaux et al 2003)

Goups of 9–16 pregnant Sprague-Dawley rats were administered 0, 1, 2, 3, 5, or 10 mg/kg PFOS in 0.5% Tween-20 daily by gavage during gestational days (GDs) 2–20. Measured final serum concentrations: 19.69, 44.33, 70.62, 79.39, & 189.4 ug/mL. EPA modeled average serum concentration = 17.6, 35.1, 52.6, 87.9, and 175 ug/mL. Rats were euthanized on GD 21 and uterine contents examined.

Maternal body weight, food consumption and water consumption were significantly decreased ( $p < 0.0001$ ) in a dose-dependent manner at  $\geq 2$  mg/kg. Liver weight was not affected in the treated rats. Serum chemistry showed significant decreases in cholesterol (decrease of 14% compared to controls) and triglycerides (decrease of 34% compared to controls) at 10 mg/kg. Serum thyroxine (T4) and T3 were significantly decreased in all treated rats when compared to controls, however, a feedback response on TSH was not observed. The number of implantations or live fetuses at term was not affected by treatment. There was a decrease in fetal weight, and birth defects such as cleft palate, ventricular septal defect, and enlargement of the right atrium were observed at 10 mg/kg, but the litter incidence rates were not given.

##### Developmental study in Pregnant Sprague-Dawley Rats (Lau et al. 2003 - - companion study to the one by Thibodeaux et al. 2003 above)

Conducted in order to examine the post-natal impact of *in utero* exposure to PFOS.

On GD 22, dams were monitored for signs of parturition. In dams administered 10 mg/kg/day, the neonates became pale, inactive, and moribund within 30–60 minutes of birth and all died. In 5 mg/kg/day dams, the neonates became moribund after 8–12 hours, with 95% dying within the first 24 hours. A 50% fetal mortality was observed in dams administered 3 mg/kg/day. Pups from dams treated with 2 mg/kg/day still had significant increases in mortality, but those from dams administered 1 mg/kg/day were similar to controls. No differences were observed in liver weight in the neonates. Pup body weight was significantly decreased in dams administered  $\geq 2$  mg/kg/day. A significant ( $p < 0.05$ ) delay in eye opening was observed at the same dose in the pups, but no differences in onset of puberty were observed at that dose. On PND 2, serum levels of both total T4 and free T4 were decreased significantly in all the treated groups, but total T4 recovered to levels similar to those of controls by weaning. No changes were observed in serum T3 or TSH. Choline acetyltransferase activity in the prefrontal lobe, which is sensitive to thyroid status, was slightly reduced in rat pups, but activity in the hippocampus was not. T-maze testing did not demonstrate any learning deficiencies. Based on the findings, the developmental LOAEL is 2 mg/kg/day PFOS for mortality, decreased body weight, and a significant 1-day delay in eye opening; the NOAEL is 1 mg/kg/day. The authors calculated a BMDL5 for a 6 day survival of 7.02 mg/kg/day.

#### Developmental Gavage Study CrI:CD(SD)IGS VAF Rats - Luebker et al 2005a

Animals (n=20/dose) were exposed 6 weeks prior to mating through LD4. Additional 8/grp in control, 1.6 & 2.0 mg/kg-d only were sac'd on GD20 for assessment. Dose groups: 0, 0.4, 0.8, 1.0, 1.2, 1.6, & 2.0 mg/kg-d. Serum concentrations were not measured, however, EPA modelled average serum concentrations were 19.9, 39.7, 49.6, 59.5, 79.4, and 99.2 ug/mL.

Maternal Effects: No mortality occurred and no effects were observed in reproductive parameters (corpora lutea, implantations, fetuses/litter) in those dams receiving C-sections. In the group sacrificed on LD 5, a significant decrease in gestation length was observed at doses  $\geq 0.8$  mg/kg. Overall absolute body weights of the dams were reduced slightly (5%–7% of that for the controls) in the 1.6 and 2.0 mg/kg/day group dams during gestation; the changes, although slight, were statistically significant. Body weight change was significantly reduced ( $p < 0.05$  or  $0.01$ ) during pre-mating, gestation and lactation. For dams allowed to deliver, the fertility index, implantations per delivered litter, gestation index, live births, and delivered pups/litter were similar between treated and control dams. Flat dose-response for decreased serum cholesterol was observed in treated animals (16\*\*, 24\*\*, 25\*\*, 23\*\*, 27\*\*, & 23%\*\*; \*\*= $p < 0.01$ ) with increased relative liver weight reported  $>0.8$  mg/kg-d.

Developmental Effects: Offspring viability was decreased starting at 0.8 mg/kg and was statistically significant at 1.6 and 2.0 mg/kg. The viability indices were 97.3%, 97.6%, 93.1%, 88.8%, 81.7%, 49.3%, and 17.1% at 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg, respectively. Lipids, glucose utilization, and thyroid hormones were similar or slightly different for treated animals compared to controls. In all treated groups, pup body weight at birth on PND 5 was significantly less than that of controls. In one male and one female pup at 2.0 mg/kg/day, the heart and thyroid were collected and examined microscopically. No lesions were found when compared to the controls.

#### DNT Gavage Study (Butenhoff et al 2009)

Female Sprague-Dawley Rats (25/dose) were exposed GD 0 to PND 20 to 0, 0.1, 0.3, or 1.0 mg/kg/day. Measured final serum levels during Gestation: 1.72, 6.245, or 26.63 ug/mL and Gestation + Postnatal: 3.16, 8.98 & 30.48 ug/mL. EPA modeled average serum concentrations: Gestation - 2, 6.0, and 20 ug/mL and Gestation+PND - 3.5, 10.5, and 34.7 ug/mL. Offspring monitored through PND72.

Maternal Effects: slight but not statistically significant decrease in body weight gain

Developmental Effects: No treatment related effects were observed on functional observational battery assessments performed on PNDs 4, 11, 21, 35, 45, and 60. Male offspring from dams administered 0.3 and 1.0 mg/kg/day had statistically-significant ( $p < 0.05$ ) increases in motor activity on PND 17, but this was not observed on PND 13, 21 or 61. No effect on habituation was observed in the 0.1 and 0.3 mg/kg/day males or in the 1.0 mg/kg/day females. On PND 17, males at 1.0 mg/kg/day showed a lack of habituation as evidenced by significantly ( $p < 0.05$ ) increased activity counts for the sequential time intervals of 16–30, 31–45, and 46–60 minutes. The normal habituation response is for motor activity to be highest when the animals are first exposed to a new environment and to decline during later exposures to the same environment as they have learned what to expect. There were no effects in males or females on acoustic startle reflexes or in the Biel swimming maze trials. Mean absolute and relative (to body weight) brain weight and brain measurements (length, width) were similar between the control and treated animals.

#### Lung Development Study (Chen et al 2012)

Sprague-Dawley Rats (N=6 offspring/grp) were exposed from GD1-21 to 0, 0.1, or 2 mg/kg/day. Offspring were sacrificed at PND21. Measured serum concentrations were reported to be 1.7 and 47.5 ug/mL. EPA modeled average serum concentrations: 1.9 and 38.5 ug/mL. Lung tissue was assessed for markers of oxidative stress and cytoplasmic protein and examined histologically. Three additional groups of 10 rats/dose were treated as described above and the number of deaths/litter recorded until PND 4.

Body weight of the pups was decreased (~20% @ PND21) and postnatal pup mortality (by PND 3) was increased significantly ( $p < 0.05$  and  $0.01$ , respectively) at 2.0 mg/kg/day, when compared to the control litters. No treatment-related findings were observed at 0.1 mg/kg/day. Postnatal pup mortality in the control, 0.1, and 2.0 mg/kg/day groups on PND 3 was approximately 4%, 3%, and 23%, respectively. Histopathological changes

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observed in pup lungs at 2.0 mg/kg/day on PND 0 included marked alveolar hemorrhage, thickened interalveolar septum, and focal lung consolidation. On PND 21, the lungs also had alveolar hemorrhage, thickened septum, and inflammatory cell infiltration. Numerous apoptotic cells were observed. An increase in biomarkers associated with oxidative stress was found in pups from the 2.0mg/kg/day dams.

#### 28 day Dietary Study in Adult Rats (Curran et al 2008)

Sprague Dawley Rats (15/sex/dose) were administered 0, 2, 20, 50, or 100 mg/kg diet. Administered doses were calculated to be 0.14/0.15, 1.33/1.43, 3.21/3.73, 6.34/7.58 (M/F) mg/kg/day. Serum concentrations at termination were measured to be 0.95/1.5, 13.45/15.4, 20.93/31.93, and 29.88/43.2 ug/mL. EPA modeled average serum concentrations were 2.7/3.7, 25.9/35.4, 62.6/92.4, and 123.7/187.5 ug/mL.

There were no treatment-related differences observed in hematology and urinalysis parameters. Statistically-significant ( $p \leq 0.05$ ) decreases in body weight and food consumption were observed in the males and females administered  $\geq 50$  mg PFOS/kg diet. Food consumption was also statistically decreased in males during week 3 of treatment in the 20 mg PFOS/kg diet group. No differences in blood pressure measurements were observed across the groups. Deformability index values in red blood cells over a range of shear stress levels were significantly lower in both males and females exposed to 100 mg PFOS/kg diet, relative to controls. Absolute and relative liver weights were statistically-significantly increased in the male and female rats at  $\geq 20$  mg PFOS/kg diet. Relative liver weight was also statistically increased in the 2 mg PFOS/kg diet females. Histopathological changes were observed in the liver of the males treated with  $\geq 50$  mg PFOS/kg diet and included hepatocyte hypertrophy and an apparent increase in cytoplasmic homogeneity. Increased hepatocyte hypertrophy and cytoplasmic homogeneity in the females was seen at  $\geq 50$  mg PFOS/kg diet. At the high doses, the serum levels of conjugated bilirubin and total bilirubin were increased significantly. Serum cholesterol was significantly decreased for males and females at  $\geq 50$  mg PFOS/kg diet. Serum T4 and T3 levels were also decreased in males and females, with T4 levels being statistically-significantly decreased at  $\geq 20$  mg PFOS/kg diet, when compared to the control levels.

#### Single dose study evaluating serum protein binding in Sprague-Dawley Rats (Chang et al 2008) –

Female Sprague-Dawley Rats (5-15) were given a single dose of 0 or 15 mg/kg PFOS. Measured final serum concentration in treated animals was 61.58 ug/mL @24 hr. EPA modeled average serum concentration was 27.75 ug/mL.

Serum TT4 decreased significantly ( $p < 0.05$ ) compared to controls after 2 hours (decrease of 24%), 6 hours (decrease of 38%), and 24 hours (decrease of 53%). The TT3 and rT3 only decreased significantly at the 24-hour time-point, while FT4 was increased significantly at 2 and 6 hours (68% and 90% over control, respectively) before becoming similar to that of controls at the 24-hour time-point.

In the second part of the study, Sprague-Dawley rats were injected intravenously with either 9.3  $\mu$ Ci (females;  $n = 5$ /group) or 11  $\mu$ Ci (males;  $n = 4$ /group) of 125I-T4 followed by a single oral dose of either vehicle or 15 mg potassium PFOS/kg bw. At the end of the 24 hours, the animals were killed and liver and serum samples collected. Serum TT4 concentration was decreased by 55% in the PFOS treated males and females compared to controls. There was also a decrease in serum 125I in the treated males. Liver 125I radioactivity decreased by 40% and 30% in males and females, respectively, but the urine and feces 125I radioactivity increased, with the males exhibiting the most activity. This indicates a loss of thyroid hormones and increased turnover.

Results suggest that oral PFOS administration results in a transiently increased tissue availability of thyroid hormones, increased turnover of T4, and a reduction in TT4, but PFOS administration does not induce a typical hypothyroid state or alter the hypothalamic-pituitary-thyroid axis.

Developmental Gavage study – glucose and lipid homeostatis (Lv et al 2013) –

The impact of gestational and lactational exposure to PFOS on glucose and lipid homeostasis in offspring was investigated. Groups of 6 pregnant SPF Wistar rats were given doses of 0, 0.5, or 1.5 mg/kg/day dissolved in 0.5% Tween 20 from GD 0 to PND 20. After birth, pups were sexed, randomly selected and cross-fostered to insure there were equal pups per litter (5 male and 5 female). Pup weights were determined on PNDs 0, 5, 10, 15, and 21. Serum and liver samples were also collected at PND 0 and 21 from an unspecified number of pups. The remaining pups were maintained for 19 weeks after weaning before final sacrifice. Blood samples were collected at 10 and 15 weeks after weaning and examined for fasting serum triglycerides, total cholesterol, and fasting blood glucose. A glucose tolerance test was administered after a 16-hour overnight fast. Other parameters evaluated included serum insulin, leptin, and adiponectin, and gonadal fat weight, pancreatic beta cell area, fat accumulation in the liver as monitored through oil red and hematoxylin and eosin staining. Body weight of pups from treated dams was significantly reduced ( $p < 0.05$ ) at birth, throughout lactation, and persisted until week 8 post-weaning. A dose-related increase in glucose intolerance was observed at 10 weeks post-weaning in pups from treated dams with statistical significance attained at 1.5 mg/kg/day. At 15 weeks, pups from the 0.5 mg/kg/day dams had significantly increased glucose intolerance, while that for high-dose pups was increased but did not attain statistical significance. At 18 weeks after weaning, pups from dams given 1.5 mg/kg/day had significant increases in serum insulin, insulin resistance index, and serum leptin. Serum adiponectin was significantly decreased in pups from both treated groups compared with that of controls. At sacrifice, pups from both treated groups had a significant increase in epigonadal fat pad weight, and fat accumulation was observed in the liver of high-dose animals.

2 year Dietary Study in Sprague-Dawley Rats (Thomford 2002, Butenhof et al 2012) –

Sprague-Dawley CrI:CD (SD)IGS BR, rats ( $n = 40-70$ ) were dosed using a PFOS containing diet for up to 105 weeks. Five per sex per dose group were sacrificed at 4 and 14 weeks. Treatment resulted in decreased body weight, with increased liver weight with hepatocellular hypertrophy. Corresponding PFOS doses were 0, 0.024, 0.098, 0.24, and 0.984 mg/kg/day, respectively, for males and 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day, respectively, for females. Five animals/sex in the treated groups were sacrificed during week 53 and liver samples were obtained for mitochondrial activity, hepatocellular proliferation rate, and determination of palmitoyl-CoA oxidase activity; liver weight was recorded. The clinical serum observations for ALT at 53 weeks were consistent with those at 14 weeks in demonstrating significant ( $p < 0.05$ ) increases for the high dose males but not females. At week 27, ALT was increased for high-dose males, but did not attain statistical significance. For males at 53 weeks in the 0, 0.5, 2, 5, and 20 ppm groups, ALT values were  $54 \pm 66$ ,  $62 \pm 52$ ,  $40 \pm 7.5$ ,  $44 \pm 8.3$ , and  $83 \pm 84$  IU/L, respectively. The large SDs were the result of high values in one animal in each of the control and 0.5 ppm groups and two animals in the 20 ppm group. Thus, some animals may be more sensitive to liver damage as a result of exposure than others. AST levels were not increased for either sex. Serum blood urea nitrogen (BUN) was significantly ( $p \leq 0.05$ ) increased at 20 ppm for males and females at weeks 14, 27, and 53 and in 5 ppm males and females at 27 and 53 weeks. The males in the 2 ppm group also had a significant ( $p \leq 0.05$ ) increase in BUN at 53 weeks. At sacrifice, males at 2 ppm had a significant ( $p < 0.05$ ) increase in hepatocellular centrilobular hypertrophy. In the males and females at 5 and 20 ppm, there were significant ( $p < 0.05$ ) increases in centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules (females only), and centrilobular hepatocytic vacuolation (males only). At the high dose, there was a significant increase in the number of animals with single cell hepatic necrosis in both males and females at 53 weeks.

1 to 5 day Gavage Study in Female Sprague-Dawley Rats (Martin et al 2007) –

10 mg PFOS/kg was administered to adult male Sprague-Dawley rats ( $n = 5$ ) for 1, 3, or 5 days by oral gavage and determined the impact of PFOS on hormone levels. Following a 1-day, 3-day, and 5-day dose, a significant decrease ( $p < 0.05$ ) was observed in total T4 (~ decrease of 47–80%) and free T4 (~ decrease of 60–82%). The total T3 was only significantly decreased after day 5 (decrease of ~ 23%). PFOS treatment caused

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hepatomegaly, hepatocellular hypertrophy, and macrovesicular steatosis. Genes associated with the thyroid hormone release and synthesis pathway included type 3 deiodinase DIO3, which catalyzes the inactivation of T3 and type 1 deiodinase DIO1, which deiodinates prohormone T4 to bioactivate T3. Treatment with PFOS caused significant ( $p < 0.05$ ) DIO1 repression and Dio3 induction only on day 5.

Developmental Study in Mice – prenatal assessment (Thibodeaux et al 2003) and postnatal assessment (Lau et al 2003) –

A two-part developmental study with PFOS was performed in mice. Groups of 20–29 CD-1 mice were administered 0, 1, 5, 10, 15, or 20 mg/kg/day PFOS during GDs 1–17. Serum concentrations were not reported. EPA modeled average serum concentrations: 33.1, 141, 218, 260, and 289 ug/mL.

Maternal body weight gain was significantly decreased at 20 mg/kg/day. Food and water consumption were not affected by treatment. PFOS treatment increased ( $p < 0.05$ ) the liver weight in a dose-dependent manner in the mice. T4 was decreased in a dose-dependent manner on GD 6 with statistical significance ( $p < 0.05$ ) attained for the 20 mg/kg/day group; levels of T3 and TSH were not affected by treatment. A significant increase in post-implantation loss was observed in animals administered 20 mg/kg/day, and reduced fetal weight ( $p < 0.05$ ) was observed from dams in the 10 and 15 mg/kg/day groups. Birth defects such as cleft palate, ventricular septal defect, and enlargement of the right atrium were observed at doses  $\geq 10$  mg/kg.

Most mouse pups from dams administered 15 or 20 mg/kg/day did not survive for 24 hours after birth. Fifty percent mortality was observed at 10 mg/kg/day. Survival of pups in the 1 and 5 mg/kg/day treated dams was similar to controls. A significant ( $p < 0.0001$ ) increase in absolute liver weight was observed at  $\geq 5$  mg/kg/day. A significant delay in eye opening was observed. No dose- or treatment-related effects were observed on T4, T3, and TSH levels in the pups.

All neonates in the 20 mg/kg/day dose group were born pale, weak, and inactive, and all died within a few hours of birth. At 10 mg/kg/day, 45% of those born died within 24 hours. Survival of the 1 mg/kg/day group was similar to that of controls. Neonatal weight was significantly decreased at 10 and 20 mg/kg/day. In the fetuses from dams treated with 20 mg/kg/day, there were large numbers of cleft palates (98.56%), sternal defects (100%), delayed ossification of phalanges (57.23%), wavy ribs (84.09%), spina bifida occulta (100%), and curved fetus (68.47%). Similar defects were observed in the fetuses from dams treated with 10 mg/kg/day except at a lower incidence. Histopathological exam showed that all fetuses examined on GD 18 from dams treated with 20 mg/kg were alive and had normal lung structures but mild to severe intracranial dilatation of the blood vessels. Neonates from the 20 mg/kg treated dams had fetal lung atelectasis (partial or complete collapse of the lung or a lobe of the lung) with reduction of alveolar space and intracranial blood vessel dilatation when examined histopathologically. Three neonates from each of the five dams treated with 10 mg/kg were examined, and 27% had slight lung atelectasis and 87% had mild to severe dilatation of the brain blood vessel.

60 day Immunotoxicity Study in Mice (Dong et al 2009) –

In order to observe chronic effects of immunotoxicity, adult male C57BL/6 mice (10/group) were administered 0, 0.008, 0.083, 0.417, 0.833, and 2.083 mg/kg/day PFOS with 2% Tween 80 in de-ionized water daily by gavage for 60 days. Measured final serum concentrations 0.674, 7.132, 21.638, 65.426, or 120.67 ug/mL. EPA modeled average serum concentrations 0.75, 7.4, 36.9, 73.6, & 180.6 ug/mL. At sacrifice, mice treated with  $\geq 0.417$  mg/kg/day had significantly lower body weight compared to the control mice, as well as significant decreases in spleen, thymus and kidney weight. Food consumption in the study was decreased in mice at 0.833 and 2.083 mg/kg/day. Liver weight was increased significantly in all dose groups compared to controls,  $5.17 \pm 0.12$  g (control),  $5.21 \pm 0.17$  g,  $5.78 \pm 0.13$  g,  $6.67 \pm 0.11$  g,  $8.17 \pm 0.21$  g.

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g, and  $11.47 \pm 0.12$  g, respectively. Serum corticosterone was decreased in mice at the two higher doses. As in the shorter-term study, thymic and splenic cellularity was decreased in a dose-dependent trend, with significant decreases observed in mice receiving  $\geq 0.417$  mg/kg/day. The CD4/CD8 marker analysis performed on splenic and thymic lymphocytes demonstrated that the numbers of T cell and B cell CD4/CD8 subpopulations were decreased starting at 0.417 mg PFOS/kg/day. Splenic NK cell activity was increased significantly compared to controls ( $31.14 \pm 1.93\%$ ) in the mice at 0.083 mg/kg/day ( $45.43 \pm 4.74\%$ ) with significant marked decreases at 0.833 mg/kg/day ( $20.28 \pm 2.51\%$ ) and 2.083 mg/kg/day ( $15.67 \pm 1.52\%$ ). The SRBC-specific IgM plaque forming cell response showed a dose-related decrease with statistical significance at 0.083 mg/kg/day and higher.

#### Developmental Immunotoxicity Study in Mice (Keil et al 2008) –

Pregnant C57BL/6N females (bred with male C3H/HeJ mice) were treated with PFOS to evaluate developmental immunity in their inbred B6C3F1 offspring. The females (10–12/group) were administered 0, 0.1, 1, or 5 mg/kg of PFOS in 0.5% Tween-20 by gavage daily on gestation days (GDs) 1–17. Pups remained with the dam for approximately 3 weeks with immunotoxicity evaluations performed at 4 and 8 weeks. One male and one female were selected from the retained litters (total of 6 male and 6 female pups) for testing of the immunotoxicity parameters; positive controls were included for each assay.

NK cell activity was not altered in any pups at 4 weeks old. At 8 weeks, however, NK cell activity was suppressed in males treated with 1 and 5 mg/kg/day (42.5% and 32.1% decreases compared to controls, respectively) and in females at 5 mg/kg/day (35.1%, compared to controls). The plaque-forming cell response for SRBC IgM production by B cells was only assessed at 8 weeks and was significantly suppressed in the 5 mg/kg/day males (53%); no effect was observed in the females. The only significant differences in lymphocyte immunophenotypes was a 21% decrease in absolute numbers of B220+ cells in 4-week-old females in the 5 mg/kg/day group compared to controls; this effect was not observed at 8 weeks. The other significant change was a 25% decrease in CD3+ and 28% decrease in CD4+ thymocytes at 5 mg/kg/day in males at the 8-week evaluation. Functional responses (nitrite production) to LPS and interferon-gamma by peritoneal macrophages were not affected with treatment in the 8-week-old mice (not evaluated at 4 weeks).

#### 21 day Immune Challenge Study in Mice (Guruge et al 2009) –

0, 5, or 25  $\mu$ g/kg PFOS (0, 0.005, or 0.025 mg/kg, respectively) was administered to 30 female B6C3F1 mice/group for 21 days and then exposed them intranasally to 100 plaque forming units (pfu; in 30  $\mu$ L of phosphate buffered saline) influenza A virus suspension. Mice were observed for 20 days past inoculation. There was not a significant change in body or organ weights (spleen, thymus, liver, kidney, and lung) of the treated mice compared to the controls. Survival rate was significantly decreased in the mice at the highest dose group after viral exposure. Survival rate in the mice on day 20 was 46% in the controls and 17% in the high-dose group.

#### 26 week Capsule Study in Cynomolgus Monkeys (Seacat et al 2002) –

PFOS administered in a capsule by gastric intubation to 6/sex/dose at 0, 0.15 or 0.75 mg/kg-d & 4/sex/dose – 0.03 mg/kg-d. Measured serum concentrations: (M/F) 15.8/13.2, 82.6/66.8, 173/171  $\mu$ g/mL. EPA modeled average serum concentration 7.7, 38.0 and 156.6  $\mu$ g/mL. Low dose animals exhibited decreased cholesterol (M/F 28\*\*/24, 3/19, & 68\*\*/49%\*\* , \*\*p<0.01) and HDL cholesterol (M/F 33\*\*/25, 24/36\*\* , & 79\*\*/63%\*\* , \*\*p<0.01). Mid dose animals exhibited increase liver weights (abs (M/F 4/12 & 55/47%\* , \*p<0.05) & rel (M/F 12.5/17 & 69\*/61%\* , \*p<0.05) as well as changes in serum thyroid hormone levels (decreased T3 (M/F 12/22 & 48\*\*/33%\*\* , \*\*p<0.01) and increased TSH (M/F 151/30 & 160\*/82% , \*p<0.05). Two of the 0.75 mg/kg/day males died; one died on day 155 and one was found moribund and was sacrificed on day 179. The monkey that died had pulmonary necrosis and severe acute recurrences of pulmonary inflammation as its cause of

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death. The specific cause of the moribund condition was not established, however, the clinical chemistry results were suggestive of hyperkalemia. Overall mean body weight gain was significantly ( $p \leq 0.05$ ) less in the 0.75 mg/kg/day males and females (lost  $8 \pm 8\%$  and  $4 \pm 5\%$ , respectively) after the treatment when compared to controls (gained  $14 \pm 11\%$  and  $5 \pm 5\%$ , respectively). Two high dose males and one high-dose female had mottled livers on gross examination at sacrifice; this was also observed in the high-dose male that died during the study. All females and 3/4 males at the high-dose had centrilobular or diffuse hepatocellular hypertrophy.

Table 6-A3. Co-critical Effects Summary

<p><b>Critical LOAEL =</b></p>	<p><b>25 ug/mL serum concentration @LOAEL from Luebker et al 2005b</b></p> <p><i>[NOTE: Not all studies have measured or calculated serum concentrations. When appropriate the oral dose vs predicted average serum concentration relationship for various strains/species/durations was used to assist in identifying whether the effects reported in studies which did not have average serum concentration likely occurred at serum concentrations at or below ~40 ug/mL (approximately 1.5-fold of the serum benchmark above.)]</i></p>
<p><b>Study (source and date) and Effects observed:</b></p>	<p><i>*Studies with EPA modelled average serum concentrations are presented first, followed by studies which were identified by extrapolating from the dose vs predicted serum concentration relationships (see relevant worksheet within the Excel file at O:\HRA\COMMON\Guidance - Water\Tox reviews-completed\Final\PFOS\EPA 2016HA PFOS AdmDoseToSerumExtrap.xlsx ]</i></p> <p><b>Rats -</b></p> <ol style="list-style-type: none"> <li>1. Developmental study in Pregnant Sprague-Dawley Rats (GD2 to~GD21) (Thibodeaux et al 2003 &amp; Lau et al 2003):                  @17.6 ug/mL [adm dose 1 mg/kg-d] - <b>Maternal effects - ↓fT4 (MDH BMD<sub>20</sub> = 10.1 ug/mL) &amp; FT4 by GD7. Developmental effects - ↑sternal defects &amp; low T4</b>                  @35.1 ug/mL [adm dose 2 mg/kg-d] -↓ Maternal BW (MDH BMD = 23 ug/mL); <b>Developmental - ↓pup BW &amp; survival &amp; delayed eye opening.</b></li> <li>2. Developmental study in Crl:CD(SD) Rats (6 wk prior to mating thru LD4) (Luebker et al 2005a)                  @19.9 ug/mL [adm dose 0.4 mg/kg-d] - <b>Maternal ↓serum cholesterol. Developmental - ↓pup BW/BWG &amp; ↓fT4</b> but no clear dose response and only seen with one of two methods used. <i>[MDH Notes: decreased fT4 observed in this study will not be included as co-critical]</i>                  @39.7 ug/mL [adm dose 0.8 mg/kg-d] - <b>Maternal - ↓gestation duration (statis signif but only 1.7% different than controls), ↓maternal BW during gestation, ↑rel liver wt.</b></li> <li>3. Gavage DNT study in Sprague-Dawley Rats (GD0-PND20) (Butenhoff et al 2009)                  @34.7 ug/mL [adm dose 1 mg/kg-d] - <b>Developmental effects - ↑motor activity with ↓ habituation on PND17</b> but not other observation days. <i>[MDH Notes: EPA identified a LOAEL based on this endpoint. In the 2014 draft HESD EPA selected this study and endpoint as the basis of the draft RfD. MDH determined that study was of sufficient quality to list observed effects]</i></li> <li>4. Gavage Lung development study in Sprague-Dawley Rats (GD1-21) (Chen et al 2012)                  @35.8 ug/mL [adm dose 2 mg/kg-d] - <b>↓pup BW &amp; survival as well as histopathological changes in pup lungs</b></li> <li>5. 28 day dietary study in Sprague-Dawley Rats (Curran et al 2008)                  @&gt;3.7 ug/mL (F) [adm dose ~0.15 mg/kg-d] - <b>12% ↑rel liver wt.,</b> however, histological changes not seen until two dose levels up (92.4 ug/mL or admin dose of 3.73 mg/kg-d]</li> </ol>

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	<p>@25.9/35.4 (M/F) ug/mL [adm dose ~1 mg/kg-d] – 12/22% ↑rel liver wt. w/ histological changes, etc. seen at next dose levels up (~2-fold higher); ↓T4 (M/F 82/48%)</p> <p>6. Single dose study evaluating serum protein binding in Sprague-Dawley Rats (Chang et al 2008) @27.75 ug/mL [adm dose 15 mg/kg-d] - ↓tT4 &amp;tT3 as well as ↑fT4</p> <p><i>Rat studies – estimated serum concentrations</i></p> <p>a. DW DNT study in pregnant Wistar Rats (GD1 – PND21) (Wang et al 2015) @0.8 mg/kg-d adm dose (based on Butenhoff et al 2009 - since dosing was via DW instead of gavage the serum levels would likely be lower than Butenhoff and therefore below the co-critical benchmark of ~40 ug/mL) – Developmental effects - ↑water maze escape latency [MDH Notes: effects at this dose level were marginal and EPA considered the response to be a NOAEL. Effects will not be included as co-critical]</p> <p>b. Gavage neurodevelopmental study in pregnant Sprague Dawley Rats (GD2-12) (Zeng et al 2011) @&gt;0.1 &amp; 0.6 mg/kg-d adm dose (based on Butenhoff et al 2009 est serum levels likely &lt; 40 ug/mL) – Effects changes in number GFAP cells, mRNA expression, etc. [MDH Notes: significance or relationship of these effects to functional effects is unclear. Not listed as critical effects at this time.]</p> <p>c. HPT 28 day gavage study in adult male Sprague Dawley Rats (Lopez-Duval et al 2014) @0.5 &amp; 1.0 mg/kg-d adm dose (based on Curran et al 2008 est serum levels likely &lt; 40 ug/mL) – reported ↓ serum LH and testosterone (flat dose-response), &amp; ↑ FSH at all doses as well as histological changes in testes @≥1 mg/kg-d). [MDH Notes: very small number of animals per dose grp and concerns regarding study design. Effects on male reproductive organs weights have been reported in other studies but at much higher dose levels. Will not include as co-critical effect at this time.]</p> <p>d. Glucose &amp; lipid homeostasis Develop Gavage study in Wistar Rats (GD0-PND20) (Lv et al 2013) @0.5 mg/kg-d admin dose (based on Butenhoff et al 2009 est serum concentrations&lt;40 ug/mL) - ↓BW &amp; glucose tolerance in offspring</p> <p>e. 2 yr dietary study in Sprague-Dawley Rats (Thomford 2002, Butenhoff et al 2012) @0.098/0.12 (M/F) mg/kg-d adm dose (based on Seacat et al 2003 est serum concentrations &lt;40 ug/mL) – histological changes in liver &amp; ↓serum glucose @0.24/0.299 (M/F) mg/kg-d adm dose (based on Seacat et al 2003 est serum concentrations &lt;40 ug/mL) - ↑macroscopic &amp; histomorphological findings in liver</p>
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	<p>f. 1 to 5 day oral gavage study in female Sprague-Dawley Rats (Martin et al 2007)  @10 mg/kg-d adm dose (based on Chang et al 2008 serum concentrations likely &lt;40 ug/mL) - ↓tT4 &amp; fT4 &amp; tT3</p> <p><b>Mice –</b></p> <p>7. Developmental study in Pregnant CD-1 Mice (GD1-GD17) (Thibodeaux et al 2003 &amp; Lau et al 2003):  @33.1 ug/mL [adm dose 1 mg/kg-d] – <b>Developmental effects – delayed eye opening</b></p> <p>8. 28 day oral Immunotox Gavage Study in B6C3F1 Mice (Peden-Adams et al 2008)  @&gt;0.083 ug/mL [&gt;0.0017 mg/kg-d adm dose] – suppression of SRBC response,  @&gt;1.8 ug/mL [0.0017 – 0.166 mg/kg-d adm dose] – ↑thymic T-cell, NK cell activity &amp; lysosome activity [MDH Notes: effects at dose levels &lt;~1ug/mL not consistent with other studies as well as concerns regarding data reporting. These effects will not be used to identify co-critical effect at this time.]</p> <p>9. 60 day Immunotox Gavage study (Dong et al 2009)  @&gt;7.4 ug/mL [0.083 mg/kg-d admin dose] - ↑ rel liver wt, ↑splenic NK cell activity, ↓SRBC specific IgM  @&gt;36.7 ug/mL [0.417 mg/kg-d admin dose] - ↓BWG &amp; ↓ relative spleen, &amp; thymus wts, ↓splenic &amp; thymic cellularity</p> <p><i>Mouse study – measured (at termination) serum concentration</i></p> <p>a. 28 day gavage study ICR adult male Mice (Qiu et al 2013)  @2.5 mg/kg-d adm dose (serum concentration @termination are reported in Figure 7.) - ↑Sertoli cell vacuolization &amp; derangement of cell layers, ↓epididymal sperm cell count and evidence of disruption of blood-testes barrier. [MDH Notes: It is difficult to accurately estimate serum concentrations from Figure 7 but concentrations appear to be close to or slightly exceed 40 ug/mL. based on other studies in mice it is likely the serum concentrations are greater than the benchmark for co-critical. In addition, MDH staff had concerns regarding study quality and methodologies used (e.g., use of formalin as a fixative). These effects will not be included as co-critical at this time.]</p> <p><i>Mouse studies – estimated serum concentrations</i></p> <p>b. Developmental Immun Gavage study C57BL/6N Mice (GD1-17) (Keil et al 2008)  @1 mg/kg-d adm dose (based on Lau et al 2003 est serum concentrations &lt;~40 ug/mL) – <b>Suppressed NK activity in M offspring</b></p> <p>c. 21 day immune challenge study in B6C3F1 (Guruge et al 2009)  @0.025 mg/kg-d adm dose (based on Peden-Adams et al &amp; Dong et al est serum concentrations &lt;40 ug/mL) -</p>
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	<p>↓survival in trt animals when challenged with Influenza A.</p> <p><b>Monkeys –</b>  10. 26 week Capsule study in Cynomolgus Monkeys (Seacat et al 2002)  @7.7 ug/mL [0.03 mg/kg-d adm dose] – ↓HDL cholesterol (F) but not clear dose response until next dose level up.  @38 ug/ mL [0.15 mg/kg-d adm dose] - ↓HDL cholesterol (F), ↑rel liver wt (MDH BMD<sub>10</sub> = 29 ug/mL)</p>
<b>Co-Critical Effects:</b>	<p><b>Rats:</b>  <u>Developmental</u> (sternal defects, ↓pup BW, decreased glucose tolerance, changes in lung development, ↓survival, delayed eye opening, ↑motor activity with ↓habituation), <u>Hepatic (liver) system</u> (↑weight w/histological changes, changes in cholesterol levels), <u>Thyroid (E)</u> (↓maternal &amp; pup tT4)</p> <p><b>Mice:</b>  <u>Developmental</u> (delayed eye opening), <u>Immune system</u> (immune suppression – e.g., SRBC response, NK cell activity, ↓ spleen and thymus weight &amp; cellularity)</p> <p>Monkeys:  <u>Hepatic (liver) system</u> (changes in cholesterol, ↑ relative liver weight)</p>
<b>Health Endpoints:</b>	Critical Endpoints – Developmental (pup body weight)
	Co-Critical Endpoints – Developmental, Hepatic (liver) system, Immune system, Thyroid (E)

**B. Non-cancer Effects - Health Standards Statute Health Effects, Specialty Study Summary (e.g., endocrine, immunologic, developmental, reproductive, neurotoxicity):**

*[Note: A complete evaluation of the toxicological literature was not conducted. MDH conducted a focused re-evaluation which relied upon EPA’s hazard assessment and key study identification (EPA 2016a).]*

Endocrine Effects	Tested:	Yes
	Observed:	<p>Yes</p> <p>Numerous human epidemiological studies have evaluated thyroid hormone levels and/or thyroid disease in association with serum PFOS. Results from these studies have provided limited support for an association. Stronger associations were found in populations at risk for iodine deficiency or positive anti-TPO antibodies (a marker for autoimmune thyroid disease).</p> <p>Studies in laboratory animals have reported decreased serum thyroid levels, in particular, thyroxin (T4) in offspring and adult</p>

		animals at exposure levels similar in magnitude to the critical effect. Changes in total and free T4 have been identified as a co-critical effects and Thyroid has been identified as an Additivity Endpoint.
Immunologic Effects	Tested:	Yes
	Observed:	<p>Yes</p> <p>A few human epidemiology studies have evaluated associations between immunosuppression measures and serum PFOS. However, no clear associations were reported between serum PFOS and rates of infectious disease.</p> <p>Studies in laboratory animals have shown that PFOS exposure alters several immunologic measures (e.g., suppression of SRBC response and/or natural killer cell activity). Some of these effects occur at exposure levels similar to the POD. The Immune System has been identified as an Additivity Health Endpoint and a database uncertainty factor has been incorporated into the RfD derivation to address the need for additional testing.</p>
Developmental Effects	Tested:	Yes
	Observed:	<p>Yes</p> <p>Human epidemiology studies have suggested an association between prenatal PFOS serum levels and lower birth weight, however, this association has not been consistent.</p> <p>Studies conducted in laboratory animals have identified several sensitive developmental effects. Decreased pup body weight appears to be among the most sensitive effects and, in part, forms the basis of the Reference Dose and corresponding serum concentration of concern. A limited number of studies have also reported changes in male reproductive development and changes in energy metabolism (e.g., glucose levels, lipid metabolism) following exposure during development. Additional effects, including increased pup death, were observed at higher exposure levels.</p>
Reproductive Effects	Tested:	Yes
	Observed:	<p>Yes</p> <p>A small number of human epidemiology studies have reported an association between preconception serum PFOS and gestational diabetes and pregnancy induced hypertension in populations with serum PFOS concentrations of 0.012-0.017 ug/mL. There has also been some evidence of associations between serum PFOS and decreased fertility, however, concerns over the possibility that this is due to reverse causation have been raised.</p>



		Studies in laboratory animals do not indicate that fertility is not a sensitive endpoint, with decreases in male reproductive organ weights, decreased epididymal sperm count, and evidence of blood-testes-barrier disruption at exposure levels higher than those causing developmental toxicity (see above). Therefore, the RfD would be protective of these effects.
Neurotoxicity Effects	Tested:	Yes
	Observed:	Yes Developmental neurotoxicity and adult neurotoxicity studies have been conducted in laboratory animals. Increased motor activity and decreased habituation of male offspring was reported following gestational and lactational exposure at levels similar to the critical effect and have been included as co-critical effects. These effects are encompassed by the Developmental Additivity Endpoint. Results from studies using water maze tests for learning and memory in animals exposed during development or as adults have yielded inconsistent results or effects only at higher dose levels.

**Other Studies/Effects/Considerations**

*NOTE: The following studies have been included because activation of PPAR has been used as rationale for why effects observed in rodent are not applicable to humans.*

**PPAR Activity – (US EPA 2016a)**

In Vitro

Studies have been conducted in order to determine if PFOS activates PPARs. The PPARs are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. These factors can alter gene expression in response to endogenous and exogenous ligands and are associated with lipid metabolism, energy homeostasis, and cell differentiation. The three types, PPAR $\alpha$ ,  $\beta/\delta$ , or  $\gamma$ , are encoded by different genes, expressed in many tissues, and have specific roles during development as well as in the adult (Takacs and Abbott 2007).

**Table 3-21. Summary of PFAS Transactivation of Mouse and Human PPAR $\alpha$ ,  $\beta/\delta$ , and  $\gamma$**

PPAR isoform	PFAS	Mouse LOEC <sup>a</sup>	Human LOEC <sup>a</sup>
$\alpha$	PFOA	10 $\mu$ mol	30 $\mu$ mol
	PFOS	120 $\mu$ mol	NA <sup>b</sup>
$\beta/\delta$	PFOA	40 $\mu$ mol	NA
	PFOS	20 $\mu$ mol	NA
$\gamma$	PFOA	NA	NA
	PFOS	NA	NA

Source: Data from Table 1 in Takacs and Abbott 2007

Notes: <sup>a</sup> LOEC = lowest observed effect concentration; lowest concentration ( $\mu$ mol) at which there was a significant difference compared to the negative control ( $p < 0.05$ )

<sup>b</sup> NA = not activated

Wolf et al. (2008) tested PFAS, including PFOS, to determine whether mouse and human PPAR $\alpha$  activity could be induced in transiently transfected COS-1 cell assays. The results were:  
 NOEC - 60  $\mu$ mol (mouse); 20  $\mu$ mol (human)  
 LOEC - 90  $\mu$ mol (48.4  $\mu$ g/mL) (mouse); 30  $\mu$ mol (human)  
 C20max - 94  $\mu$ mol (mouse); 262  $\mu$ mol. (human)

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Zhang et al. (2014) examined the direct binding properties of PFOS and other PFASs using the ligand binding domain of human PPAR $\gamma$ . Authors concluded that PFASs induce disruption of lipid homeostasis and inflammation by the PPAR $\gamma$  pathway as well as the PPAR $\alpha$  pathway. Among the three members of the sulfonate family tested (4, 6, and 8 carbons), PFOS displayed the strongest activation potency.

#### In Vivo -

Martin et al. (2007) administered PFOS to male Sprague-Dawley rats by oral gavage at doses of 0 or 10 mg/kg/day for 1, 3, or 5 consecutive days. PFOS exhibited peroxisome proliferator-activated receptor alpha agonist-like effects on genes associated with fatty acid homeostasis. PFOS was poorly correlated with peroxisome proliferators in the global gene expression patterns and indicated weak matches with hepatotoxicity related signatures and weak correlation to PPAR $\alpha$  agonist treatment. Expression of HMG-CoA reductase was significantly upregulated, and cholesterol biosynthesis was downregulated in a manner suggesting a mechanism distinct from the statins. They also noted that PFOS exhibited similarities to compounds that induce xenobiotic metabolizing enzymes through PPAR $\gamma$  and constitutive androstane receptor (CAR).

Wang et al. (2010) dosed albino Wistar female rats with 3.2 mg PFOS/kg diet from GD 1 to weaning (PND 21). Gene expression changes in pups were examined on PNDs 1, 7, and 35. Significant effects were observed on genes involved in neuroactive ligand-receptor interaction, calcium signaling pathways, cell communication, the cell cycle, and peroxisome proliferator-activated receptor (PPAR) signaling.

In a 4-week study in rats, the hepatic effects of PFOS, WY-14,648 and phenobarbital (PB) were compared (Elcombe et al. 2012). Groups of 30 male Sprague-Dawley rats were administered either 20 ppm PFOS, 100 ppm PFOS, 50 ppm WY-14,648, or 500 ppm PB in the diet ad libitum for either 1, 7, or 28 days. The study showed that PFOS exhibits the combined effects of WY-14,648 and PB, behaving as a combined peroxisome proliferator and phenobarbital-like enzyme inducer. The data suggested that PFOS may activate not only PPAR $\alpha$ , but also CAR and PXR.

To assess PPAR involvement in developmental effects of PFOS, Abbott et al 2009 bred male and female 129S1/SvIm wild-type and PPAR $\alpha$  knockout (KO) mice. As the results from the wild-type and KO pups were similar, the author concluded that PFOS-induced neonatal lethality and delayed eye opening were not dependent on the PPAR $\alpha$  activation.

Qazi et al. (2009a) tested the effects of 0, 0.005%, or 0.02% PFOS on wild-type and PPAR $\alpha$ -null 129/Sv mice. The study indicated that the immunomodulation was partially dependent on PPAR $\alpha$  activation.

Changes in gene expression were examined in wild-type and PPAR $\alpha$ -null mice administered PFOS by gavage at 0, 3, or 10 mg/kg/day for 7 days (Rosen et al. 2010). Study findings suggest that there are PPAR $\alpha$ -independent effects in null mice that also occur in wild-type mice. Thus, some of the liver effects in the wild-type animals are not necessarily a reflection of PPAR $\alpha$  activation. The results also support those from other studies that indicate PFOS exposure results in metabolic changes both linked to, and independent of, PPAR- $\alpha$ .

#### **ToxCast Assays –**

PFOS was tested in 1,087 assays and was active in 175. Some of the data from the ToxCast assays such as the interactions with PPAR and CAR support the experimental data for PFOS and PFOA. In cases where effects were only observed at concentrations greater than those causing cytotoxicity, attributing the outcome to PFOS rather than the cytotoxicity is less certain.

Four different estrogen receptor (ESR) assays reported activation following PFOS treatment, all of which were Estrogen Receptor 1- (ESR1-) related, suggesting that PFOS has the ability to induce ESR1. PFOS antagonized the androgen receptor (AR). Although there is no direct cellular cytotoxicity value to compare, PFOS rat AR antagonism

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occurred at lower concentrations than the minimum cytotoxicity value. Thyroid receptor (TR) antagonism AC50 was higher than its respective cell specific cytotoxicity.

PFOS activated a variety of genes related to immunotoxicity in the ToxCast database. These genes include: chemokine ligand (CXCL) 10, CXCL8, collagen type II alpha (COL3A), interleukin-1 alpha (IL-1 $\alpha$ ), plasminogen activator (PLA), plasminogen activator urokinase (PLAUR), vascular cell adhesion molecule (VCAM1), and the TNF receptor subfamily gene CD40 (CD40). All of the immunological assays were performed by the vendor BioSseek. The vendor did not have a cytotoxicity AC50 for every cell type utilized. Given the limited cytotoxicity reference values it is difficult to determine if all gene activity can be attributed to PFOS.

PFOS activated PPARs, PXR, constitutive adrostane receptor (CAR), and retinoic acid receptor (RAR) in assays conducted under the ToxCast program. PFOS induced the DNA sequences for PPAR alpha (PPAR $\alpha$ ), peroxisome proliferator hormone response elements (PPRE), and PPAR gamma (PPAR $\gamma$ ) and antagonized the PPAR $\gamma$  receptor. The only PPAR assay AC50 that was above the cell-specific cytotoxicity AC50 was PPAR $\gamma$  antagonism. PFOS induced DNA sequences for PXR at concentrations lower than the cell-specific cytotoxicity AC50. CAR and RAR alpha antagonism were also observed but not at levels below the cell specific cytotoxicity values.

### C. Duration Specific Health-based Water Criteria Derivation

Relative Source Contribution (RSC)		
Henry's Law Constant (atm m3/mol)	Pure water: $3.05 \times 10^{-9}$ Fresh water: $4.7 \times 10^{-9}$	3M report 1999 as cited by OECD 2002 (Based on the above water solubility values and utilizing the vapor pressure of $3.27 \times 10^{-9}$ atm.
What is the volatility <sup>1</sup> ?	No	
Is there documentation to justify the use of an RSC other than the defaults <sup>2</sup> ?	A non-standard approach was utilized. See discussion below.	

<sup>1</sup> Nonvolatile (<  $3 \times 10^{-7}$  atm m3/mol); Low ( $3 \times 10^{-7}$  to  $10^{-5}$  atm m3/mol); Moderate ( $10^{-5}$  to  $10^{-3}$  atm m3/mol) or High (>  $10^{-3}$  atm m3/mol)

<sup>2</sup> Non-volatile/low volatility/moderate volatility – 0.5 for acute/short-term, 0.2 for subchronic/chronic

<sup>3</sup> High volatility – 0.2 for acute/short-term/subchronic/chronic

#### **RSC evaluation from EPA (USEPA 2016d) (See Section 8.5 for more information):**

From a national perspective, the dominant source of human exposure to PFOS is expected to be from the diet; indoor dust from carpets and other sources also is an important source of exposure, especially for children. EPA's Health Advisory (HA) was calculated using a relative source contribution (RSC) of 20%, which allows for other PFOS exposure sources (e.g., dust, diet, air) to make up 80% of the RfD. EPA used an RSC of 0.2 and the 90<sup>th</sup> percentile intake rate for lactating women (0.054 L/kg-d) to calculate a lifetime HA for PFOS of 0.07  $\mu$ g/L, and recommends that it apply to both short-term (i.e., weeks to months) scenarios during pregnancy and lactation, as well as to lifetime-exposure scenarios.

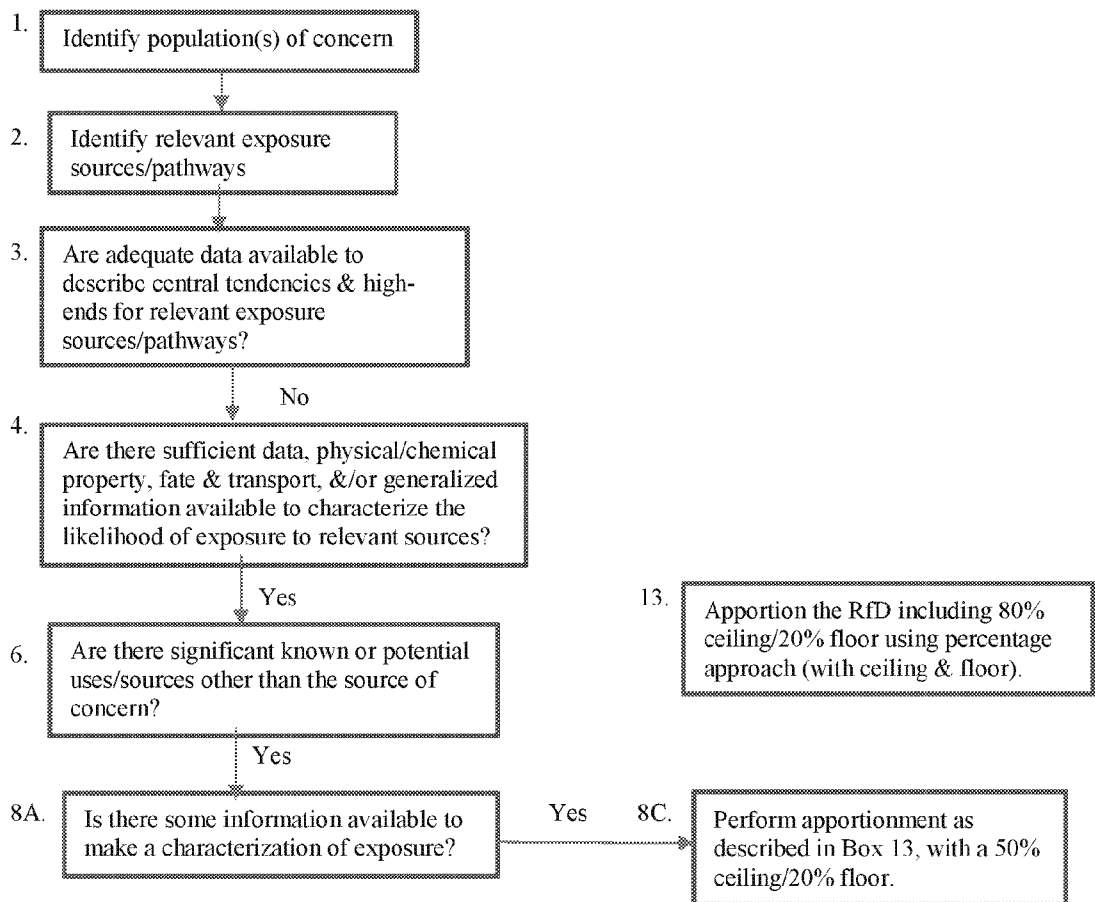
#### **MDH RSC Approach:**

The RSC is applied to account for all routes of exposure and allocates only a portion of the RfD to water ingestion, with the remaining portion allocated for non-water exposures, including inhalation and ingestion from food. The values of the duration specific default RSCs (0.5, 0.2, and 0.2 for short-term, subchronic, and chronic, respectively) are based on the magnitude of contribution of these other exposures that occur during the relevant exposure duration (MDH 2008). In the case of PFOS, the RSC concept needed to be applied in a framework recognizing the long

elimination half-life of PFOS, such that a person’s serum concentration at any given age is not only the result of his or her current or recent exposures within the duration of concern, but also from exposure from years past.

Egeghy and Lorber (Egeghy PP and M Lorber 2011) examined the relative impact of non-water exposures using a two pronged approach: 1) based on serum concentrations reported in the 2003-04 National Health and Nutrition Examination Study (NHANES) and 2) exposure media concentration data from multiple sources. For the second approach Egeghy and Lorber selected exposure media concentration data from multiple sources in the literature to estimate daily median and 95th percentile exposure intakes for young children and adults from dust, diet, water, and air. Because of the sparseness of media-specific data, the authors deemed the resulting intake estimates adequate for screening-level intake assessment but subject to considerable uncertainty. This uncertainty was greater for the upper percentile estimates than for the median values. MDH has chosen to use recent NHANES biomonitoring data (2013-2014) and East Metro new resident biomonitoring data (2014), to estimate upper-end non-water exposures (similar to option 1 in Egeghy and Lorber, 2011).

MDH uses the Exposure Decision Tree process as presented in EPA’s Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (US EPA 2000). The Decision Tree presents a series of decision points at which the quality and quantity of available exposure data are evaluated and at which the derivation of the RSC is ultimately steered toward one of several conclusions indicating an appropriate RSC. MDH has relied upon the percentage method, which is intended to reflect relative portions of other (non-water ingestion) routes of exposure and the likelihood for changing levels within those multiple sources. The relevant portions of the Exposure Decision Tree are presented below.



The 80 percent ceiling within the Decision Tree is to ensure that the health-based goal will be low enough to provide adequate protection for individuals whose total exposure is, due to any of the exposure sources, higher than currently indicated by the available data (US EPA 2000). This also increases the margin of safety to account for possible unknown sources of exposure.

It has been acknowledged that serum concentrations are the best measure of exposure. These values can be used in place of the RfD in the Decision Tree process. The serum concentration at the POD selected by MDH (and EPA) is 6.26 µg/mL. The serum concentration associated with the resulting RfD, which incorporated a total UF of 100, is 0.063 µg/mL (or 63 µg/L). Background (i.e., exposure from non-water ingestion routes) data for infants, the population of concern, are not available, however, given the long half-life the biomonitoring results from the East Metro (new residents) and NHANES can be used to provide insight into the magnitude of non-water exposures.

MDH’s East Metro PFC biomonitoring project sampled a subset of people living in the East Metro region who were connected to a contaminated public water supply (Nelson 2016). Treatment to remove PFCs was added to the public water system (PWS) and volunteer participants had blood levels measured at three time points: 2008, 2010 and 2014:  
 2008 – 35.7 geo mean (CI 31.4 – 40.5) µg/L (95<sup>th</sup> percentile 100 µg/L, range 3.2 – 448 µg/L)  
 2010 – 24.9 geo mean (CI 22.1 – 28.0) µg/L (95<sup>th</sup> percentile 69.5 µg/L, range 1.6 – 234 µg/L)  
 2014 – 18.5 geo mean (CI 16.1 – 21.3) µg/L (95<sup>th</sup> percentile 70.0 µg/L, range 1 – 180 µg/L)

As part of the last biomonitoring effort new Oakdale residents (N=156) were also sampled in 2014. Since these individuals did not have historical exposure to the contaminated water their serum samples can be considered representative of Minnesota non-water exposures: 7.2 geo mean µg/L (CI 6.5-8.0); 95<sup>th</sup> percentile 21 µg/L (range 0.34-30). These levels are higher than the NHANES 2013-14 geometric mean and within the confidence interval of the 95<sup>th</sup> percentile values (shown below) but are noticeably lower than the East Metro population that were historically exposed to contaminated water.

It is important to note that the general population (NHANES) serum levels have been decreasing over time, with 3 to 4-fold drop since 2003-04 (the serum levels used in Egeghy and Lorber 2011). The 2013-14 data provide the most recent data regarding ‘background’ serum levels in the US general populations.

General population (NHANES) serum levels have been decreasing over time (CDC 2017)

Year	Geometric Mean (µg/L) (95% CI)	50 <sup>th</sup> Percentile (µg/L) (95% CI)	95 <sup>th</sup> Percentile (µg/L) (95% CI)
1999 – 2000	30.4 (27.1-33.9)	30.2 (27.8-33.9)	75.7 (58.1-97.5)
2003-2004	20.7 (19.2-22.3)	21.2 (19.8-22.4)	54.6 (44.0-66.56)
2005-2006	17.1 (16.0-18.2)	17.5 (16.8-18.6)	47.5 (42.7-56.8)
2007-2008	13.2 (12.2-14.2)	13.6 (12.8-14.7)	40.5 (35.4-47.4)
2009-2010	9.32 (8.13-10.7)	9.70 (8.50-10.8)	32.0 (22.6-48.5)
2011-2012	6.31 (5.84 – 6.82)	6.53 (5.99 – 7.13)	21.7 (19.3 – 23.9)
2013-2014	4.99 (4.50-5.52)	5.20 (4.80-5.70)	18.5 (15.4-22.0)

While data on infants is not available there are publications regarding the serum levels in young children:

- (Schechter 2012) sampled children in Dallas, Texas between August and November 2009. Reported median and maximum PFOS serum concentrations were: 2 and 10.6 ug/L, respectively, in children less than three years of age. Reported median and maximum PFOS serum concentrations were: 3.7 and 23.3 ug/L, respectively, in children older than three years of age but less than six years of age.
- (Wu 2015) sampled children two to eight years of age in California between December 2007 and November 2009. Reported geometric mean and 95<sup>th</sup> percentile PFOS serum concentrations were: 6.28 and 13.1 ug/L, respectively.

- (Harris 2017) recently published serum concentrations in six to ten year old children sampled between 2007 and 2010 in the Boston area. Reported geometric mean and 90<sup>th</sup> percentile PFOS serum concentrations were: 6.2 and 13.7 ug/L, respectively.

These data support the use of upper-end percentile values from NHANES and the East Metro new resident as conservative representatives of 'background' non-water ingestion routes of exposure.

The apportionment to water ingestion can be calculated by taking a ceiling of 80% and subtracting a conservative (high end) serum value from the recent biomonitoring data from the East Metro new residents or NHANES. Eighty percent of the serum concentration associated with the RfD would be 50.4 ug/L (63 ug/L x 0.8). Subtracting the 95<sup>th</sup> percentile values, as a high-end estimate of background, non-water exposures, from the new East Metro residents (21 ug/L) or 2013-14 NHANES (18.5 ug/L) produces a residual serum concentration of roughly 30 ug/L, or approximately 50% of the serum concentration at the RfD (63 ug/L) and approximately 60% of the 80% ceiling value (50.4 ug/L). Given the conservative nature of the calculation, selection of an RSC of 50% for water ingestion is appropriate and consistent with box 8C of the Decision Tree. It should be noted that the results of this analysis do not support raising the apportionment of water ingestion sources to 80 percent.

### C.1 Develop Non-Cancer Water Guidance Value

The most appropriate dose metric for PFOS is serum concentration. PFOS is a bioaccumulative chemical, with a half-life of over five years. Criteria for bioaccumulative contaminants focuses on long-term exposures. However, high, short-term exposures can result in internal body burdens that take years to eliminate. Infants, whether formula-fed or breast-fed consume a much greater volume of liquid on a per body weight basis than older children and adults. In addition, PFOS crosses the placenta and is transferred to breastmilk. Empirical data from the published literature indicates that breastfeeding can result in significant exposures, resulting in higher serum concentrations in infants compared to their mothers.

Serum concentrations can be calculated if the rate of elimination (derived from half-life), the dose (water concentration x water intake rate) and volume of distribution are known. The following equation (also used by EPA to calculate HEDs) provides the simple relationship between dose and average serum concentration.

$$Dose \left( \frac{mg}{kg \cdot day} \right) = \frac{Serum \ Concentration \left( \frac{\mu g}{L} \right) \times \frac{1 \ mg}{1000 \ \mu g}}{Clearance \ Rate \left( \frac{L}{kg \cdot day} \right)}$$

Where:

*Clearance Rate = Volume of Distribution (L/kg BW) x (Ln2/half-life, days)*

and

*Dose (mg/kg - day) = Water Intake Rate (L/kg BW/day) x Water Concentration (ug/L) x (1 mg/1000 ug)*

This equation can be rearranged to calculate serum concentration based on dose and clearance.

$$Serum \ Concentration \left( \frac{\mu g}{L} \right) = \frac{Water \ IR \left( \frac{L}{kg \cdot day} \right) \times Water \ Concentration \left( \frac{\mu g}{L} \right)}{Clearance \ Rate \left( \frac{L}{kg \cdot day} \right)}$$

Two exposure scenarios were examined: 1) an infant fed with formula reconstituted with contaminated water starting at birth and continuing ingestion of contaminated water through life; and 2) an infant exclusively breast-fed for 12 months, followed by drinking contaminated water. In both scenarios the simulated individuals began life with a pre-existing body burden through placental transfer. The serum concentration of the mother were calculated to be at steady state, using the equation presented above, at the time of delivery. Upper percentile intake rates were used for the breastfed infant scenario and 95th percentile intake rates were used for water intake to simulate a reasonable maximum exposed (RME) individual.

According to the 2016 Breastfeeding Report Card (CDC 2016) nearly 66 percent of mothers in Minnesota report breastfeeding at six months, with 31.4 percent exclusively breastfeeding. The percent breastfeeding dropped to 41% at twelve months. MDH has selected an exclusive breastfeeding duration of one year for the breast-fed infant scenario.

A summary of the model parameters is presented in the table below. For details on the basis of each of the parameters and the selection of input value(s) please refer to the Background Document: MDH Toxicokinetic Model and Derivation of Human Health-Based Water Guidance located at: [O:\HRA\COMMON\Guidance - Water\Tox reviews-completed\Final\PFOA\ExpoScenarioCalc\FinalTeamReviewMaterials\Backgrd\\_DrftMarchFinal\\_TKModel.docx](O:\HRA\COMMON\Guidance - Water\Tox reviews-completed\Final\PFOA\ExpoScenarioCalc\FinalTeamReviewMaterials\Backgrd_DrftMarchFinal_TKModel.docx) (MDH 2017b)

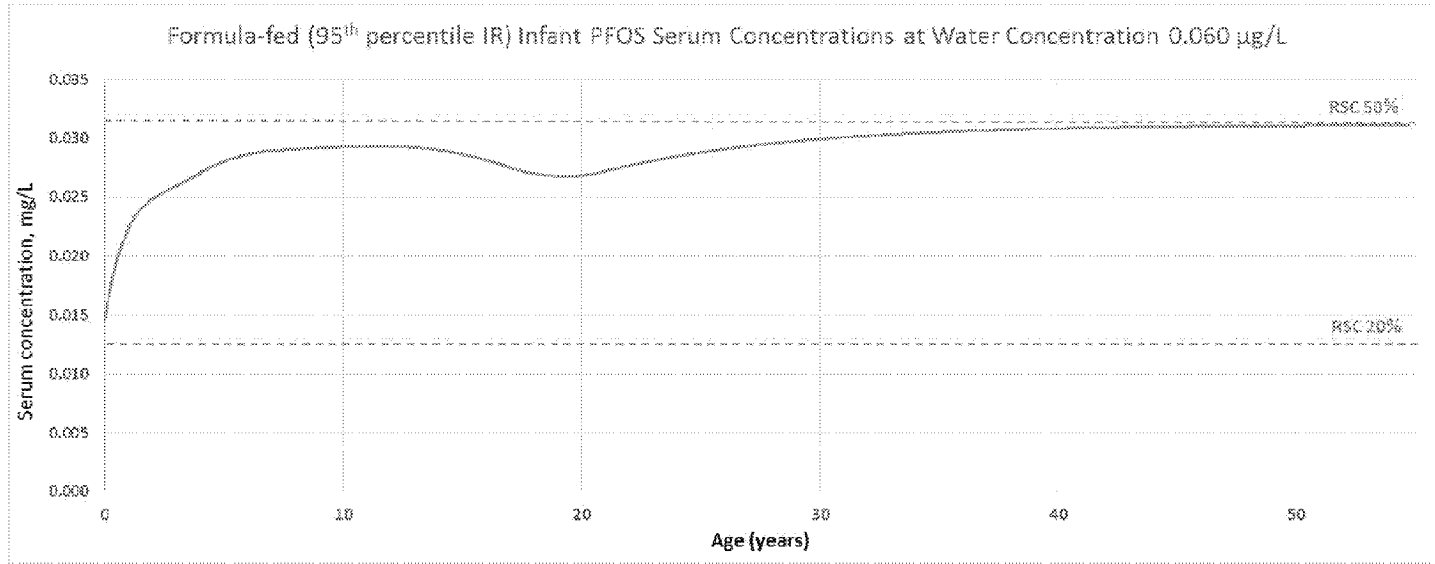
Model Parameter	Value(s)
Half-life (days)	1971 days
Volume of distribution (Vd)	0.23 L/kg
Vd Age Adjustment Factor (Vd AF)	Range from 2.1 @age 1-30 days to 1.2 @age 5 – 10 years. Value of 1 used for ages >10 years.
Clearance Rate (CR)	$0.23 \text{ L/kg} \times (\ln 2/1971 \text{ days}) = 0.000081 \text{ L/kg-d}$
Placental transfer factor	46% (% of maternal serum level)
Breastmilk transfer factor	1.3% (% of maternal serum level)
Water Intake (L/kg-d)	95 <sup>th</sup> percentile for Consumers Only (default intake rates used by MDH. Table 3-1 & 3-3, EPA 2011)
Breastmilk Intake (L/kg-d)	Upper percentile (approximates 95 <sup>th</sup> percentile) for exclusively breastfed infants (Table 15-1, EPA 2011)
Body weight (kg)	Calculated from water and breastmilk intake tables listed above.

**Water Concentration Calculation Results:**

Scenario #1 - Formula formula-fed Infant

The water concentration that keeps the serum concentration attributable to drinking water (solid line below in Figure 1) below an RSC of approximately 50% (0.0.063 mg/L serum x 0.5 = 0.0315 mg/L) throughout life is 0.060 µg/L. Because of the long half-life the serum concentration curve is very flat, and even a small increment increase in the water concentration (0.061 µg/L) raises the serum concentration above the 50 percent threshold for nearly 9 years.

Figure 1. Strictly formula-fed infant serum concentrations over a lifetime, based on 95<sup>th</sup> percentile water ingestion rate and an RSC of 50%.

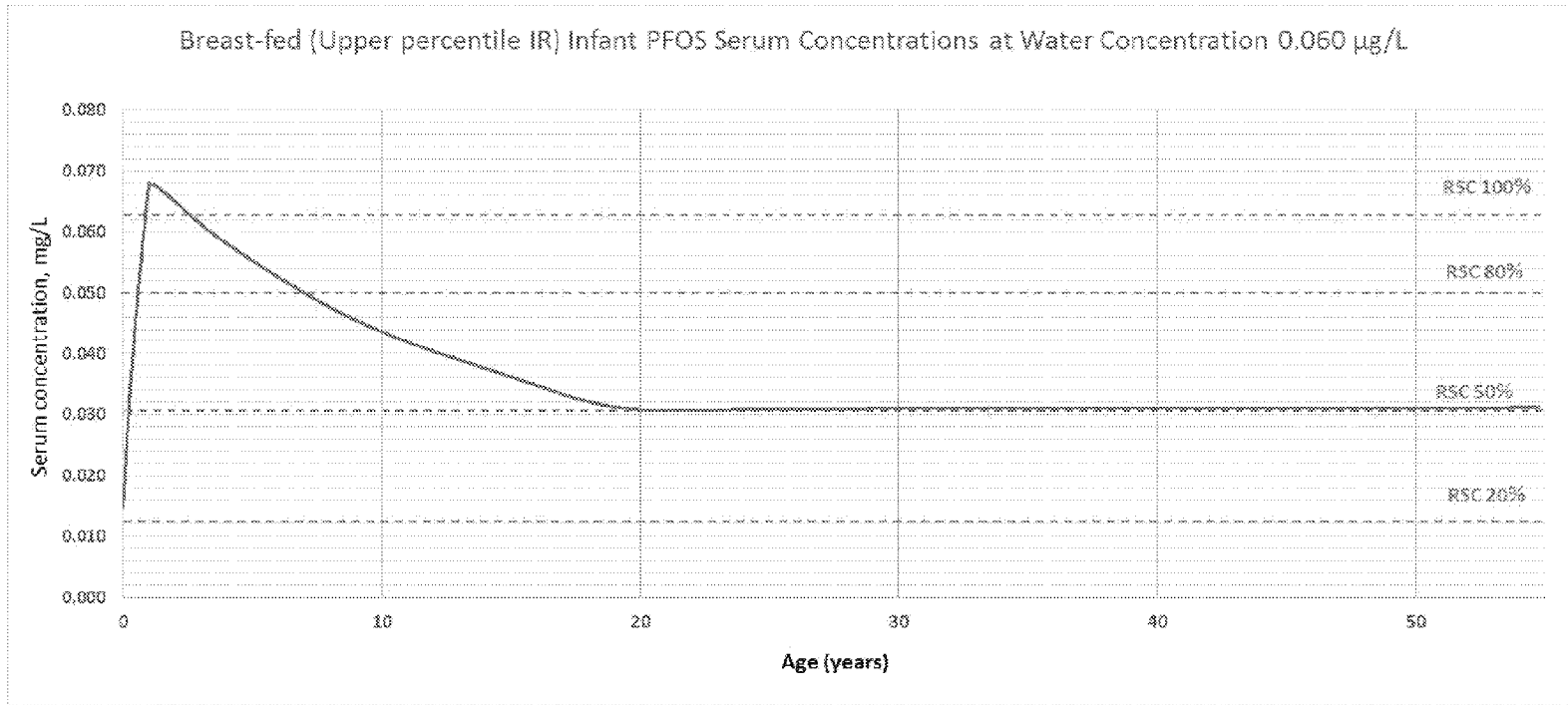


Scenario #2 - Breast-fed Infant

While a water concentration of 0.060 µg/L is protective of individuals directly exposed to contaminated water it is not sufficiently protective for infants who are exclusively breastfed for a year by mothers who have been chronically exposed to 0.060 µg/L in water. Under scenario #2 infant PFOS serum levels exceed the serum concentration at the reference dose for nearly 20 months and the 50% RSC threshold for nearly 18.5 years. See Figure 2.

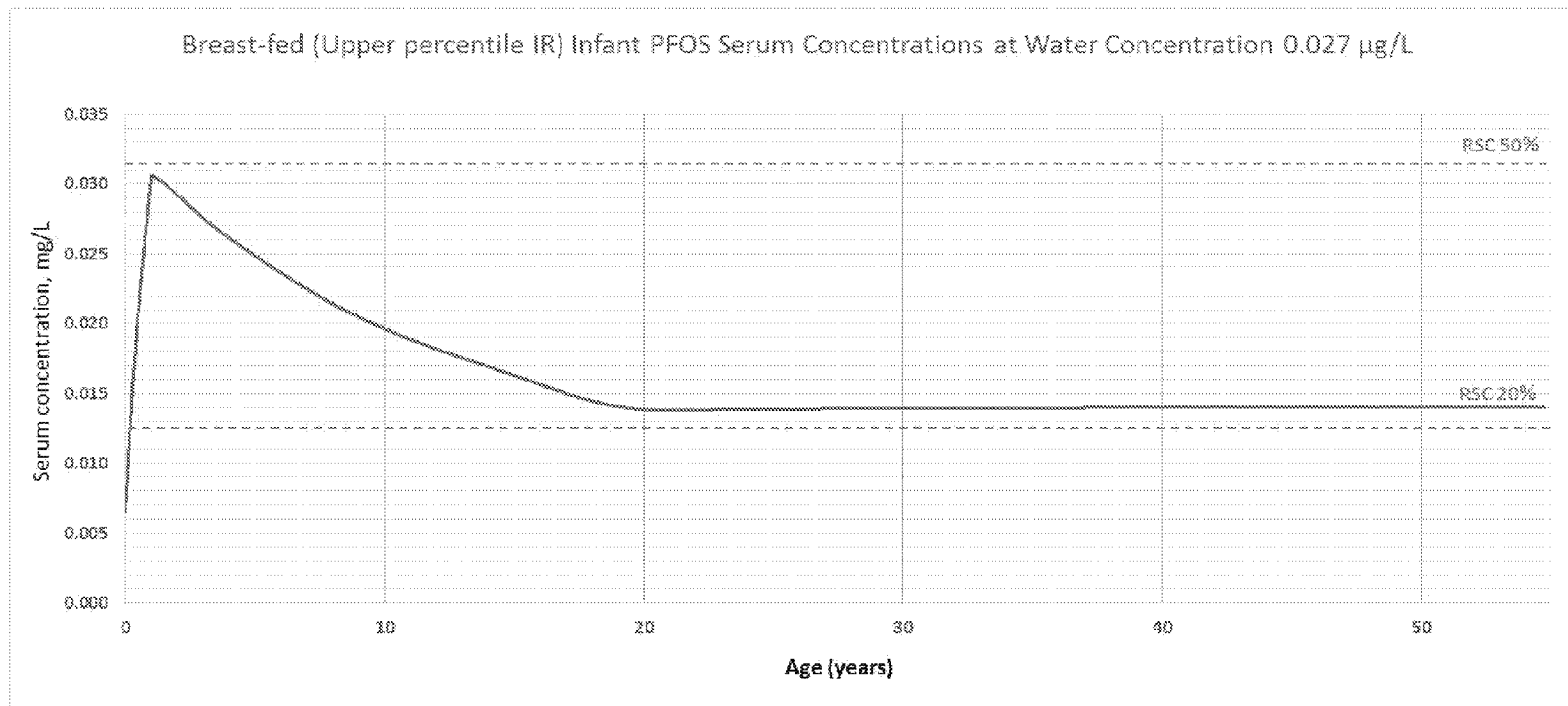


Figure 2. Serum concentrations for an exclusively breast-fed for 1 year, followed by water ingestion, based on upper/95<sup>th</sup> percentile ingestion rate and an RSC of 50% at a water concentration of 0.060 µg/L.



In order to maintain serum concentrations below an RSC threshold of 50% ( $0.063 \times 0.5 = 0.0315$  mg/L) for infants exclusively breast-fed for one year the water concentration must be lowered to 0.027 µg/L. See Figure 3.

Figure 3. Serum concentrations for an exclusively breast-fed for 1 year, followed by water ingestion, based on upper/95<sup>th</sup> percentile ingestion rate and an RSC of 50% at a water concentration of 0.027 µg/L.



Even a small incremental increase in the water concentration (0.028 µg/L) raises the serum concentration above the 50 percent threshold for more than three months. Given the health endpoints of concern include developmental concerns and the duration of exceeding the 50% threshold constitutes a subchronic period of time, the acceptable water concentration was set at 0.027 µg/L and not rounded to one significant digit.

\*\*\* 7. Cancer Effects \*\*\*

**A. Relevant Cancer Studies Summary Table**

Cancer Study Description – duration, route, species/strain, age at dosing, N/sex/group, early life exposure?, etc.	Administered Dose (mg/kg-d)	Tumor Incidence Rate Per Tumor Site at Each Dose Level (by sex, statistical significance)	Study POD mg/kg/d	Slope Factor (mg/kg-d) <sup>-1</sup>	Reference (note limitations in comment filed)*
2-yr dietary study – Rats 0, 0.5, 2, 5, or 20 ppm in diet. Observations were made at 4, 14 and 53 weeks of treatment	0/0, 0.024/0.029, 0.098/0.120, 0.24/0.299, or 0.984/1.251 (M/F) mg/kg-d	See Table 6-A1 above for noncarcinogenic effects.  <i>Neoplastic effects:</i> Males – hepatocellular adenoma (Ms 0, 6, 6, 2, & 12% - positive trend) thyroid follicular cell adenoma & carcinoma (10, 12, 10, 10, & 8.5%)  Females – hepatocellular adenoma (0, 2, 2, 2, & 8*% - positive trend), 1 carcinoma in highest dose grp; thyroid follicular adenoma & carcinomas (0, 0, 0, 6* & 2%) & C-cell adenoma & carcinoma (20, 14, 12, 16 & 8*%); mammary fibroadenoma/adenoma (38, 60*, 46, 52, & 25*%) & carcinomas (18, 24, 31, 22, & 23%) [high backgrd in controls]			(Thomford 2002) and (Butenhoff 2012) and aci (US EPA 2016a)

\*Note: exposure included early life stages; maximum tolerated dose level was not achieved; and time-to-tumor (latency) information if available.

**B. Oral Cancer-related Study Summaries:**

**Human Carcinogenicity Data:**

(USEPA 2016d):

Several human epidemiology studies evaluated the association between PFOS and cancers including bladder, colon, and prostate (Alexander et al. 2003; Alexander and Olsen 2007; Mandel and Johnson 1995). A large increase in mortality risk from bladder cancer was demonstrated, and a subsequent study of bladder cancer incidence in the same cohort found rate ratios of 1.5 to 1.9 in the two highest cumulative exposure categories, compared to an internal referent population (Alexander et al. 2003; Alexander and Olsen 2007). The risk estimates lacked precision because the number of cases were limited. Smoking prevalence was higher in the bladder cancer cases, but the analysis did not control for smoking because data were missing for deceased workers, and therefore positive confounding by smoking is a possibility in this analysis. No elevated bladder cancer risk was observed in a nested case-control study in a Danish cohort with plasma PFOS concentrations at enrollment

between 0.001 and 0.0131 µg/mL (Eriksen et al. 2009). Other studies that evaluated cancer risk for specific sites (e.g., prostate, breast) in the general population were inconsistent (Bonefeld-Jørgensen et al. 2011, 2014; Hardell et al. 2014; Innes et al. 2014).

**Animal Carcinogenicity Data:**

(USEPA 2016d):

A single chronic cancer bioassay in animals is available for PFOS (Thomford 2002/Butenhoff et al. 2012).<sup>5</sup> Increased incidence of hepatocellular adenomas in the male (12% at the high dose) and female rats (8% at the high dose) and combined adenomas/carcinomas in the females (10% at the high dose) were observed, but did not display a clear dose-related response. In males only, the serum alanine transaminase (ALT) levels were increased at 14, 27, and 53 weeks. At 105 weeks there was an increase in eosinophilic clear cell foci, and cystic hepatocellular degeneration in males given 2, 5, and 20 parts per million PFOS. Thomford et al. (2002) identified low levels of single cell necrosis in all dose groups (males and females) with a significant increase in incidence at the high dose for males and females. Thyroid and mammary gland tumors were also observed but did not exhibit dose response. Mammary gland tumors had a high background incidence in all dose groups and showed no response to dose.

Under EPA's Guidelines for Carcinogen Risk Assessment (USEPA 2005a), there is *Suggestive Evidence of Carcinogenic Potential* for PFOS. In the only chronic oral toxicity and carcinogenicity study of PFOS in rats, liver and thyroid tumors (mostly adenomas) were identified in both the controls and exposed animals at levels that did not show a direct relationship to dose. The evidence for cancer in animals was judged to be too limited to support a quantitative cancer assessment (i.e., no dose-response).

**Genotoxicity Data:**

(USEPA 2016d)

All genotoxicity studies including an Ames test, mammalian-microsome reverse mutation assay, an *in vitro* assay for chromosomal aberrations, an unscheduled DNA synthesis assay, and mouse micronucleus assay were negative.

**C. Critical Cancer Study Information:**

Cancer Classification (source & date):	Suggestive Evidence of Carcinogenic Potential (USEPA 2016d)
Slope Factor Source, Date of Development:	Not Applicable
Slope Factor Study Quality:	Not Applicable
Describe the Basis for the Toxicity Value:	Not Applicable
Supporting Study Description:	In the only chronic oral toxicity and carcinogenicity study of PFOS in rats, liver and thyroid tumors (mostly adenomas) were identified in both the controls and exposed animals at levels that did not show a direct relationship to dose. (Thomford 2002) (Butenhoff 2012). The evidence for cancer in animals was judged to be too limited to support a quantitative cancer assessment (i.e., no dose-response).

#### **D. Mode of Action Information:**

**1. Is there evidence of mutagenic mode of action or another mode of action expected to be linear at low doses?**

No.

**2. Is there evidence of a nonlinear mode of action (e.g., no evidence of linearity and sufficient information supporting a nonlinear mode of action)?**

(USEPA 2016d) [See Section 4.2.3 for more details]

The mode of carcinogenic action of PFOS is not clearly understood. Some have concluded based on available data that liver tumors observed in the cancer bioassays can be attributed mostly to the impact of PFOS on peroxisome proliferation based on a hypothesized lower sensitivity of humans to this MOA. Some data support the hypothesis that PPAR $\alpha$  agonism MOA could be responsible for observed liver tumors in animals. Several studies have demonstrated that PFOS can activate PPAR $\alpha$ ; however, data are generally lacking for increased cell proliferation. Specifically, no increase in hepatic cell proliferation was detected in the subchronic study (Seacat et al. 2003) or the cancer bioassay (Thomford 2002) of PFOS. Limited necrosis was present in these studies, but did not demonstrate a response to dose. In addition, no subchronic or longer-term studies revealed evidence of preneoplastic foci in the liver.

Short-term genotoxicity assays suggested that PFOS is not a DNA-reactive compound, with negative results from five *in vitro* studies, as well as from an *in vivo* bone marrow micronucleus assay.

Other possible MOAs for carcinogenicity have been explored, including mitochondrial biogenetics and gap junctional intercellular communication (GJIC). These are not clearly defined MOAs, and their importance relative to PFOS exposure is not certain.

**3. Is there evidence that the mode of action is not relevant to humans?**

Not Available (NA)

**4. Is there evidence of life-stage sensitivity?**

NA

**5. Are there structure-activity correlations available?**

NA

**6. Is route-to-route extrapolation used? Not applicable**

**E. Develop a Cancer Guidance Value**

$$\frac{(\text{Additional Lifetime Cancer Risk, } 1 \times 10^{-5}) \times (\text{Conversion Factor, } 1000 \text{ ug/mg})}{[(\text{SF} \times 10 \times 0.125 \text{ L/kg-d} \times 2) + (\text{SF} \times 3 \times 0.045 \text{ L/kg-d} \times 14) + (\text{SF} \times 1 \times 0.041 \text{ L/kg-d} \times 54)] / 70}$$

where: SF = cancer slope factor (per mg/kg-d)

SF*	Cancer Guideline [ug/L]
x	#VALUE!
*Enter in Slope Factor	

=ug/L

OR

$$(\text{Additional Lifetime Cancer Risk, } 1 \times 10^{-5}) \times (\text{Conversion Factor, } 1000 \text{ ug/mg}) \times (\text{Slope Factor, per mg/kg-d}) \times (\text{Lifetime Adjustment Factor}) \times (\text{Lifetime Intake Rate, } 0.044 \text{ L/kg-d})$$

=ug/L

Comments: Not applicable

## References Consulted During Re-Review:

ASTSWMO (2015). Association of State and Territory Solid Waste Management Officials. Perfluorinated Chemicals (PFCs): Perfluorooctanoic Acid (PFOA) & Perfluorooctane Sulfonate (PFOS) Information Paper.

ATSDR. (2015). "Agency for Toxic Substances and Disease Registry. Draft Toxicological Profile for Perfluoroalkyls." Retrieved August 15, 2015, from <http://www.atsdr.cdc.gov/toxprofiles/tp200.pdf>.

Australian Health Protection Principal Committee, e. (2016). "enHealth Statement: Interim national guidance on human health reference values for per- and poly-fluoroalkyl substances for use in site investigations in Australia." from <http://www.health.nsw.gov.au/environment/factsheets/Documents/pfas-interim-health-values-ahppc.pdf>.

Bijland, S., PCN Rensen, EJ Pieterman, ACE Mass, JW van der Hoorn, MJ van Erk, KW van Dijk, SC Chang, DJ Ehresman, JL Butenhoff, HMG Princen. (2011). "Perfluoroalkyl Sulfonates Cause Alkyl Chain Length-Dependent Hepatic Steatosis and Hypolipidemia Mainly by Impairing Lipoprotein Production in APOE\*3-Leiden CETP Mice." *Toxicological Sciences* **123**(1): 290-303.

Butenhoff, J., SC Chang, GW Olsen, PJ Thomford. (2012). "Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctane sulfonate in Sprague Dawley rats." *Toxicology* **293**: 1-15.

Cariou, R., B Veyrand, A Yamada, A Berrebi, D Zalko, S Durand, C Pollono, P Marchand, J-C Leblanc, J-P Antignac, B Le Bizec. (2015). "Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and chord serum of French women and their newborns." *Environment International* **84**: 71-81.

CDC (2016). Centers for Disease Control and Prevention. Breastfeeding Report Card.

CDC (2017). Centers for Disease Control and Prevention (CDC). Fourth National Report on Human Exposure to Environmental Chemicals. Updated Tables, January 2017, Volume One.

Chang, S., BC Allen, KL Andres, DJ Ehresman, R Falvo, A Provencher, GW Olsen, JL Butenhoff. (2016). "Evaluation of serum lipid, thyroid, and hepatic clinical chemistries in association with serum perfluorooctanesulfonate (PFOS) in cynomolgus monkeys after oral dosing with potassium PFOS." *Toxicological Sciences* (Submitted manuscript).

Chen, T., L Zhang, J-q Yue, Z-q Lv, W Xia, Y-j Wan, Y-y Li, S-q Xu. (2012). "Prenatal PFOS exposure induces oxidative stress and apoptosis in the lung of rat off-spring." *Reproductive Toxicology* **33**: 538-545.

Danish Ministry of the Environment (2015). Perfluoroalkylated substances: PFOA, PFOS and PFOSA. Evaluation of health hazards and proposal of a health based quality criterion for drinking water, soil and ground water. Environmental project No. 1665, 2015.

Dong, G.-H., Y-H Zhang, L Zheng, W Liu, Y-H Jin, Q-C He, (2009). "Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/5 mice." *Archives of Toxicology* **83**: 805-815.

Dong, G., MM Liu, D Wang, L Zheng, ZF Liang, YH Jin, (2011). "Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice." Archives of Toxicology **85**: 1235-1244.

EFSA (2008). European Food Safety Authority. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific Opinion of the Panel on Contaminants in the Food chain. (Question No EFSA-Q-2004-163).

Egeghy PP and M Lorber (2011). "An assessment of the exposure of Americans to perfluorooctane sulfonate: A comparison of estimated intake with values inferred from NHANES data." Journal of Exposure Science and Environmental Epidemiology. **21**: 150-168.

Fromme, H., C Mosch, M Morovitz, I Alba-Alejandre, S Boehmer, M Kiranoglu, F Faber, I Hannibal, O Genzel-Boroviczeny, B Koletzko, W Volkel. (2010). "Pre- and Postnatal Exposure to Perfluorinated Compounds (PFCs)." Environmental Science & Technology **44**: 7123-7129.

German Ministry of Health. (2006). Assessment of PFOA in the drinking water of the German Hochsauerlandkreis. Statement by the Drinking Water commission (Trinkwasserkommission) of the German Ministry of Health at the Federal Environment Agency June 21, 2006/revised July 13, 2006. Provisional Evaluation of PFT in Drinking Water with the Guide Substances Perfluorooctanoic acid (PFOA) and Perfluorooctane Sulfonate (PFOS) as Examples.

Harris, M., SL Rifas-Shiman, AM Calafat, X Ye, AM Mora, TF Webster, E Oken, SK Sagiv. (2017). "Predictors of Per- and Polyfluoroalkyl Substance (PFAS) Plasma Concentrations in 6–10 Year Old American Children." Environmental Science & Technology **Advance Access: DOI: 10.1021/acs.est.6b05811**

Health Canada (2010). Drinking Water Guidance Value Perfluorooctane sulfonate (PFOS).

Health Canada. (2016a). "Health Canada's Drinking Water Screening Values for Perfluoroalkylated Substances (PFAS)." Retrieved May 27, 2016, from <http://s3.documentcloud.org/documents/2756386/Health-Canada-PFAS-Screening-Values-Fact-Sheet.pdf>.

Health Canada. (2016b). "Perfluorooctane Sulfonate (PFOS) in Drinking Water. Draft for Public Consultation Document.", from <http://healthycanadians.gc.ca/health-system-systeme-sante/consultations/perfluorooctane-sulfonate/document-eng.php>.

Karrman, A., I Ericson, B van Bavel, PO Darnerud, M Aune, A Glynn, S Lignell, G Lindstrom. (2007). "Exposure of Perfluorinated Chemicals through Lactation: Levels of Matched Human Milk and Serum and a Temporal Trend, 1996-2004, in Sweden." Environmental Health Perspectives **115**: 226-230.

Kim, S.-K., KT Lee, CS Kang, L Tao, K Kannan, KR Kim, CK Kim, JS Lee, PS Park, YW Yoo, JY Ha, YS Shin, JH Lee. (2011). "Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures." Environmental Pollution **159**: 169-174.

Lau, C., JR Thibodeaux, RG Hanson, JM Rogers, BE Grey, ME Stanton, JL Butenhoff, LA Stevenson. (2003). "Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation." Toxicological Sciences **74**: 382-392.



Liu, J., J Li, Y Liu, HM Chan, Y Zhao, Z Cai, Y Wu. (2011 ). "Comparison on gestation and lactation exposure of perfluorinated compounds for newborns." Environment International **37**: 1206-1212.

Lopez-Doval, S., R Salgado, A Lafuente. (2016). "The expression of several reproductive hormone receptors can be modified by perfluorooctane sulfonate (PFOS) in adult male rats." Chemosphere **155**: 488-497.

Luebker, D., MT Case, RG York, JA Moore, KJ Hansen, JL Butenhoff. (2005b). "Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats." Toxicology **215**: 126-148.

Luebker, D., RG York, KJ Hansen, JA Moore, JL Butenhoff. (2005a). "Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats:dose-response and biochemical and pharmacokinetic parameters." Toxicology **215**: 149-169.

Lv, Z., G Li, Y Li, C Ying, J Chen, T Chen, J Wei, Y Lin, Y Jiang, Y Wang, B Shu, B Xu, S Xu. (2013). "Glucose and Lipid Homeostasis in Adult Rat Is Impaired by Early-Life Exposure to Perfluorooctane Sulfonate." Environmental Toxicology **28**: 532-542.

MDH (2008). Minnesota Department of Health. Statement of Need and Reasonableness (SONAR) in the Matter of Proposed Rules Relating to Health Risk Limits of Groundwater.

MDH (Minnesota Department of Health). (2017a). "MDH Health Risk Assessment Methods to Incorporate Human Equivalent Dose Calculations into Derivation of Oral Reference Doses (May 2011, revised 2017)." from <http://www.health.state.mn.us/divs/eh/risk/guidance/hedrefguide.pdf>.

MDH (Minnesota Department of Health) (2017b). Background Document: Toxicokinetic Model for PFOS and PFOA and Its Use in the Derivation of Human Health-based Water Guidance Values. .

Nelson, J. (2016). Personal Communication regarding MDH MN (East Metro) PFC biomonitoring project data based on June 9, 2015 Meeting Agenda and Materials for the Advisory Panel to the Environmental Health Tracking and Biomonitoring Program.  
<http://www.health.state.mn.us/divs/hpcd/tracking/panel/2015Junematerials.pdf>.

NTP (2016a). National Toxicocology Program. Draft Systematic Review of Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS).

NTP (September 2016). National Toxicocology Program Monograph - Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate.

Qiu, L., X Zhang, X Zhang, Y Zhang, J Gu, M Chen, Z Zhang, X Wang, S-L, Wang. (2013). "Sertoli Cell Is a Potential Target for Perfluorooctane Sulfonate-Induced Reproductive Dysfunction in Male Mice." Toxicological Sciences **135**(1): 229-240.

RIVM (2010). (National Institute for Public Health and the Environment) Environmental risk limits for PFOS. A proposal for water quality standards in accordance with the Water Framework Directive. Report 601714013/2010.

Schechter, A., N Malik-Bass, AM Calafat, K Kato, JA Colacino, TL Gent, LS Hynan, TR Harris, S Malla, L Birnbaum. (2012). "Polyfluoroalkyl Compounds in Texas Children from Birth through 12 Years of Age." Environmental Health Perspectives **120**: 590-594.

Scher D, P. C. (2016). Personal Communication. PFCs in FDL Study.

Seacat, A., PJ Thomford, KJ Hansen, GW Olsen, MT Case, JL Butenhoff. (2002). "Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys." Toxicological Sciences **68**: 249-264.

Seacat, A., PJ Thomford, KJ Hansen, LA Clemen, SR Eldridge, CR Elcombe, JL Butenhoff, (2003). "Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats." Toxicology **183**: 117-131.

TCEQ. (2016). "Texas Commission on Environmental Quality. Texas Risk Reduction Program (TRRP) - Protective Concentration Levels (PCLs).", from <https://www.tceq.texas.gov/remediation/trrp/trrppcls.html>.

Thibodeaux, J., RG Hanson, JM Rogers, BE Grey, BD Barbee, JH Richards, JL Butenhoff, LA Stevenson, C Lau, (2003). "Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations." Toxicological Sciences **74**: 369-381.

Thomford, P. (2002). 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Rats. Final Report. Volumes I-IX. Covance Study No. 6329-183.

United Kingdom. Drinking Water Inspectorate (2007). Guidance on the Water Supply (Water Quality) Regulations 2000/01 specific to PFOS (perfluorooctane sulphonate) and PFOA (perfluorooctanoic acid) concentrations in drinking water.

US EPA (2000). US Environmental Protection Agency . Office of Water. Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. EPA-822-B-00-004. October 2000.

US EPA (2011). US Environmental Protection Agency "Exposure Factors Handbook. Office of Research and Development." from <https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252>.

US EPA. (2016c). "US Environmental Protection Agency - Office of Water. Health Effects Support Document for Perfluorooctane Sulfonate (PFOS)." Retrieved May 19, 2016, from [https://www.epa.gov/sites/production/files/2016-05/documents/hesd\\_pfos\\_final-plain.pdf](https://www.epa.gov/sites/production/files/2016-05/documents/hesd_pfos_final-plain.pdf).

US EPA. (2016d). "US Environmental Protection Agency - Office of Water. Drinking Water Health Advisory for Perfluorooctane Sulfonate (PFOS)." Retrieved May 19, 2016, from [https://www.epa.gov/sites/production/files/2016-05/documents/pfos\\_health\\_advisory\\_final-plain.pdf](https://www.epa.gov/sites/production/files/2016-05/documents/pfos_health_advisory_final-plain.pdf).

Wambaugh, J., RW Setzer, AM Pitruzzello, J Liu, DM Reif, NC Kleinstreuer, N Ching, Y Wang, N Sipes, M Martin, K Das, JC DeWitt, M Strynar, R Judson, KA Houck, C Lau, (2013). "Dosimetric anchoring of *in vivo* and *in vitro* studies for perfluorooctanoate and perfluorooctanesulfonate." Toxicological Sciences **136**: 308-327.

Wang, L., Y Wang, Y Liang, J Li, Y Liu, J Zhang, A Zhang, J Fu, G Jiang, (2014). "PFOS induced lipid metabolism disturbances in BALB/c mice through inhibition of low density lipoproteins excretion." Scientific Reports **4**: 4582.

Wu, X., DH Bennett, AM Calafat, K Kato, M Stryner, E Andersen, RE Moran, DJ Tancredi, NS Tulse, I Hertz-Picciotto, (2015). "Serum concentrations of perfluorinated compounds (PFC) among selected populations of children and adults in California." Environmental Research **136**: 264-273.