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 ***

Perfluorooctanoic Acid

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 (located at: \\Data3fb\eh\HRA\COMMON\Guidance - Water\Tox reviews-completed\Final\PFOA\PFOA_2007Review\PFOA_Final_Nov07.pdf) developed in 2007 for additional information

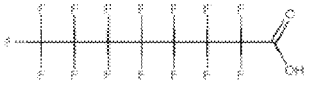
CAS # 335-67-1(free acid)

335-66-0 (acid fluoride)
3825-26-1 (ammonium salt, APFO)
2395-00-8 (potassium salt)
335-93-3 (silver salt)
335-95-5 (sodium salt)
[Note: perfluorooctanoate anion does not have a specific CAS number.]

Synonyms: *PFOA*
IUPAC name (PubChem):
2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid

Chemical Formula: C8-H-F15-O2

Structure:



	Initials	Date Started	Date Completed
Initial Primary Re-Review (Partial) Final Primary Re-Review (Final) Final Re-Review	HMG	7/22/2016 11/7/2016	9/5/2016 11/08/2016
Initial Secondary Re-Review (partial) Final Re-Review (final) Final Secondary Re-Review	JAJ	9/6/2016 11/9/2016	9/12/2016 11/16/2016
Initial Team Re-Review (partial) Final Re-Review (final) Final Secondary Re-Review	Tox Team	9/16/2016 11/23/2016 3/31/2017	10/5/2016 12/22/2016 4/20/2017

*** 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, Immune system)

* 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 Health-Based Guidance Evaluation

Perfluorooctanoic acid (PFOA) is a synthetic, fully fluorinated, organic acid used in a variety of consumer products and in the production of fluoropolymers and generated as a degradation product of other perfluorinated compounds. PFOA is one of a large group of perfluoroalkyl substances (PFASs) that are used to make products more resistant to stains, grease, and water. Major U.S. manufacturers voluntarily agreed to phase out production of PFOA by the end of 2015.

Because of strong carbon-fluorine bonds, PFOA is stable to metabolic and environmental degradation. Exposure to PFOA in the United States remains possible due to its legacy uses, existing and legacy uses on imported goods, degradation of precursors, and extremely high persistence in the environment and the human body.

PFOA 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 2016b) along with a Health Effects Support Document (HESD) (USEPA 2016a) for PFOA 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.

PFOA is a bioaccumulative chemical, with an average half-life of 2.3 years in humans. High, short-term exposures result in an internal body burden that can take several 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 bottle-fed and breast-fed infants as well as long-term exposures.

Noncancer HBV = 0.035 ug/L (Developmental, Hepatic (liver), Immune, and Renal (kidney) systems)
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 2016b) Based on RfD derived from a developmental tox study in mice (reduced ossification of proximal phalanges & accelerated puberty in male pups), RSC of 0.2, and lactating women intake rate (0.054 L/kg-d). HA is protective of short as well as lifetime exposure. A cancer-based value was also calculated (0.5 ug/L) but since it was greater than the noncancer value it was not used. [previous provisional HA was 0.4 ug/L (2009)]	5/19/2016
0.4 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
0.4 ug/L	Drinking water guideline value	Delaware Dept of Resources and Environmental Control aci (USEPA 2016b)	
0.4 ug/L (Class I) 2 ug/L (Class II)	Provisional Groundwater Remediation Objective	Illinois EPA aci (ASTSWMO 2015). Based on RfD from MDH.	
0.06 ug/L	Drinking water guideline value	Maine Department of Health and Human Services aci (ASTSWMO 2015)	
0.42 ug/L	Drinking water guideline value	Michigan Department of Environmental Quality 2011 aci (USEPA 2016b)	
0.04 ug/L 0.014 ug/L	Drinking water guideline value Draft Health-based MCL	(New Jersey Department of Environmental Protection. 2007) Based on 'target' human blood level of 0.018 mg/mL, total UF of 100 (10A, 10H), DW-to-blood concentration factor of 100, and RSC of 0.2 (New Jersey Drinking Water Quality Institute 2016) health-based MCL recommendation is based on target human serum level of 0.0145 ug/mL based on increase liver wt in mice. A total UF of 300 (10H, 3A, 10DB) was applied. A clearance factor of 0.00014 was applied, resulting in an RfD of 2 ng/kg-d (or 0.000002 mg/kg-d). The draft MCL is based on the RfD, 2 L/70 kg-d intake rate and an RSC of 0.2. Recommendation was finalized in March 2017. NJ also calculated a cancer slope factor of 0.021 per mg/kg-d based on increased incidence of testicular tumors. The health-based MCL based on cancer effects @1 in a million lifetime cancer risk level is 0.014 ug/L – same as the noncancer value.	
2 ug/L	Drinking water guideline value	North Carolina Division of Water Quality aci (United States Environmental Protection Agency (EPA) - Office of Water 2016b)	
0.29 ug/L	Groundwater used as drinking water	(TCEQ 2016) Based on RfD 0.000012 mg/kg-d	
0.02 ug/L	Drinking water guideline value	Vermont Agency of Natural Resources aci (United States Environmental Protection Agency (EPA) - Office of Water 2016b)	

5 ug/L 50 ug/L	enHealth interim Drinking water quality guideline Recreational water quality guideline	(Australian Health Protection Principal Committee. enHealth 2016) http://www.health.nsw.gov.au/environment/factsheets/Documents/pfas-interim-health-values-ahppc.pdf Based on TDI of 0.0015 mg/kg-d.	6/29/2016
0.2 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 30 ug/L. Noncancer value based on POD_{HEQ} of 0.000625 mg/kg-d (Perkins et al 2004 rat study) and composite UF of 25 resulting in a TDI of 0.000025 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 PFOA was 0.7 ug/L]	
0.3 ug/L	Drinking Water (and ground water used for drinking water)	(Danish Ministry of the Environment 2015) Based on TDI of 0.0001 mg/kg-d, 'RSC' of 0.1, and intake rate of 0.03 L/kg-d. 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. Water guidance for PFOS and PFOSA is 0.1 ug/L	
0.3 ug/L >0.1-0.6 ug/L >0.6-1.5 ug/L >1.5-5.0 ug/L 5.0 ug/L 0.5 ug/L	Lifelong precautionary value Precautionary Action Values (PAV) PAV_{10} PAV_3 PAV_1 PAV_0 PAV for infants & pregnant women	(Health. 2006) Drinking Water value - lifelong health tolerable guidance value for all populations groups (from 2003) 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.	1/5/2007
0.09 ug/L		(Sweden) Livsmedelsverket (2014), aci (Danish Ministry of the Environment 2015). A maximal tolerable level of 0.09 µg/L was derived for PFOS. 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: Perfluoroctane sulfonate (PFOS); Perfluorhexane sulfonate (PFHxS); Perfluorobutane sulfonate (PFBS); Perfluoroctanoic 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 = 10 ug/L (Level 1 + put measures in place to reduce to below 10 ug/L) Level 3 = 90 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.

Value and/or Type of Review	Type/Description (Year of Publication)	Source [Refer to the General HRL EndNote Library]	Date Obtained
0.00002 mg/kg-d	RfD (2016)	(USEPA 2016a) Health Effects Support Document for Perfluorooctanoic Acid (PFOA)	5/49/2016
0.00002 mg/kg-d	Draft intermediate MRL (Draft Toxicological Review 2015)	(ATSDR 2015) http://www.atsdr.cdc.gov/toxprofiles/tp200.pdf 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). $BMDL_{10}$ for liver weight (Butenhoff et al 2002 study in monkeys) used to generate a HED POD of 0.00154 mg/kg-d. Total UF 90 (3A, 10H, 2 DB) resulted in intermediate MRL of 0.00002 mg/kg-d	9/15/2015
0.000006 mg/kg-d	RfD	(Prevention. 2014) Based on POD of 0.0018 mg/kg-d (geometric mean of 6 HEDs for liver effects) and total UF of 300 (3A, 10H, 10DB)	
0.0000153 mg/kg-d	Intermediate RfD	(Michigan Department of Environmental Quality 2011) Based on LOAEL of 3 mg/kg-d (monkey study by Butenhoff et al 2002). Adjusted for differences in half-life = 0.046 mg/kg-d. Divided by 3,000 total UF (3A, 10H, 10L, 10S)	
0.000002 mg/kg-d 0.021 per mg/kg-d	Draft RfD Draft CSF	(New Jersey Drinking Water Quality Institute 2016) draft target human serum level of 0.0145 ug/mL based on increase liver wt in mice. A total UF of 300 (10H, 3A, 10DB) was applied. A clearance factor of 0.00014 was applied, resulting in an RfD of 2 ng/kg-d (or 0.000002 mg/kg-d). NJ also calculated a cancer slope factor of 0.021 per mg/kg-d based on increased incidence of testicular tumors.	
0.0015 mg/kg-d	Interim TDI	(Australian Health Protection Principal Committee 2016) [adopted 2008 EFSA TDI] http://www.health.nsw.gov.au/environment/factsheets/Documents/pfas-interim-health-values-ahppc.pdf	6/29/2016

0.000025 mg/kg-d	Draft TDI	(Health Canada 2016a) Screening Value and draft proposed drinking water guideline (Health Canada 2016b). Draft document included calculation of a cancer based TDI of 0.003 mg/kg-d (resulting in DW guidance level of 30 ug/L), which was less conservative than the noncancer TDI. Noncancer value based on POD_{HEQ} of 0.000625 mg/kg-d (Perkins et al 2004 rat study) and composite UF of 25 resulting in a TDI of 0.000025 mg/kg-d. Documents are expected to be finalized in 2017. [The previous Drinking Water Guidance Value of 0.7 ug/L (Health Canada 2010) was based on HED of 0.000077 mg/kg-d (based on monkey study by Butenhoff et al 2002 and serum level of 77 ug/mL @LOAEL)].	6/30/2016
0.0001 mg/kg-d	TDI	(Danish Ministry of the Environment 2015) Based on $BMDL_{10}$ of 0.456 mg/kg-d from Palazzolo et al 90 day rat study. Converted to HED of 0.003 mg/kg-d by ~142 factor for TK, UFA 3 and UFH of 10.	6/2/2016
0.0015 mg/kg-d	TDI	(EFSA 2008) Administered dose NOAEL of 0.06 mg/kg-d (subchronic study in rats) and administered dose $BMDL_{10}$ from a number of rat and mouse studies of 0.3 – 0.7 mg/kg-d. Admin dose $BMDL_{10}$ value of 0.3 mg/kg-d 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 0.0015 mg/kg-d.	1/14/2009

*** 5. Toxicokinetic and Toxicodynamic Information ***

Toxicokinetics:

Source: (USEPA 2016a) (See Chapter 2 for additional information) and (USEPA 2016b) as well as MDH 2007 review worksheet.

NOTE: *Toxicokinetic profiles and the underlying mechanism for half-life differences across species/genders are not completely understood, although many of the differences appear to be related to elimination kinetics and factors that control membrane transport. To date, three transport families appear to play a role in absorption, distribution, and excretion: organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and multidrug resistance-associated proteins (MRPs).*

Absorption: Absorption data are available for oral, inhalation, and dermal exposure in laboratory animals, and extensive data are available from humans demonstrating the presence of PFOA in serum. PFOA is moderately soluble in aqueous solutions and oleophobic (i.e., minimally soluble in body lipids), movement across the apical and basal membrane surfaces of the lung, gastrointestinal tract, and skin involves transporters or mechanisms other than simple diffusion across the lipid bilayer. As discussed above, there are three transport families that appear to play a role (i.e., OATs, OATPs, and MRPs) in enterocytes in uptake of PFOA. Together they function in the uptake of organic anions from gastrointestinal contents and transport of those anions into the portal blood supply.

Based on animal data, PFOA is well absorbed following oral exposure, with several studies reporting >90% total absorbed. An inhalation study in rats resulted in measurable serum concentrations following repeated exposure demonstrating absorption of PFOA, however percent

absorption was not reported. There is evidence that PFOA is absorbed following dermal exposure. The results of *in vitro* percutaneous absorption studies of PFOA through rat and human skin have been reported by Fasano et al 2005 and suggest only a small portion (1.44 ± 1.13%) of the total AFPO applied penetrated through rat skin and a negligible amount (0.048 ± 0.01%) penetrated human skin after 48 hours. The calculated permeability coefficients were $3.25 \pm 1.51 \times 10^{-5}$ centimeters per hour (cm/h) and $9.49 \pm 2.86 \times 10^{-7}$ cm/h in rat and human skin, respectively. A dermal toxicity study by Kennedy (1985) indicated that application of an aqueous paste of APFO could produce toxicity at high doses.

Distribution: It has been suggested that PFOA circulates in the body by noncovalently binding to plasma proteins. Protein binding in plasma from cynomolgus monkeys, rats, and humans was evaluated via *in vitro* methods - rat, human, and monkey plasma proteins were able to bind 97–100% of the PFOA added at concentrations ranging from 1 to 500 ppm. Human serum albumin (HSA) carried the largest portion of the PFOA among the protein components of human plasma. Serum albumin is a common carrier of hydrophobic materials in the blood, including short- and medium-chain fatty acids, thyroxine (T4), heme, inorganic ions, and some pharmaceuticals. Approximately 60% of the serum protein in humans and rats is albumin. A variety of measurements of the albumin/PFOA complex suggest a conformational change in the protein as a result of the PFOA binding as well.

The binding of PFOA to human TTR (thyroid hormone transport protein) has also been evaluated *in vitro* using a radioligand-binding assay. PFOA demonstrated a high binding affinity for TTR with 949 nmol, causing a 50% inhibition of T4 binding to the TTR. It also is possible that PFOA will display nonspecific binding to proteins within the cellular matrix as well as in the serum but little work has been done to investigate that probability.

No clinical studies are available that examined tissue distribution in humans following administration of a controlled dose of PFOA. However, samples collected in biomonitoring and epidemiology studies provide data showing distribution of PFOA within the body.

The highest tissue concentrations are usually in the liver. Liver accumulation in males is greater than in females. Other tissues with a tendency to accumulate PFOA are the kidneys, lungs, heart, and muscle, plus the testes in males and uterus in females. Post-mortem studies in humans have found PFOA in liver, lungs, bone, and kidneys, but only low levels in brain.

During pregnancy, PFOA is present in the placenta and amniotic fluid in both animals and humans. Post-delivery, PFOA is transferred to offspring through lactation in a dose-related manner.

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 ~90% of maternal serum concentrations and levels in breast milk are typically ~5% of maternal serum concentrations. One study (Fromme 2010) also measured serum concentrations in mothers and breastfed infants at 6 months after delivery and reported ~5-fold higher serum concentrations in infants than in their mothers.*

Metabolism: PFOA is stable to metabolic and environmental degradation because of strong carbon-fluorine bonds. It also is resistant to metabolic biotransformation.

Elimination: Excretion data are available for oral exposure in humans and laboratory animals. Several studies have investigated the elimination of PFOA in humans, Cynomolgus monkeys, and rats. In human females, elimination pathways include pregnancy (cord blood) and lactation (breast milk).

Elimination half-lives differ among species. There are also significant gender differences in humans and some laboratory animal species. Information from humans does not, at this time, provide sufficient data to determine the magnitude of inter-individual and gender differences in excretory half-lives. The transporters appear to play an important role in renal excretion of PFOA and possibly its biliary elimination as well.

Reported half-life in humans typically range from 2.3 – 3.8 years. Half-lives from animals included: monkeys (M/F 30/21 days); rats (M/F 11.5 days/3.4 hours); and mice (M/F 27.1/15.6 days). The gender difference between male and female rats is not seen in mice. Several studies have evaluated the impact of developmental age on gender differences in rats and found that PFOA plasma concentrations were 35–65-fold higher in males than in females at > 5 weeks of age but not at 4 weeks. It appears that maturation of the transport features responsible for the gender difference in elimination occurs between the ages of 3 and 5 weeks in the female rat and appears to be related to hormonal control.

Dose level also impact excretion. Rigden et al (2015) evaluated urinary levels of PFOA following doses of 0, 10, 33 & 100 mg/kg-d for 3 days. The urinary levels at 33 and 100 mg/kg-d were 500 and 3,200 times greater than at 10 mg/kg-d suggesting that there is a threshold limit on resorption (e.g., saturation of resorption). As a consequence, half-life for continuous low-dose exposure would be longer than for single or short-term high-dose exposures.

Several studies evaluating the role of transporters in the kidney tubules have been conducted. Most studies have examined the organic anion transporters (OATs) located in the proximal portion of the descending tubule. OATs are found in other tissues as well and were discussed earlier for their role in absorption and distribution. In the kidney, they are responsible for delivery of organic anions, including a large number of medications from the serum into the kidney tubule for excretion as well as reabsorption of anions from the glomerular filtrate. The transporters are particularly important in excretion of PFOA because it binds to surfaces of serum proteins (particularly albumin), which makes much of it unavailable for removal during glomerular filtration. Other transporter families believed to be involved in renal excretion are the OATPs and the multidrug resistance-associated proteins (MRPs). However, they have not been evaluated as extensively as the OATs for their role in renal excretion.

Knowledge about specific OAT, OATP, and MRP transporters in the kidney is evolving. Studies to date regarding the gender specific elimination rates in rats indicate that female rats possess an active secretory mechanism that male rats do not possess. Sex hormones were also observed to have an effect on elimination rates in rats. Male sex hormones (e.g., testosterone) appear to decrease the presence of OATs in the renal basolateral membranes while female sex hormones (e.g, estradiol) appear to increase the transporters.

Much work remains to be done to explain the gender differences between male and female rats and to determine whether it is relevant to humans. Similarities are possible because the long half-life in humans suggests that they might be more like the male rat than the female rat. There is a broad range of half-lives in human epidemiology studies suggesting a variability in the unbound fraction of PFOA in serum or in human transport capabilities resulting from genetic variations in structures and consequently in function. Genetic variations in human OATs and OATPs are described in a review by Zaïr et al. (2008).

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 (Nelson 2016). Treatment to remove PFCs was

added to the PWS and volunteer participants had blood levels measured at three time points: 2008, 2010 and 2014:
2008 – 14.9 ug/L geo mean (CI 12.9 – 17.3); 95th percentile 60 ug/L (range 1.6 – 117 ug/L)
2010 – 11.2 ug/L geo mean (CI 9.7 – 13.1); 95th percentile 48.7 ug/L (range 0.94 – 110.5 ug/L)
2014 – 5.5 ug/L geo mean (CI 4.6 – 6.4); 95th percentile 26 ug/L (range <LOD – 47 ug/L)

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 – 1.8 geo mean ug/L (CI 1.6-2.0); 95th percentile 5 ug/L (range 0.17-8.1).

Personal communication with Deanna Scher 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 PFOA from 2003 to 2004 collected by NHANES. PFOA was detected in a high percentage 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 2017(CDC 2017):

Geometric mean ug/L (95th% CI) and 95th Percentile ug/L (95th% CI) from 1999 through 2014 were:
1999 – 2000: 5.21 (4.72-5.74) and 11.9 (10.9-13.5) ug/L
2003-2004: 3.95 (3.65-4.27) and 9.80 (7.40-14.1)
2005-2006: 3.92 (3.48-4.42) and 11.3 (8.80-14.5)
2007-2008: 4.12 (4.01-4.24) and 9.60 (8.90-10.1)
2009-2010: 3.07 (2.81-3.36) and 7.50 (6.20-9.70)
2011-2012: : 2.08 (1.95-2.22) and 5.68 (5.02-6.49)
2013-2014: 1.94 (1.76-2.14) and 5.57 (4.60-6.27)

Taken together, the data suggest that PFOA concentrations in human serum in the U.S. declined between 1999 and 2012. Over the course of the study, the geometric mean concentration of PFOA in human serum decreased from 5.21 µg/L to 2.08 µg/L and the 95th percentile concentration decreased from 11.9 µg/L to 5.68 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.

Toxicodynamics:
Source: (USEPA 2016a) (See Chapter 2 for additional information) and (USEPA 2016b) as well as MDH 2007 review worksheet.

Mode/Mechanism of Action Information:

Noncancer Effects –
Since PFOA is metabolically stable it is the toxicity of the parent compound that is of concern.

Human epidemiology data report associations between PFOA exposure and high cholesterol, increased liver enzymes, decreased vaccination response, thyroid disorders, pregnancy-induced hypertension and preeclampsia, and cancer (testicular and kidney). Epidemiology studies examined workers at PFOA production plants, a high-exposure community population near a production plant in the United States (i.e., the C8 cohort), and members of the general population in the United States, Europe, and Asia.

For PFOA, oral animal studies of short-term subchronic and chronic duration are available in multiple species including monkeys, rats, and mice. These studies report developmental effects, liver and kidney toxicity, immune effects, and cancer (liver, testicular, and pancreatic). Developmental effects observed in animals include decreased survival, delayed eye opening and

reduced ossification, skeletal defects, altered timing of on-set of puberty, and altered mammary gland development.

Because of its impact on cellular receptors and proteins, PFOA possesses the ability to impact the biotransformation of dietary constituents, intermediate metabolites, and other xenobiotic chemicals by altering enzyme activities and transport kinetics. PFOA is known to activate PPAR pathways by increasing transcription of mitochondrial and peroxisomal lipid metabolism, sterol, and bile acid biosynthesis and retinol metabolism genes. Based on transcriptional activation of many genes in PPARα-null mice, however, indicate that it also can activate the CAR, FXR, and PXR and metabolic activities linked to these nuclear receptors.

Cancer Effects –
Under EPA's Guidelines for Carcinogen Risk Assessment (USEPA 2005a), there is "suggestive evidence of carcinogenic potential" for PFOA. Epidemiology studies demonstrate an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population. Two chronic bioassays of PFOA support a positive finding for its ability to be tumorigenic in one or more organs of male rats, including the liver, testes, and pancreas. *[The half-life in female rats is very short so it is possible that carcinogenic potential in female has not adequately been tested. No cancer bioassays are available in other species.]*

Supplement to Original Review Completed on 12/10/2007.

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

Relevant Epidemiology Studies or Human Information:

Sources: (USEPA 2016a) [See Section 3.1] *[reviewed by MDH epi staff – no suggested edits]*

Epidemiology studies of effects of PFOA have been conducted in three types of populations:

- workers exposed in chemical plants producing or using PFOA (~serum concentration range of 0.010 – > 2.0 (means around 1–4 µg/mL),
- high-exposure communities (i.e., an area in West Virginia and Ohio that experienced water contamination over a period of more than 20 years) (~serum concentration range 0.010–0.100 µg/mL), and
- general population studies with background exposures (~serum concentration range below LOD to < 0.010 µg/mL).

Although moderate-to-high correlations between PFOA and PFOS are often seen in general populations (r > 0.5), the correlation is lower in the West Virginia and Ohio high-exposure area (r=0.3). In evaluating and synthesizing results from these studies, it is important to consider differences in the exposure range within the study population and the exposure level within the referent group, as differences (or inconsistencies) can be expected depending on the shape of the exposure-response curve and the exposure range encompassed by different studies. In addition, the optimal choice of an exposure metric (e.g., cumulative or a time-specific) depends on the specific outcome being examined. Health outcomes assessed include blood lipid and clinical chemistry profiles, thyroid effects, diabetes, immune function, birth and fetal and developmental growth measures, and cancer.

Serum lipids –

The association between PFOA and serum lipids has been examined in several studies in different populations. Cross-sectional and longitudinal studies in occupational settings and in the high-exposure community (the C8 Health Project study population) generally observed positive associations between serum PFOA and total cholesterol (TC) in adults and children (aged 1–< 18 yrs); most of these effect estimates were statistically significant. Although exceptions to this pattern are present (e.g., some of the analyses examining incidence of self-reported high cholesterol based on medication use, the results are relatively consistent and robust. Similar associations were seen in analyses of LDL, but were not seen with HDL. The range of exposure in occupational studies is large (with means varying between 0.4 and > 12 µg/mL), and the mean serum levels in the C8 population studies were around 0.08 µg/mL. Positive associations between serum PFOA and TC (i.e., increasing lipid level with increasing PFOA) were observed in most of the general population studies at mean exposure levels of 0.002–0.007 µg/mL. The interpretation of results for these general population studies is limited, however, by the moderately strong correlations (Spearman r > 0.6) and similarity in results seen for PFOS and PFOA. Additionally, many of the C8 studies do not appear to have controlled for the impact of diet on serum lipids.

Liver disease and liver function –

Few studies of the relationship between PFOA and liver disease are available, but the C8 Health Project did not observe associations with hepatitis, fatty liver disease, or other types of liver disease. In the studies of PFOA exposure and liver enzymes (measured in serum), positive associations were seen. The results of the occupational studies provide evidence of an association with increases in serum AST, ALT, and GGT, with the most consistent results seen for ALT. The associations were not large and might depend on the covariates in the models, including BMI, use of lipid

lowering medications, and triglycerides. Two population-based studies of highly exposed residents in contaminated regions near a fluorochemical industry in West Virginia have evaluated associations with liver enzymes, and the larger of the two studies reported associations of increasing serum ln ALT and ln GGT levels with increasing serum PFOA concentrations. A cross sectional analysis of data from the NHANES, representative of the U.S. national population, also found associations with ln PFOA concentration with increasing serum ALT and ln GGT levels. Serum bilirubin was inversely associated with serum PFOA in the occupational studies. A U-shaped exposure-response pattern for serum bilirubin was observed among the participants in the C8 Health Project, which might explain the inverse associations reported for occupational cohorts. Overall, an association of serum PFOA concentration with elevations in serum levels of ALT and GGT has been consistently observed in occupational, highly exposed residential communities, and the U.S. general population. The associations are not large in magnitude, but indicate the potential of PFOA to affect liver function.

Immune function –
Associations between prenatal, childhood, or adult PFOA exposure and risk of infectious diseases (as a marker of immune suppression) have not been consistently seen, although there was some indication of effect modification by gender (i.e., associations seen in female children but not in male children). Three studies have examined associations between maternal and/or child serum PFOA levels and vaccine response (measured by antibody levels) in children (mean 0.004 ug/mL) and in adults (mean 0.032 ug/mL). The study in adults was part of the high-exposure community C8 Health Project. A reduced antibody response to one of the three influenza strains tested after subjects received the flu vaccine was seen with increasing levels of serum PFOA; these results were not seen with PFOS. The studies in children were conducted in general populations in Norway and in the Faroe Islands. Decreased vaccine response in relation to PFOA levels was seen in these studies, but similar results also were seen with correlated PFASs (e.g., PFOS).

[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. The panel agreed that:

- *The scientific evidence for suppression of the antibody response from experimental animal studies and human studies of PFOA support a high and moderate level of evidence, respectively.*
- *Moderate level of evidence in experimental animal studies for increased hypersensitivity-related outcomes, and low level of evidence in humans. [Note – draft monograph had proposed high level of evidence for experimental animals but following peer review discussion this was changed to moderate.]*

The draft monograph was finalized in September 2016 (NTP 2016a)

Thyroid -
Three large studies provide support for an association between PFOA exposure and incidence or prevalence of thyroid disease in women or children with background exposure (mean 0.026 – 0.123 ug/mL), but not in men. In addition, associations between PFOA and TSH were seen in pregnant females with anti-TPO antibodies. In contrast, generally null associations were found between PFOA and TSH in people who had not been diagnosed with thyroid disease.

Diabetes -

No associations were observed between serum PFOA levels and type II diabetes incidence rate in general or worker populations with mean serum PFOA up to 0.0913–0.113 µg/mL. PFOA was not associated with measures of metabolic syndrome in adolescents or adults. However, one study found an increased risk for developing gestational diabetes in females with mean serum PFOA (measured preconception) of 0.00394 µg/mL.

Fertility, pregnancy, and birth outcomes –
There are no occupational exposure or general population studies examining pregnancy-related hypertension and preeclampsia in relation to PFOA exposure. The only data available come from the high-exposure C8 Health Project study population. Several studies, using different designs and exposure measures, have examined that outcome in this population. There is a progressively greater refinement and reduction in misclassification (or exposure and outcome) among this set of studies. Each of the studies provides some evidence of an association between PFOA exposure and risk of pregnancy-induced hypertension or preeclampsia (0.01 – 0.02 ug/mL), with the most robust findings from the methodologically strongest study.

The association between PFOA and birth weight was examined in numerous studies. Most studies measured PFOA using maternal blood samples taken in the second or third trimester or in cord blood samples. Studies on the high-exposure C8 community population did not observe associations between PFOA and either birth weight among term births or the risk of low birth weight among all (singleton) births. In contrast, several analyses of general populations indicate a negative association between PFOA levels and birth weight, while others did not attain statistical significance. A meta-analysis of many of these studies found a mean birth weight reduction of 19 g (95% CI: -30, -9) per each one unit (ng/mL) increase in maternal or cord serum PFOA levels. It has been suggested that GFR can impact birth weight. A meta-analysis based on PBPK simulations found that some of the association reported between PFOA and birth weight is attributable to GFR and that the actual association could be closer to a 7-g reduction (95% CI: -8, -6). Verner et al. (2015) showed that, in individuals with low GFR, there are increased levels of serum PFOA and lower birth weights. While there is some uncertainty in the interpretation of the observed association between PFOA and birth weight given the potential impact of low GFR, the available information indicates that the association between PFOA exposure and birth weight for the general population cannot be ruled out. In humans with low GFR (which includes females with pregnancy-induced hypertension or preeclampsia), the impact on body weight is likely due to a combination of the low GFR and the serum PFOA.

Two studies examined development of puberty in females in relation to prenatal exposure to PFOA as measured through maternal or cord blood samples in follow-up of pregnancy cohorts conducted in England. The results of these two studies are conflicting, with no association (or possible indication of an earlier menarche seen with higher PFOA) in one study, and a later menarche seen with higher PFOA in the second study. Another study examined PFOA exposure measured concurrently with the assessment of pubertal status. An association between later age at menarche and higher PFOA levels was observed, but the interpretation of this finding is complicated by the potential effect of puberty on the exposure biomarker levels (i.e., reverse causality).

Studies found a positive association with ADHD in children in the highly exposed community and the general population. No other behavior endpoints in children were associated with maternal PFOA levels in either population. Limited data suggest a correlation between higher PFOA levels (>0.02 µg/mL) in females and decreases in fecundity and fertility, but there are no clear effects of PFOA on male fertility endpoints (0.0035–0.005 µg/mL).

C8 Science Panel conclusions –

As part of the C8 Health Project, the C8 Science Panel used epidemiological and other data available to them to assess probable links between PFOA exposure and disease. Analyses conducted by the C8 Science Panel used historical serum PFOA estimates over time, which were developed based on estimated intake of contaminated drinking water. The panel concluded that a probable link existed between PFOA exposure and ulcerative colitis, high cholesterol, pregnancy-induced hypertension, and thyroid disease.

The C8 Science Panel found no probable link between PFOA exposure and multiple other conditions, including birth defects, other autoimmune diseases (e.g., rheumatoid arthritis, lupus, type 1 diabetes, Crohn's disease, MS), type II diabetes, high blood pressure, coronary artery disease, infectious disease, liver disease, Parkinson's disease, osteoarthritis, neurodevelopmental disorders in children (e.g., ADHD, learning disabilities), miscarriage or stillbirth, chronic kidney disease, stroke, asthma or COPD, and preterm birth or low birth weight.

Cancer –
Evidence of carcinogenic effects of PFOA in epidemiology studies is based on studies of kidney and testicular cancer. These cancers have relatively high 5-year survival rates of 73% for kidney cancer and 95% for testicular cancer (based on National Cancer Institute [NCI] Surveillance, Epidemiology, and End Results data for 2005–2011). Thus studies that examine cancer incidence are particularly useful for these types of cancer. The high-exposure community studies also have the advantage for testicular cancer of including the age period of greatest risk, as the median age at diagnosis is 33 years. The two occupational cohorts in Minnesota and West Virginia do not support an increased risk of these cancers, but each of them is limited by a small number of observed deaths and incident cases. Two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrollment of 0.024 µg/mL) and kidney and testicular cancers. There is some overlap in the cases included in these studies. None of the general population studies examined kidney or testicular cancer, but no associations were found in the general population between mean serum PFOA levels up to 0.0866 µg/mL and colorectal, breast, prostate, bladder, or liver cancer. As part of the C8 Health Project, the C8 Science Panel concluded that a probable link existed between PFOA exposure and testicular and kidney cancer.

A group of independent toxicologists and epidemiologists critically reviewed the epidemiological evidence for cancer based on 18 studies of occupational exposure to PFOA and general population exposure with or without co-exposure to PFOS. The project was funded by 3M, but the company was not involved in the preparation or approval of the report. The authors evaluated the published studies based on the study design, subjects, exposure assessment, outcome assessment, control for confounding, and sources of bias. They followed the Bradford Hill guidelines on the strength of the association, consistency, plausibility, and biological gradient in reaching their conclusion. They found a lack of concordance between community exposures and occupational exposures one or two magnitudes higher than those for the general population. The discrepant findings across the study populations were described as likely due to chance, confounding, and/or bias (Chang et al. 2014).

PFOA is a 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 PFOA 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 PFOA 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 PFOA from the model were used to determine the HED associated with the study NOAEL and LOAEL. Wambaugh et al. (2013) used a model 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 PFOA 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 PFOA 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 at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF**[2]	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
Animal Studies: **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 POD multiplied by estimated human Clearance rate (0.00014 L/kg bw/day)[1].						
Reproductive & Developmental Studies:						
2-Gen Gavage Study – Sprague-Dawley Rats (F0 30/sex/dose; F1 60/sex/grp) F0 – dosed 10 wks prior to mating & until sac (M after mating; F after weaning) Study duration 84 days (F0 M) F1 - in utero, lactation, & through	0, 1, 3, 10 or 30 *Sexually mature Fs have very short half-life. As a result serum concentrations are low (i.e., measured final serum concen at 10 & 30 mg/kg-d were 0.37 & 1.02 ug/mL) and more episodic. As a result utility of developmental*	F0 – Females: no effects F0 – Males ≥ 45.9 ug/mL - ↑ rel liv wt (Ms – 21, 47, 61, & 84%, p<0.01); ↑ rel kidney wt (Ms – 16-17, 22-23, 21-22, 23-27%, p<0.01)# *[Note: histological assessment does not appear to have been conducted.]* ≥ 101.2 ug/mL - ↓ BW (7, 12, & 26%, p<0.01); ↑ mean rel to bw food consumption (Ms- 105, 110 & 118% of controls) ≥ 171.1 ug/mL - ↑thickness & prominence of the zona glomerulosa & vacuolation in the cells of the adrenal cortex (Ms – 2/10 & 7/10)	F0 & F1 (adult) Ms NA EPA NOAEL 45.9 ug/mL EPA LOAEL based on ↓BW & ↑ rel kidney wt	300 (3A, 10H, 10L) EPA	0.00002 EPA	(Butenhoff 2004a) and aci EPA 2016a #*[EPA indicates that ↑ kidney wts at lower doses can be regarded as adaptive response to transport challenge, however no supporting*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
mating for Ms & weaning for Fs. Study duration 16 wks (M) (112 days); 10 wks (F) (70 days) F2 – through PND22	*studies in rats are limited.* *Males F0 - Measured final serum concentration: NA, NA, 51.5, & 45.3 ug/mL* Predicted AUC ug/mL*h 92500, 204000, 345000, & 412000 mg/L-h (EPA 2016a Table 4-3) Average serum concentration = predicted M AUC ug/mL-hr/(84 d x 24 hr/d) = 45.9 ug/mL 101.2 171.1 204.4	204.4 ug/mL – sac of 1M @day 45 duc to adverse clinical signs; clinical signs in other Ms including dehydration, urine-stained abdominal fur, & ungroomed coat; ↓ absol food consumption (~91% of controls) <u>*MDH BMD modeling:*</u> *F0 M BW @termination (BMDL/BMD) – 75.6/87.2 ug/mL (BMR_{10}) lowest value but modeling identified as "questionable".* *F0 M rel right kidney wt @ termination (BMR_{10}) – 6.56/16.0 ug/mL* *F0 M rel liver wt @ termination (BMR_{10}) – models unusable or questionable (lowest values 16.5/20.4 ug/mL)* F1 – pups *[remember F rats – very short $t_{1/2}$]* Highest dose grp – –↓mean pup wt/litter (~8-9.5%) ↑ mortality shortly after weaning; delayed sexual maturation (Ms ave 3.7 days & Fs ave 1.7 days longer) F1 – adult offspring ≥ 45.9 ug/mL - ↓ BW (Ms @termination 6, 6, 11, & 22%); ↑ absol & rel (Ms 20, 40, 53, & 76%) liver wts (Ms); ↑ rel kidney wt (Ms – 11-13, 18-19, 17, & 16-17%) ≥ 101.2 ug/mL – discolored areas in liver (Ms 6/60, 10/60, & 9/60), diffuse hepatocellular hypertrophy & scattered incidence of focal-to-multifocal necrosis & inflammation in liver (Ms); ↓ absol & rel pituitary wt (no histological changes)	[NOAEL/LOAEL$_{HED}$ NA/0.0064 mg/kg-d] F0 Males 6.56 (F0) ug/mL Ms MDH BMDL 16.0 (F0) ug/mL Ms MDH BMD based on ↑ rel kidney wt [F0 NOAEL/LOAEL$_{HED}$ 0.00092/0.0022 mg/kg-d] F1 pups 171.1 ug/mL EPA NOAEL 204.4 ug/mL EPA LOAEL based on ↓pup BW & ↑ number of dead pups	*30 (3A, 10H) MDH*	*0.000031 MDH <u>for compariso n purposes only</u>*	*rationale was provided]*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		≥ 171.1 ug/mL - ↓ absol food intake but rel food consump signif ↑ (Ms) 204.4 ug/mL - ↑ incidence of urine-stained abdominal fur, abdominal distention; hypertrophy & vacuolation of the zona glomerulosa of the adrenal gland (7/10); ↓ motor activity (Ms); ↓ BW/BWG (Fs) during cohabitation; ↑ ave number of estrous stages (5.4 vs 4.7 per 21 days) but upon further evaluation found not to be signif different *MDH BMD modeling of adult F1 Males:* *F1 M BW @termination (BMR_{10}) – 90.0/111 ug/mL but modeling identified as Questionable.* *F1 M rel right kidney wt @ termination (BMR_{10}) – 19.7/45.3 ug/mL* *F1 M rel liver wt @ termination (BMR_{10}) – all models unusable (lowest values 16.9/21.6 ug/mL)* F2 Pups *[remember F rats – very short $t_{1/2}$]* – 101.2 & 171.1 ug/mL – ↑ mortality PND1 but independent stats analysis by EPA found no differences btwn grps. *Note: pups were killed at weaning, therefore post-weaning effects observed in F1 were not evaluated.*	**MDH notes that given the very short half-life in female rats this animal model may not be adequate to assess developmental toxicity* F2 204.4 ug/mL/NA EPA NOAEL/LOAEL **see MDH note above*			
Reported details of male repro organ histology from above study		No evidence of altered testicular and sperm structure & function trt F0 rats w/mean grp serum concen of up to ~45 µg/mL. Significant dose-related ↑ in seminal vesicle wt ($p<0.05$)				York et al 2010 aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		w/ & w/o fluid in F1 Ms, but fertility of trt Ms in all generations comparable to the controls.				
Neurodevelopmental dietary study – C57BL/6/Bkl pregnant mice 6/grp Exposed GD1 to end of pregnancy. The behavior of the weaned offspring was analyzed in locomotor, circadian activity, elevated plus maze, and forced swim tests at 5–8 weeks of age. Muscle strength and motor coordination tests were given at 3–4 mons of age.	0 or 0.3	Locomotor activity, anxiety-related behavior, depression-like behavior, or muscle strength were not altered in offspring. In circadian activity tests, M offspring were significantly more active ($p = 0.013$) & F offspring were significantly less active ($p = 0.036$) than control offspring during the first hour of the test. Trted M offspring were significantly more active ($p<0.05$) than controls from the dark phase of day 1 through the dark phase day 2. Both M & F trt offspring had signif less inactive periods ($p<0.05$) during the light phase compared to their respective controls. In the accelerating rotarod test, trted F offspring exhibited ↓ fall latency over the four trials compared to control Fs, but no effect of trt was observed in M offspring. *Authors concluded that prenatal exposure to 0.3 mg/kg/day resulted in gender-related postnatal alterations in offspring behavior & motor function at 3–4 mons of age.*				Onishchenko et al 2011 aci EPA 2016a *Concerns: very small number of animals for neurological assessment, not clear if litter effect is controlled for, mice were housed 3-4/cage – this can impact behavior, and single dose grp prohibits full dose response evaluation .*
Developmental Gavage Study – Pregnant CD-1 Mice Gavage on GD 1-17 (a portion of dams were terminated at end of gestation &	0, 1, 3, 5, 10, 20, or 40 *Measured final serum concentrations: 21.9, 40.5, 71.9,*	*Maternal –* ≥ 38 ug/mL - ↑ liver weight (rel wts: 49*, 77*, 89*, 118*, 132*, & 159*% compared to controls, *$p<0.05$) *[Note: histological assessment does not appear to have been conducted.]*	*Maternal* NA EPA NOAEL 38 ug/mL EPA LOAEL			(Lau 2006) *Authors supplied numerical data for Figures 1 & 2 containing maternal*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF²**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
remainder allowed to deliver) For Teratology Study (dams killed @ GD18): N=9-27 dams/group and 45 dams/control. e.g.: Controls: n= 45 (42) 1 – n=17 (15) 3 – n=17 (16) 5 – n=27 (20) 10 – n = 26 (14) 20 – n = 42 (5) 40 – n= 9 (0) (in parentheses: excluding dams w/ 100% FLR) For the Postnatal Development Study: N differed by endpoint, e.g.: Postnatal survival: N = 8-22 litters Pre-weaning BW: N = 7-30 litters Post-weaning BW: N = 4-11individ/group Eye opening: N = 3-22 litters Vaginal opening & estrous: N = 8-47 individ from 2-20 litters	*116, 181, & 271 ug/mL* Predicted AUC ug/mL*h 16400, 33600, 40700, 49600, 61400, & 80100 mg/L-h (EPA 2016a Table 4-3) Average serum concentration = predicted AUC ug/mL-hr/(18 d x 24 hr/d) = 38 ug/mL 77.8 94.2 115 142 185	≥ 94.2 ug/mL –↓ BWG [but often not clear DR & not statis sign until ≥ 20 mg/kg-d]; ↑ incidence of dams with full litter resorptions (FLR) (25.9*, 46.1*, 88.1* & 100*%); ↓ postnatal survival (↑stillbirths & neonatal mortality) ≥ 115 ug/mL - ↑ time to parturition ≥ 142 ug/mL – 20% ↓ live fetal BW @term; ↑prenatal loss; 185 ug/mL– complete loss of pregnancies *Authors – BMDL/BMD_5 for ↓BWG 3.58/6.76 mg/kg-d; ↑liver wt 0.17/0.20 mg/kg-d.* *MDH BMD modeling:* *↑re; liver wt - BMDL/BMD_{10} – all model results unusable* *% FLR – BMDL/BMD_{05} 68.0/75.7 ug/mL* *↓BWG - BMDL/BMD_{10} – all model results unusable.* *Gest length BMDL/BMD_{1SD} – 93.3/142 ug/mL* *Development –* 38 ug/mL – ↓ ossification of forelimb proximal phalanges @ all doses except 94.2 ug/mL but not clear DR; ↓ ossification of hindlimb proximal phalanges @all doses except 77.8 & 94.2 ug/mL, not clear DR; acceleration of sexual maturation Ms ('inverse' dose response – 4 d earlier @38 ug/mL, 2-3 d earlier @77 - 115 ug/mL, & slightly delayed @142 ug/mL)	based on ↑ liver wt [NOAEL/$LOAEL_{HED}$ NA/0.0053 mg/kg-d] *Developmental* NA EPA NOAEL 38 ug/mL EPA LOAEL based on ↓ossification & accelerated puberty (M) [NOAEL/$LOAEL_{HED}$	300 (3A, 10H, 3L, 3 DB) MDH 300 (3A,	**0.000018 MDH** [0.00002 Selected	*BW/BWG and liver wts.* *See spreadsheet sent by author*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
PPS: N = 4-56 individ from 2-22 litters Litter was the statistical unit for fetal effects, neonatal survival, pup pre-weaning bw, eye-opening. But individual animal was statistical unit for post-weaning bw, vaginal opening, PPS. Study duration 18 days (dams) & 17 days (pups)		≥ 77.8 ug/mL – dose dependent growth delays (statis signif ↓BW preweaning) (post-weaning BW @control levels by 6 wk (F) or 13 wk (M)) ≥ 94.2 ug/mL - ↑minor limb & tail defects; ↓ postnatal survival (↑stillbirths & neonatal mortality); delayed eye opening ≥ 115 ug/mL - ↓ live births; ↓ ossification in supraoccipital bone ≥ 142 ug/mL - ↓ fetal weight; delay vaginal opening & time to estrous *Authors BMDL/BMD_5 for ↑mortality 1.09/2.84 mg/kg-d; ↓pup weight 0.86/1.07 mg/kg-d; delayed eye opening = 2.1/2.64 mg/kg-d. MDH estimates (based on dose/serum ratio)* corresponding serum concentrations to be for mortality 41/74 ug/mL; pup BW 33/41 ug/mL; & eye opening 61/68 ug/mL* *MDH modeling:* *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.* *Neonatal survival BMDL/BMD_{05} on PND2 or PND6 - all models unusable* *Pup BW BMDL/BMD_{05} all models unusable.* *Eye opening BMDL/BMD_{1SD} = 38.9/54.5 ug/mL*	NA/0.0053 mg/kg-d]	10H, 10L) EPA 300 (3A, 10H, 3L, 3 DB) MDH	by EPA as RfD] **0.000018 MDH**	**admin dose to average serum concentration relationship is not linear. Therefore used the ratio for doses most closely corresponding to the BMD/BMDL calculated value rather than regression equation.*
Developmental gavage study – ICR mice	0, 1, 5, or 10 mg/kg-d	*Maternal* ≥1 (admin dose) - ↑rel maternal liver (35*, 115*, & 185**%) & kidney (16**, 14.5*, & 27**%) wt; hepatic hypertrophy; renal cells in outer medullar & proximal tubule	*Maternal* - NA NOAEL			(Yahia 2010) and aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
Dosed on GD0-17 (prenatal study, N=5-9 dams/grp) Dosed GD0-18 (postnatal study, N=5 dams/grp) Pups only assessed until PND4		were slightly hypertrophic (no incidence data or dose-response data); ↑ BUN (27.8*, 25.4 & 20.5 vs control 22.6 – no clear dose response) & phosphorus (no clear dose response) ≥5 (admin dose) - ↓maternal BW (12* & 17**%); ↑absol maternal liver wt, ↓total serum protein (15* & 22**%) & globulin (22** & 32**%); ↑AST (311 & 813**%), ALT (150 & 372**%), & ALP (32 & 296**%); ↓ albumin (11 & 15*%), triglycerides (47 & 82**%), phospholipids (10 & 33*%), TC (8 & 32*%),& free fatty acids (34 & 44*%); 10 (admin dose) - ↓ GGT; delayed parturition w/ ~58% of all pups born stillborn and death occurring w/i 6 hrs of birth in remaining pups. *Developmental –* ≥5 (admin dose) - ↓live fetal birth wt (9.5** & 28**%); ↓pup BW (8** & 29**%); ↓ survival on PND4 (84.4** & 0**%); ↑incidence cleft sternum, reduced phalanges ossification, & delayed eruption of incisors [all statis signif at next dose level up]	1 mg/kg-d (admin dose) LOAEL *Developmental* 1 mg/kg-d (admin dose) NOAEL 5 mg/kg-d (admin dose) LOAEL based on ↓ pup BW & survival			
Developmental Gavage Study – Pregnant CD-1 Mice (N=10/grp) Treated GD11-16	0, 2, 10 or 25 mg/kg-d	≥2 (admin dose) – ↓placental wt, ↑ incidence of resorption & dead fetuses; post-implantation loss 8.83, 30.98 & 55.41% compared to 3.87% in controls; parietal & S-TGC & GlyT cell frequency in the	NA NOAEL			(Suh 2011) and aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
Dams were sac'd on GD 16		placental junctional & labyrinth zones was ↓ in dose dependent manner; ≥10 (admin dose) - ↓fetal wt & number of live fetuses; placentas displayed necrotic changes 25 (admin dose) - ↓maternal BW *MDH BMD modeling:* *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)* *Number of live fetuses: all model results unusable.* *Fetal BW – $BMDL/BMD_{05}$ – 2.29/2.56 (admin dose) mg/kg-d*	2 mg/kg-d (admin dose) LOAEL based on ↑resorptions, placental effects & fetal death			
Targeted Developmental Gavage Study – Pregnant CD-1 Mice N=17-21 dams/dose Animals dosed GD1-17 Focused assessment of liver effects. Only F pups assessed after weaning. Impact of high fat diet (HFD) was also assessed – On PND35 offspring were placed on HFD with 60% kcal% fat or	0, 0.01, 0.1, 0.3, or 1	≥ 0.01 (admin dose) – ↑ chronic active periportal inflammation @PND21 (still observed @PND91 in ≥0.3 mg/kg-d); ↑hepatocellular hypertrophy @PND91 ≤ 0.3 (admin dose) – LDL, HDL & TC levels in PFOA + HFD were lower than controls + HFD; dose related ↑ rel liver wt (@PND 21) *Transmission electron microscopy (TEM) was only conducted on control & high dose grps. TEM of liver sections on PND91 showed cellular damage & mitochondrial abnormalities w/no evidence of peroxisome proliferation. W/i hypertrophied hepatocytes, mitochondria were ↑ in number but also exhibited altered morphologies suggestive of ↑ &/or uncontrolled fission & fusion reactions.	0.01 mg/kg-d (adm dose) EPA NOAEL 0.3 mg/kg-d (adm dose) EPA LOAEL based on ↑TC @PND91 in HFD animals (gestational & lactational exposure only)			(Quist 2015) and aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
control diet with 10% kcal% fat (1 pup from 7-10 dams/dose grp). After 6 wks animals were returned to Purina 5001 diet.		Authors state: *findings confirm that developmental exposures to PFOA induce alterations in cholesterol biosynthesis and fatty acid metabolism, and demonstrate that those responses may vary when animals are challenged with a high-fat diet.. . . at PND 91, when PFOA is only detected at a low, residual level in the serum, these mice exhibited a dose-dependent increase in hepatocellular hypertrophy. Ultrastructural examination confirms that the hypertrophy was not due to peroxisome proliferation or SER induction as would be expected with PFAAs or enzyme inducers, respectively.* *Rather, hypertrophied cells contain increased numbers of dividing and proliferating mitochondria either as the result of impaired mitochondrial function or, possibly, a mitochondrial defect due to developmental PFOA exposure.*				
4 wk gavage study in BALB/C or C57BL/6 female weanling mice N= 5/grp Gavaged 5d/wk for 4 wks	0, 1, 5 or 10 mg/kg-d 5 d/wk Time adjusted: 0, 0.71, 3.6 or 7.1 mg/kg-d	<u>BALB/C mice:</u> ≥ 1 (adm dose) -↑absol & rel liver wt; ↓absol & rel uterine wt; delayed vaginal opening (VO) VO did not occur at 5 or 10 mg/kg-d (adm dose) ≥ 5 (adm dose) - ↓mammary gland development (↓ ductal length, number of terminal buds, stimulated terminal ducts, BrdU revealed signif lower number of proliferating cells) 10 (adm dose) - ↓BW	<u>BALB/C mice:</u> NA/1 mg/kg-d (adm dose) EPA NOAEL/LOAEL based on delayed VO, ↑ liver wt & ↓uterine wt			Yang et al 2009 aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF^2**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		C57BL/6 mice: ≥ 1 (adm dose) -↑absol & rel liver wt; ↑absol & rel uterine wt but signif ↓@10 mg/kg-d) ≥ 5 (adm dose) – stimulatory effect on mammary gland development; delayed VO (VO did not occur at 10 mg/kg-d (adm dose)) 10 (adm dose) - ↓BW; inhibition of mammary gland development	C57BL/6 mice: NA/1 mg/kg-d (adm dose) EPA NOAEL/LOAEL based on ↑ liver & uterine wts			
Developmental Gavage Study – CD-1 Pregnant Mice Study 1: 28-48/grp GD1-17 & pups were cross fostered. No pups remained with their original birth mother Study duration 18	Study 1 – 0, 3, or 5 on GD1-17 Pups cross fostered resulting in 7 trt grps: C, 3U, 5U, 3L, 5L, 3U+L, & 5U+L *Serum concen. were measured at weaning & also in F pups @ 6 & 9 wks of age* Predicted AUC ug/mL*h 33700 & 40700 mg/L-h (EPA 2016a Table 4-3) Average serum concentration = predicted AUC	Study 1 Cross-fostering study to determine if postnatal BW deficits, neonatal lethality, & develop delays were the result of gestational exposure, lactational exposure, or a combination of gestational & lactational exposure *Maternal* – 77.9 ug/mL - ↑liver wt; delayed lactational morphology 94.2 ug/mL – ↑BW/BWG, ↑ whole litter loss *Develop* – Rel liver wt ↑ & reduced mammary gland development @PND63 (F) in all exposed offspring 77.9 ug/mL - ↓pup BW (M/F pup U – 4/8 & 13/15%, U+L- 15/21 & 21/25%); ↓eye opening & body hair growth (U & U+L); ↑liver wt in all trt grps (25 – 46%); 94.2 ug/mL - ↓pup birth BW; ↓postnatal survival (U+L); ↓eye opening & body hair growth (U & U+L)	Study 1 *Maternal* NA EPA NOAEL 77.9 ug/mL EPA LOAEL based on ↑absol & rel maternal liver wt *Developmental* NA EPA NOAEL 77.9 ug/mL EPA LOAEL based on delayed eye opening & hair growth, ↑rel liver wt, ↓BW, delayed			(Wolf 2007) and White et al 2009 aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF**[2]	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
	ug/mL-hr/(18 d x 24 hr/d) = 77.9 ug/mL 94.2	↑Rel liver ratios in pups exposed lactationally @serum levels ~15 ug/mL; in pups exposed *in utero* 65-70 ug/mL. *MDH did not attempt BMD modeling due to the small number of dose grps and lack of nested data (which would require individual animal data).*	mammary gland development (F) [NOAEL/LOAEL$_{HED}$ NA/0.0109 mg/kg-d]	300 (3A, 10H, 10L) EPA	0.00004 EPA	
Study 2: 12-14/grp Restricted Exposure Study Study duration 12 (GD7-17)	Study 2 0 or 5 GD7-17 (N=12/14) GD10-17 (N=14) GD13-17 (N=12) GD15-17 (N=12) *Measured final serum concentration GD 7-17: 24.8 ug/mL* Predicted AUC ug/mL*h 25400 mg/L-h (EPA 2016a Table 4-3) Average serum concentration = predicted AUC ug/mL-hr/(12 d x 24 hr/d) = 87.9 ug/mL	Study 2 Dams & 1 M & 1 F pup necropsied on PND22. Mammary gland development in F offspring assessed at various interval up to 18 months of age. BW of 1 pup/sex/dose weighed weekly from PND29-245. *Maternal –* ↑BWG in dams exposed GD 7-17 or 10-17; ↑ liv wt all grps except GD15-17. Qualitatively mammary glands for trt dams appeared immature compared to controls. Control dams nursing offspring exposed to PFOA in utero also had delayed lactational morphology presumably the result of exposure to the control dam from maternal grooming of in utero exposed offspring. *Pups –* ↓birth BW (Ms) in dams dosed GD7-17 or 10-17. By PND78 M offspring BW had recovered. Offspring of dams dosed GD13-17 weighed significantly more on PND161. Rel liver wt ↑ in all grps. Eye opening & body hair growth delayed in pups expos GD7-17 & 10-17	Study 2 *Maternal* NA EPA NOAEL 87.9 ug/mL EPA LOAEL based on ↑maternal rel liver wts *Developmental* NA EPA NOAEL 87.9 ug/mL EPA LOAEL based on delayed eye opening & hair growth, ↑rel			

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF**[2]	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		Mammary gland development scores (ductal elongation & branching, appearance of TEBs) signif ↓ in all exposed grps @PND29 & 32. Mammary tissues not scored at 18 mons of age due to lack of protocol for mature animals, however, dark foci (composition unknown) in mammary tissue occurred at higher frequency in exposed animals.	liver wt, ↓BW (M), delayed mammary gland development (F) [NOAEL/LOAEL$_{HED}$ NA/0.0123 mg/kg-d]	300 (3A, 10H, 10L) EPA	0.00004 EPA	
Mammary development gavage study – CD-1 Pregnant Mice Blood, liver, brain, & 4th & 5th mammary glands were collected from female pups. Study duration 18 days GD1-17 (13/grp) 6 offspring/grp sac on PNDs 7, 14, 21, 28, 42, 63, & 84	0, 0.3, 1.0, or 3.0 *F offspring serum concen. measured on PND7(earliest time point) 4.98, 11.026, & 20.7 ug/mL & PND14 (peak levels) 4.535, 16.95, & 26.525 ug/mL* EPA modeled ave serum value @0.3 mg/kg-d was 12.4 ug/mL (Table 4-8). Values for other doses not reported. Using the ave serum concen calculated for pregnant CD-1 from Lau et al & Wolf et al the ave serum concen for 1 & 3 mg/kg-d	≥ 0.3 [12.4 ug/mL] - ↑ rel liver wt PND7 (M/F: 26*/19*, 59*/38* , & 97*/76*%, p≤0.05); delayed mammary gland development (F pups) @PND14 & 21 (however, developmental scores did not show dose-related trend – e.g., PND21: 1.9, 1.3, & 1.6 vs 3.4 for controls) ≥ 1 - ↑ rel liver wt PND14 (M/F: 17/26* & 41*/58*%); delayed mammary gland development (F pups) @PND7 to 84 3 - ↑ rel liver wt PND14, 21 & 28 (M&F) <u>*MDH BMD Modeling using serum concen:*</u> *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)* *$BMR_{10\%}$- all model results either questionable or unusable. Questionable result w/lowest AIC 0.779/1.61 ug/mL* *BMR_{1SD} - all model results either questionable or unusable. Questionable result w/lowest AIC 0.994/1.98 ug/mL*	NA EPA NOAEL 12.4 ug/mL EPA LOAEL based on ↑liver wt & delayed mammary gland development @PND14 [NOAEL/LOAEL$_{HED}$ NA/0.0017 mg/kg-d]			(Macon 2011) and aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
	would be 38 & 77.8 ug/mL					
- - - - - - - - - - - -	- - - - - - - - - -	- -	- - - - - - - - - -			
Study duration 8 days GD 10-17 (3-5/grp)	0, 0.01, 0.1 or 1.0 *F offspring serum concen. measured on PND1(earliest time point) 0.0226 (control), 0.2845, 2.3035, & 16.3055 ug/mL*	≥ 0.01 – statis signif decrease in qualitative developmental scores @PND21 for mammary gland (2.2, 1.8 & 1.6 vs 3.3 in controls). Quantitative scores only statis signif @ highest dose ≥ 0.1 – statis signif decrease in number of terminal end buds (TEB) 1 - ↑liver wts *Using the serum concentrations reported on PND 1 (Post 2012) calculated BMDL/$BMD_{10\%}$ serum concentrations of 0.0247/0.0257 ug/mL for ↓ qualitative mammary gland development score @PDN21 & 0.0229/0.0251 ug/mL for ↓ # TEBs @PND21.* *MDH modeling using PND1 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) 0.0836/0.379 ug/mL for ↓ # TEBs @PND21: BMDL/BMD_{1SD} 0.0836/0.379 ug/mL (lowest BMDL) 0.15/0.685 ug/mL (viable alternate)*	NA/0.01 mg/kg-d (adm dose) EPA NOAEL/LOAEL based on qualitative mammary gland development score LOAEL of 1 mg/kg-d (adm dose) based only on quantitative score			
Multigenerational Gavage Study – CD-1 Mice Examination of extended consequence of altered mammary gland development.	$P0_A$ – 0 (N 10), 1 (N 12), or 5 (N 11) mg/kg-d GD1-17. $P0_B$ – 0 (N 7) or 1 (N 10) mg/kg-d	*Measured serum levels (ug/mL) @PND22: P0: Control 0.0040; control + 5ppb DW 0.0748; 1 mg/kg-d 6.658; 1 mg.kg-d – 5 ppb DW 4.772; & 5 mg/kg-d 26.98 ug/mL. F1 pups: control 0.0006; control –DW 0.0213; 1 mg/kg-d 2.4438; 1 mg/kg-d+DW 2.7439; & 5 mg/kg-d 10.045 ug/mL*	NA EPA NOAEL 1 mg/kg-d (adm dose) EPA LOAEL			(White 2011)and aci EPA 2016 *Also see study summary of Albrecht et al below in Other Studies section*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
Mammary gland whole-mounts were scored on a developmental scale *(quantitative measures, as in study above, do not appear to have been conducted)* Lactational challenge substudy – dams were separated from offspring for 3 hr on PND 10 (peak of lactation) & then returned to litter & allowed to nurse for 30 min.	GD1-17 + 5 ppb in DW starting GD7 for duration of study. F1 females & F2 continued to receive DW until end of study (except during F1 breeding & early gestation). *So 5 'dose' grps:* • *0,* • *0+DW(~0.054 ug/day gestation & 0.105 ug/day lactation),* • *1 mg/kg-d (gest only)* • *1 mg/kg-d (gestation) +DW (37+0.051 ug/day gestation & 37+0.30 ug/day lactation), &* • *5 mg/kg-d (gestation)*	*F1 dams: control 0.002; control + DW 0.0869; 1 mg/kg-d 0.0093; 1 mg/kg-d 0.1733, & 5 mg/kg-d 0.0187 ug/mL* *F2 pups: control 0.0004; control –DW 0.0266; 1 mg/kg-d 0.0046; 1 mg/kg-d+DW 0.0285; & 5 mg/kg-d 0.0078 ug/mL* ≥ 0 + 5 ppb DW &1 mg/kg-d (admin dose) – ↓mammary gland score in dams & F1 pups @all assessment time points & F2 (PND42 only) 1 mg/kg-d (admin dose, gestation) - ↑F2 BW & ↓mammary developmental score @PND63 (but no effect in other dose grps) 1 & 5 mg/kg-d (gestation expo) - ↑rel liver wt F1 pups 5 mg/kg-d (admin dose) - ↑prenatal loss, ↓ number of live offspring & postnatal survival in $P0_A$; F1 exposed *in utero* had significantly fewer implants; ↓ F1 BW ~PND42 F2 – control females exhibited unusually low mammary gland scores & developmental delays in trt grps relative to controls were not statis signif. F1 maternal lactational challenge - no significant effects on milk produced in 30 min or in time to initiate. *Authors: Despite striking morphological abnormalities in lactating glands no clear evidence of diminished nutritional support provided by dams as measured by F2 BW was found.*	based on delayed mammary gland develop in dams during lactation			*which evaluated role of PPARα using PPARα-humanized mice. (only 0 & 3 mg/kg-d trt grps) Mammary gland ductal length & terminal buds were quantified (i.e., did not use qualitative developmental score). No signif difference in either parameter was observed btwn control & trt grps. In addition, expression of PPARα target genes that modulate lipid metabolism was ↑ in both wild-type & humanized mice coincident w/↑ liver wt & microscopic lesions. Neonatal mortality was observed only in wild-type offspring*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
Mammary Develop Gavage study - Female CD-1 & C57BL6 Mice Goal - examination of mammary gland development sensitivity across mouse strains. Due to space limitations study was conducted in 3 blocks. 4-8 CD-1 litters/trt block 3-7 C57BL/6 litters/trt block Endpts measured: BW/BWG, Absol/rel liver wt; neonatal develop; serum estradiol & progesterone (P); & qualitative mammary gland develop scores Study duration 17 days	0, 0.01, 0.1, 0.3 & 1 mg/kg-d admin GD 1-17 via gavage Serum measured @PND 21, 35 & 56 in CD-1 And PND21 & 61 for C57BL/6 *Measured serum levels (ug/mL) reported @PND21 (earliest timept but ~22 days after last exposure):* *CD-1 - 0.0748, 0.4573, 0.9048, & 3.119 ug/mL* *C57BL/6 – 0.0261, 0.2471, 0.8913, & 2.14167 ug/mL*	CD-1 mice – ≥ 0.01 (adm dose) - ↓qualitative mammary gland develop score @PND35 (2.3, 2.2, 2.3, & 1.9 vs 3.1 in controls) but inconsistent dose-response @PND56; nonstatis signif ↑ progesterone levels ≥ 0.1 (adm dose) - ↓qualitative mammary gland develop score @PND21 (2.3, 2.0 & 1.7 vs 2.9 in controls) 1 (adm dose) - ↓rel liv wt @PND21 (12%); ↓net BW @PND21 & 35 C57BL/6 mice – ≥ 0.3 (adm dose) - ↓qualitative mammary gland develop score @PND21 (1.8 & 1.8 vs 2.9 in controls) and PND61 (2.1 & 1.7 vs 2.8 in controls) *Authors notes:* *TEBs, lateral & longitudinal branching & secondary branching were all ↓w/↑ PFOA dose, resulting in a much smaller gland. By PND 35, in addition to the growth defects already described, PFOA caused a delay in the fourth and fifth glands growing together.* *Scoring is based on the level of development compared to controls & maybe based on entirely different criteria that can still result in similar scores across strains.* *CD-1 mouse appear to be more sensitive re: effects on mammary development. This is likely a reflection of the higher & longer circulating PFOA levels in CD-1 mice. We suggest that it*	CD-1 Mice NA EPA NOAEL 0.01 mg/kg-d (adm dose) EPA LOAEL based on delayed mammary gland development @PND56 (gestational exposure only) C57BL6 Mice 0.1 mg/kg-d (adm dose) EPA NOAEL 0.3 mg/kg-d (adm dose) EPA LOAEL based on delayed mammary gland development @PND61 (gestational exposure only)			(Tucker 2015) and aci EPA 2016a *Pregnancy rates in CD-1 were >60% but much lower (~27%) in C57BL/6 mice*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		is the peak serum PFOA concentration that regulates these effects, which may have occurred between birth & w/i the first two weeks of life),rather than the serum PFOA level at the time of evaluation. Based study effects here and in Macon et al (full gestation or late gestation dosing) observed effects are likely the result of in utero exposure, followed by exacerbation of effect from the exposure during lactation.				
Latency & PPARα MOA Evaluation of Tumor Development Resulting from Developmental (gestation & lactation; dams directly dosed via gavage from GD1-17; pup lactation exposure presumed due to long t ½ in dams) Exposure – CD-1, SV-129WT & SV-129 PPARαKO Mice. Animals were from separate experiments published at Hines et al 2009 & Abbott et al 2007. CD-1 21-37 female offspring/grp. [from 12-14 dams/group & 6 dam/group @ HDT]	CD-1: 0, 0.01, 0.1, 0.3, 1, & 5 mg/kg-d SV-129: 0, 0.1, 0.3, 0.6, & 1 mg/kg-d SV-129KO: 0, 0.1, 0.3, 1, & 3 mg/kg-d	CD-1: ≥1 (adm dose) - ↑severity (but not incidence) chronic active inflammation; ↑lto cell hypertrophy (sign trend but statis sign from controls only at highest dose (5 mg/kg) – 14% controls & 3%/27%/19%/ 29%/81% treated); 5 mg/kg-d (adm dose) - sign ↑ trend for oval cell hyperplasia (3.5% controls vs. 14% @ 5 mg/kg-d) In addition to lto cell hypertrophy, mice exposed to PFOA developed centrilobular hepatocyte hypertrophy (sign trend & sign from controls @ HDT; 17% (controls) and 17%/35%/35%/10%/81% in treated groups) – indicating that a PPARα-independent mechanism was responsible. 129/Sv Wild-Type: Centrilobular hepatocyte hypertrophy (60% (controls) and 50%/100%/50%/88% in treated (not sign from controls up to 1 mg/kg-d- but sign ↑ severity) @ ≥ 0.3 mg/kg-d)	1 mg/kg-d (adm dose) EPA NOAEL 5 mg/kg-d (adm dose) EPA LOAEL based on ↑lto cell hypertrophy @18 months from gestational & lactational exposure			(Filgo 2015) aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
Only 6-10 female offspring/dose grp for SV-129 grps [from 5-9 dams/group]. Dosing duration 38 days and female offspring evaluated @ 18 months		129/Sv PPARα-Knock-Out: Centrilobular hepatocyte hypertrophy (sign trend: 17% (controls) and 0/10%/11%/ 44% in treated); sign ↓ Ito cell hypertrophy; sign ↑ trend for hematopoietic cell proliferation (33% controls & 10%/ 80%/ 67%/ 78% treated); sign ↑ bile duct hyperplasia @ 3 mg/kg-d; and sign ↑ trend for bile duct hyaline droplet accumulation Authors state that this study was NOT designed as a carcinogenesis study but was designed as a result of liver tumors found in PPARα knock-out mice in a previous study. Difficult to draw conclusions regarding the carcinogenicity of PFOA based on the data collected because of the small number of animals evaluated in both studies of SV-129 mice & the lack of PFOA exposure between PND 21& 18 months for all dose groups. Similar to Butenhoff et al 2012 lack of dose-response for total liver tumors, although the four hepatocellular adenomas seen at 0.3 mg/kg/day in CD-1 mice were signif greater ($p<0.05$) than the control. Tumor types varied across the dose groups. The authors also reported on preneoplastic basophilic, and eosinophilic foci were observed in the CD-1 mice but did not show a response to dose. An interesting histological finding in both the CD-1 and SV-129 mice was a trend for ↑ Ito cell atrophy& lesion severity across the doses. Ito cells accumulate fat in the liver sinusoids - this observation provides additional support for hepatic steatosis as a condition of concern following developmental PFOA exposure.				

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		There was an ↑ in severity with dose for the Ito cell fat deposits for all but the high-dose group. The Ito cell lesion was present in the SV-129 mice, but was not associated with tumors. CD-1 mice had a significant ↑ in Ito cell hypertrophy at 5 mg/kg/day compared to controls, but there was a lack of dose-response. The authors concluded that liver damage from PFOA exposure occurring early in development is not totally linked to PPAR-α & could progress as animals aged w/o continued dosing, thus compromising liver function & possibly leading to tumor development.				
14 day Gavage Study – Adult Male Crl:CD BR Rats (15/grp) Focus – determine impact of PFOA on aromatase activity Study duration 14 days	0, 0-pair-fed, 0.2, 2, 20, & 40 mg/kg-d	≥0.2 (adm dose) - ↑protein yield of hepatic microsomes, ≥2 (adm dose) - ↑absol & rel liver wt; ↑hepatic aromatase activity, total hepatic aromatase activity adjusted for liver & BW effects; serum estradiol ≥20 (adm dose) - ↓BW (pair-fed controls also had ↓BW)	0.2 mg/kg-d (adm dose) EPA NOAEL 2 mg/kg-d (adm dose) EPA LOAEL based on ↑ liver wt, serum estradiol & hepatic aromatase			Liu et al 1996 *(also see reproductive hormone studies in Other Study Section below)*
14 day Gavage study – Male Klunming mice 8 wks of age 6/grp for testes wts and 4/grp for other assays	0, 2.5, 5 or 10	There was no effect on rel testes wt at any dose. Some effects were observed on testicular morphology at the lowest dose, including atrophy of the seminiferous tubules, depletion of spermatogonial cells, detachment of germ cells from the seminiferous epithelium, & ↓ sperm production. The severity of the testicular morphological changes ↑ w/dose. The ↑ in MDA & hydrogen peroxide accompanied by	NA/2.5 mg/kg-d (adm dose) Author NOAEL/ LOAEL based on ↓sperm count, testicular SOD, catalase, NRF2 & BAX			Liu et al 2015 aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
Evaluated effects on testes and epididymis		the ↓ in SOD & carnitine acyltransferase (CAT) activity & NRF2 expression indicate that oxidative stress played a major role in the observed toxicity. NRF2 plays an important role as a messenger that upregulates genes involved in response to oxidative stress. @LDT (2.5 mg/kg-d adm dose) statis sign ↓sperm count (clear dose response based on Fig 3), ↓SOD & CAT activity (clear dose response based on Fig 4), and statis sign ↓folding change in NRF2 expression (clear dose response based on Fig 5)	expression and ↑MDA, hydrogen peroxide 2.5/5 mg/kg-d (adm dose) EPA NOAEL/ LOAEL based on ↓sperm count, changes in testicular morphology, evidence of ↑free radical oxidation			
28 day Gavage Study – 14 day old Male BALBL/c Mice 3-5/grp Study 1: Evaluate testicular effects of PFOA on the blood testes barrier	Study 1 0, 1.25, 5 or 20	Study 1: > 1.25 – weakening of the blood testes barrier (dose-dependent manner) as indicated by passage of red fluorescent dye > 5 - ↑tumor necrosis factor actin protein	Study 1: 1.25/5 mg/kg-d (adm dose) Author NOAEL/LOAEL for blood testes barrier NA/1.25 mg/kg-d (adm dose) Author NOAEL/LOAEL for protein biomarkers of cellular intercommunication			Lu et al 2015 aci EPA2016a *Also see Li et al 2011 summarized below under Other Studies re: assessment of wild, null and humanized PPAR mice and testicular toxicity.*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
Study 2: Impact on male fertility 6-8 wks of age 15/grp Study duration 28 days	Study 2 0 or 5	Study 2: ↓ number of mated females per trt male and pregnant females per trt male mouse ($p<0.001$); ↓number of pups per litter (10.2±0.72 vs 11.89±0.54, but not statis sign); ↓average litter weight (16.17±1.63 vs 19.95±0.80, $p<0.05$)	Study 2 NA EPA NOAEL 5 mg/kg-d (adm dose) EPA LOAEL based on ↓fertility & ↓pup birth wt			
Immune Studies (also see Loveless et al studies in following section as well)						
10 day Immune dietary study – C57BL/6 Mice Diet concen 0.001, 0.003, 0.01, 0.02 & 0.05%	0, 2, 6, 20, 40 & 100 mg/kg-d	≥2 (adm dose) -↑lauroyl-CoA & palmitoyl-CoA (measures of peroxisome proliferation) ≥20 (adm dose) - ↓spleen & thymus wts ≥40 (adm dose) -↑liver wts	~6/20 mg/kg-d (adm dose) NOAEL/LOAEL for spleen & thymus			(Yang 2001) and aci EPA 2016a
Immune DW study – Adult Female C57BL/6N Mice Dose-response studies 16/grp Study duration 15 days *Note: Dose-response study is summarized here. See publication for info on the single dose (30 mg/kg-d) antibody synthesis study.*	0, 0.94, 1.88, 3.75, 7.5, 15, or 30 mg/kg-d *Measured final serum concentrations @day 1post-dosing: NA,NA, 74.9, 87.2, 128.1. or 162.6 ug/mL* Predicted AUC ug/mL*h 7300, 13800, 22400, 30500,	≥ 20.2 ug/mL - ↑ rel liver wt (51-70% one day post trt & 45-61% 15 days post trt) (data not shown) ≥ 61.9 ug/mL - ↓ absol & rel (16*, 18, 31*, & 40*%, * $p<0.05$) spleen wt post dosing (PD) day 1 (by PD day 15 return to ~control); ↓IgM response to SRBC challenge (7-11%, increasing to 29% @ highest dose), IgG response was ↑@ this dose level & 84.4 ug/mL but not higher doses, DTH response were not signif altered. ≥ 84.4 ug/mL - ↓ absol & rel (rel – 10, 30*, & 49*%) thymus wt post dosing (PD) day 1 (by PD day 15 return to ~control) ≥ 111 ug/mL - ↓BW	38.2 ug/mL EPA NOAEL# 61.9 ug/mL EPA LOAEL based on ↓ IgM (1 day post-dose), ↑IgG (15 days post-dose), ↓absol & rel spleen wt (1 day post-dose) [NOAEL/LOAEL$_{HED}$	300 (3A,	0.00002 EPA	(DeWitt 2008) and aci EPA 2016a #*Note: although changes in liver wt were observed at the LDT EPA did not use this effect as the basis for selection of their NOAEL/LOAEL*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
	40100, & 56000 mg/L-h (EPA 2016a Table 4-3) Average serum concentration = predicted AUC ug/mL-hr/(15 d x 24 hr/d) = 20.2 ug/mL 38.2 61.9 84.4 111 155	155 ug/mL - ↓6 to 15% BW (Figure 2A) *Authors BMD modeling of IgM serum titers - $BMDL/BMD_{1SD}$ 1.75/3.06 mg/kg-d. [MDH based on dose:ave serum ratio estimated corresponding serum levels: ~34/53 ug/mL]* *MDH unable to model since data is only reported in figure.*	0.0053/0.0087 mg/kg-d]	10H, 10S) EPA		
Immunotox 21 day DW study – 4 wk old Male ICR Mice n=10/group 0, 2, 10, 50 & 250 mg PFOA/L	0, 0.49, 2.64, 17.63 & 47.21 mg/kg-d	≥0.49 (adm dose) - 50%↓ in splenic CD8+ lymphocytes ≥17.63 (adm dose) - ↑ interleukin-1β in spleen; ↑c-myc expression in thymus; ↑splenic CD4+ lymphocytes 943 & 106%); ↓thymic CD4+CD8+ populations; ↑ rel spleen wt (28%); ↑ rel thymus wt (46%) 47.21 (adm dose) – spleen: enlargement w/marked hyperplasia of the white pulp & ↑cellular density of the lymphoid follicles, ↑tumor necrosis factor-α, interleukin-1β, interleukin-6 & c-myc expression; thymus: ↓cortex & medulla thickness & densely arranged cortex lymphoid cells, 110% ↑thymic CD8+ lymphocytes; ↑ rel spleen wt (53%); ↑ rel thymus wt (53%)	NA NOAEL 0.49 mg/kg-d (adm dose) LOAEL based CD4- and CD8+ splenocytes			Son et al 2009 aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
Immunotox 15 d DW study – PPARα evaluation. Female PPARαKO and C57BL/6-Tac WT Study duration 14 days	Study 1 (T-cell depend) 0, 7.5 or 30 mg/kg-d Injected w/SRBC day 11 Study 2 (T-cell indepen) 0, 0.94, 1.88, 3.75 & 7.5 mg/kg-d Injected w/dinitrophenyl ficol day 11	Study 1: 7.5 (adm dose) - ↓rel thymus wt in WT (but not at highest dose) 30 (admin dose) - ↓BW in WT mice; ↓rel spleen wt in WT; ↓IgM both WT & KO Study 2: ≥1.88 (admin dose) - ↓antibody respon (9.4 – 10.7%) *Authors looked at changes in lymphocyte populations & saw no dose-depend changes, concluding that both sets of antigen responses were due to changes in cellular function rather than lymphocytotoxicity.*	Study 1: 7.5/30 mg/kg-d (adm dose) EPA NOAEL/LOAEL based on ↓ sheep RBC IgM response (PPAR KO mice) Study 2: 0.94/1.88 mg/kg-d (adm dose) EPA NOAEL/LOAEL based on T-cell independent response			(DeWitt 2015) and aci EPA 2016a
Other systemic endpoints -						
21 day Drinking Water Study – ICR male mice N = 10	0, 2, 10, 50 & 250 mg PFOA/L 0, 0.49, 2.64, 17.63 or 47.21 mg/kg-d	≥ 0.49 - ↑rel liver wt (27%) ≥ 2.64 - ↑plasma ALT (50%) ≥17.63 - ↓BWG, ↑plasma AST, enlarged hepatocytes w/acidophilic cytoplasm & presence of eosinophils, ↓ tumor necrosis factor-α expression 47.21 - ↓food & water consumption, ↓ interleukin-β expression, ↑transforming growth factor-β expression	NA EPA NOAEL 0.49 mg/kg-d (adm dose) EPA LOAEL based on ↑liver wt NOAEL/LOAEL for ↑ ALT 0.49/2.64 mg/kg-d (adm dose)			Son et al 2008 aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
28 day Gavage Study – Male Sprague-Dawley rats 10/grp	0, 5 or 20	≥ 5 - hypoactivity, ↓ food consumption, cachexia, & lethargy by 3rd week of study; ↑ visceral index (i.e., hepatic, renal, gonad wt/BW) used to evaluate hyperplasia, swelling, or atrophy; hepatic hypertrophy, fatty degeneration, & acidophilic lesions as well as angiectasis (gross dilation) & congestion in the hepatic sinusoid or central vein; pulmonary congestion & focal or diffuse thickened epithelial walls. 20 – sensitivity to external stimuli; turbidness & swelling in the epithelium of the proximal convoluted tubule	NA EPA NOAEL 5 mg/kg-d (adm dose) EPA LOAEL based on ↑ visceral indices & liver & pulmonary lesions			Cui et al 2009 aci EPA 2016a
29 day Gavage Study – Male CD-1 mice 20/grp Linear PFOA Injected with SRBC on day 24 Study duration 29 days	0, 0.3, 1, 10, or 30	≥ 0.3 - ↑incidence of microscopic lesion in the liver including mild hepatocellular hypertrophy; ↑ absol (25, 84*, 240*, & 230*%, $p<0.05$) & rel (33, 179*, 292*, 317*%) liver wt; ↓ absol (1, 11, 44*, & 56*%) & rel (3, 14*, 35*, & 45*%) spleen & absol (10, 2, 50*, & 50*%) & rel (10, 6, 66*, & 39*%) thymus wts. ≥ 1 - ↓ HDL (29*, 39*, & 56*%); moderate-to-severe hypertrophy & individual cell necrosis (11/20, 20/20, 19/20 vs. 0/19 in controls); liver focal necrosis (3/20, 4/20, 7/19 vs. 0/19 in controls) ≥ 10 - ↓BW; ↑neutrophils and monocytes & ↓ eosinophils; ↑serum corticosterone; ↓ total serum cholesterol (TC) (31* & 49*%) & triglycerides (53* & 68*%), ↑ hepatocellular mitotic figures, fatty changes, & bile duct hyperplasia; ↓spleen & thymus cell counts, minimal-to-severe lymphoid depletion/atrophy of the thymus,	0.3 mg/kg-d (adm dose) EPA NOAEL 1 mg/kg-d (adm dose) EPA LOAEL based on ↑ absol & rel liver wt, w/ moderate-severe hypertrophy w/single cell & focal necrosis, ↓rel spleen wt			(Loveless 2008) and aci EPA 2016a *RE: serum lipids - - see Tan et al 2013 study under Other Studies below which examined whether dietary fat content is an important variable. Only 1 dose level (5 mg/kg-d) was used. Study indicated that PFOA intensified damage to liver tissues when*

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		↓IgM titers; ↑serum corticosterone (CORT) levels *Note: negative correlation btwn serum CORT & IgM was found. Authors hypothesized that portion of thymic response was due to physiological stress. However, DeWitt et al 2009 investigated this hypothesis (see pages 3-118 to 3-119 of EPA 2016a) & found that stress-related CORT production did not have a major impact on IgM response to the SRBC inoculation.*				*given in the presence of a high fat diet (HFD)*
29 day Gavage Study – Male CD rats 20/grp Linear PFOA Animals received dose of SRBC on day 23 Study duration 29 days	0, 0.3, 1, 10, or 30	≥0.3 - ↑ absol (9, 30*, 63*, & 42*%, *p<0.05) & rel (10, 35, 83*, or 91*%, p<0.05) liver wt; ↓total (36*, 31*, 19, & 16%) & non-HDL (43*, 38*, 15, & 13%) cholesterol, HDL cholesterol (25*, 21*, 25*, & 21%), & triglycerides (31*, 25, 32*, & 34*%); minimum to mild hepatocellular hypertrophy ≥10 - ↓BW/BWG, hematocrit & hemoglobin; moderate hepatocellular hypertrophy & focal necrosis; 30 - ↑ reticulocutes & hematopoiesis No differences in total spleen & thymocyte cell & organ wts, microscopic exam of thymus, mesenteric lymph nodes or poplutcal lymph nodes, or IgM titers between trt & control	1 mg/kg-d (adm dose) EPA NOAEL 10 mg/kg-d (adm dose) EPA LOAEL based on ↑absol & rel liver wt & histological changes			(Loveless 2008) and aci EPA 2016a
13 week Dietary Study – Male ChR-CD Rats	0, 0.06, 0.64, 1.94 or 6.5	≥31.6 ug/mL – ↑ rel liver wt @wk 4 (13, 45, & 70%) & 13 (4.5, 19, & 56%) w/hepatocellular hypertrophy; ↑ hepatic palmitoyl CoA oxidase activity @ wk 4	3.3/31.6 ug/mL Author NOAEL/LOAEL	30 (3A, 10H)	[0.000015] *MDH, for compariso n purposes*	(Perkins 2004) and aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
0, 1, 10, 30 or 100 ppm (45-55/group) 2 control grps – nonpair-fed and pair-fed 15/grp sac @ 4, 7 & 13 wk of trt. 10/grp sac @ 13 wks & 8-wk recovery period Study duration 90 days	*Measured final serum concentrations: 7.1, 41, 70, 138 ug/mL* Predicted AUC ug/mL*h 7230, 69100, 168000, & 326000 mg/L-h (EPA 2016a Table 4-3) Average serum concentration = predicted AUC ug/mL-hr/(90 d x 24 hr/d) = 3.3 ug/mL 31.6 76.9 149.3	(75*, 200* & 363*#%, *p<0.05 vs ad lib controls, #p<0.05 pair-fed controls), @wk7 (128,357*, 671*#%), & @13 wk (25, 75#, & 113*#%) 76.9 ug/mL - ↑ hepatic palmitoyl CoA oxidase activity @ wk 7 through 13 (357 & 671% @ wk7, 75 & 113% @ wk 13); mild to slight coagulative necrosis in liver (control to hi dose: 0/45, 1/45, 0/45, 2/45, & 3/44) 149.3 ug/mL – ↓ BW (~8%)/BWG (~14-17%) vs nonpair fed controls [BWG was still ↓at end of recovery period); ↓ food consumption in wk 1 & 2 (~18%, w/~5% ave over 13 wks); indication of elevated estradiol @ wk 4 (very few animals assessed)	[NOAEL/LOAEL$_{HED}$ 0.00045/0.0044 mg/kg-d] 31.6 EPA NOAEL 76.9 ug/mL EPA LOAEL based on ↑ absol & rel liver wt w/hepatocellular hypertrophy accompanied by slight (not stats signif) ↑ heptatic coagulative necrosis [NOAEL/LOAEL$_{HED}$ 0.0044/0.0108 mg/kg-d]	30 (3A, 10H) EPA	0.00015 EPA	*Note: Table 4 – very few animals assessed for estradiol levels at many time points*
90 day Gavage Study – Rhesus monkey. PFOA in 0.5% Methocel7 for 7 d/wk (N = 2/sex/group) *Note: very small number of animals* Study duration 90 days	0, 3, 10, 30 or 100	≥ 3- GI upset (diarrhea, frothy emesis); dose-related trend ↓ alkaline phosphatase levels; ↑ pituitary wt but not accompanied by morphological changes (M) 10- 1 anorexic animal; ↑ SGPT; ↓ absol heart & brain wts & ↓ rel liver wt (F) but not accompanied by morphological changes ≥ 30 – ↑ mortality* (3 animals); ↓ BW; moderate to severe ↓activity; changes in hematological values (e.g., ↓ RBC, Hb,	NA (M)/3 (F) mg/kg-d (adm dose) EPA NOAEL 3 (M)/10 (F) mg/kg-d (adm dose) EPA LOAEL based on			Goldenthal 1978 (unpublished study)

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		Hct, ↑ prothrombin time); ↓ SGOT; ↑ cholesterol, total protein & albumin; slight to moderate hypocellularity of bone marrow; moderate atrophy of lymphoid follicles in the spleen 100 – 100% mortality* between wk 2-5, with clinical signs beginning in wk 1 **all animals that died had marked diffuse lipid depletion in the adrenal glands*	pituitary wt (M) & heart & brain wt (F)			
26 Week Oral Capsule Study – Male Cynomolgus Monkeys 6/group, except 3 mg/kg-d had 4/group Study duration 26 weeks (182 days)	0, 3, 10 or 30/20 (dose was ↓ from 30 to 20 at day 22) *Measured steady state serum concen (Butenhoff 2004b):81±40, 99±50, & 156±103 ug/mL Authors state that steady-state appears to have been attend w/I ~4-6 wks of dosing.* Predicted AUC ug/mL*h 380000, 553000, & 710000 mg/L-h (EPA 2016a Table 4-3)	≥ 87.0 ug/mL – ↑ absolute & relative liver weight (20, 27, 60*%, *p≤0.01); evidence of mitochondrial proliferation in livers; 1 death (cause undetermined); ↑ triglycerides (@wk5 – 16, 73*, & 145*%; wk10 – 37, 77*, & 56%; wk14 – 41, 120*, & 148%; wk27 – 16, 64, & 109*%) *p≤0.05); ↓ tT4 ((@wk5 – 15, 37.5*, & 22%; wk10 – 24, 35*, & 30*%; wk14 – 16, 31*, & 11%; wk27 – 33*, 29*, & 32*%), ↓ fT4 ((@wk5 –8, 32*, & 23*%; wk10 – 9, 27*, & 27%; wk14 – 11, 29, & 10%; wk27 – 33, 38*, & 42*%); tT3 & fT3 was also ↓but dose response was not consistent. 162.5 ug/mL - ↑ mortality (only 2 tolerated dose for duration of treatment) dose was decreased to 20 mg/kg-d after 12days; ↓ BW; marked to moderate ↑serum enzyme concentrations (e.g., ALT); *3M liv-to-brain wt ratio BMDL = 3.9 mg/kg-d (corresponding serum conc. 23 ug/mL)* *ATSDR (draft 2015) ↑absol liver wt BMD/$BMDL_{10}$ 22.01/15.53 ug/mL*	NA EPA NOAEL 87.0 ug/mL EPA LOAEL based on ↑absol & rel liver wt (hepatocellular hypertrophy) *[LOAEL HED ~0.012 mg/kg-d]*		*[for compariso n purposes only. LOAEL HED based 0.000041 If BMDL based 0.00007]*	Thomford 2001; (Butenhoff 2002) and aci EPA 20126a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
	Average serum concentration = predicted AUC ug/mL-hr/(182 d x 24 hr/d) = 87.0 ug/mL 126.6 162.5	*(HED=0.00154 mg/kg-d); ↑rel liver wt BMD/$BMDL_{10}$ 53.04/46.31 ug/mL* *MDH BMD modeling:* *Absol liv wt – BMDL/BMD_{10} – 24.1/33.2 ug/mL* *Rel liver wt – BMDL/BMD_{10} – questionable modeling results 38.8/64.7 ug/mL (lowest AIC) & 29.3/39.2 ug/mL (lowest BMDL)* *Triglycerides BMDL/BMD_{1SD} – 29.3/45.9 ug/mL* *fT4 – all models unusable*				
2 year Dietary Study – Crl:CDBR Rats 50/sex/dose Dietary levels 0, 30 or 300 ppm	M/F 0/0, 1.3/1.6, or 14.2/16.1 mg/kg-d Add'l grp of 15/sex for 0 & 300ppm evaluated @1yr interim sac	>1.3/1.6 (adm dose) - ↑ALT(e.g., M @12 mons 132* & 217*% vs control levels, *p≤0.05), AST (e.g., M @ 12 mons 57* & 68*% vs control levels) & ALP (e.g., M @ 12 mons 21 & 57*% vs control levels) from 3 to 18 months, but only at 24 mons in high dose grp (M); testicular vascular mineralization (6 & 18*% vs 0% in controls) 14.2/16.1 (adm dose) - ↓BW/BWG, slight ↓ in food consumption, ↑survival rate (likely due to lower BW); ↑incidence liver lesions: cystoid degeneration (M – 14 or 56*% vs 8% in controls); hepatocellular hypertrophy (M/F: 12/2 or 80*/16*% vs 0% in controls); mononuclear cell infiltrate (M-64 & 96*% vs 74% in controls); ↑incidence lung lesions: alveolar macrophages (M: 32 & 62*% vs 20% in controls), hemorrhage (M: 28 & 44*% vs 20% in controls); ↑incidence ovarian lesions: tubular hyperplasia (14* & 32*% vs 0% in controls); ↑incidence testicular	1.3/1.6 (M/F) mg/kg-d (adm dose) EPA NOAEL 14.2/16.1 (M/F) mg/kg-d (adm dose) EPA LOAEL based on ↓BWG (M/F), lesions in liver, testes, & lungs (M) NA/1.6 (M/F) mg/kg-d (adm dose) MDH NOAEL 1.3/16.1 (M/F) mg/kg-d (adm dose) MDH LOAEL			Sibinski et al 1987 published as (Butenhoff 2012) and aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		lesions: vascular mineralization (6 & 18*% vs 0% in controls). *Neoplastic findings:* Males: liver hepatocellular carcinoma 2 & 10% vs 6% in controls; Leydig cell adenomas 4 & 14*% vs 0% in controls *[4% was indicated to be within historical controls by authors & EPA 2016]*; Thyroid C-cell adenoma 4 & 9% vs 0% in controls Females: mammary gland fibroadenoma 42 & 48*% vs 22% in controls *[all considered to be within the norm for background variation. Re-evaluation found no statis signif difference for fibroadenoma, adenocarcinoma, total benign neoplasms, or total malignant neoplasms]* **[Neoplastic findings are discussed further in Table 7-A below]**				
2 yr Mechanistic dietary study – Crl:CD BR Male Rats (156/grp) (follow-up to study above) 0 or 300 ppm	0 or 13.6 mg/kg-d Interim sac conducted every 3 months up to 21 months	13.6 (adm dose) - ↓BW, ↑rel liver wts & hepatic β-oxidation activity; ↑absol testes wts. ↑incidence Leydig cell hyperplasia (46% vs 14% in controls); ↑pancreatic acinar cell hyperplasia (39% vs 18% in controls). No signif difference in serum testosterone or prolactin. Serum FSH was signif ↑@6 months & LH @ 6 & 18 months. Serum estradiol ↑@1,3,6,9,& 12 months. *Neoplastic findings:*	NA NOAEL 13.6 mg/kg-d (adm dose) LOAEL			Biegel et al 2001 aci EPA 2016a

Study Description – duration, route/ vehicle, species/ strain, age at dosing, N/sex/group, etc.	**Admin Dose (mg/kg/d)** [ave serum concen]#	**Effect(s) Observed at each HED dose level and earliest time point observed**	**Study POD_{HED} (mg/kg/d)** [1] [serum concen]#	**UF[2]**	**Candidate RfD mg/kg-d**	**Reference** (note limitations in comment filed)
		Liver adenomas 3% (ad libitum controls), 1% (pair-fed controls) & 13% Leydig cell adenomas – 11% in trt animals compared to 3% in pair-fed controls & 0% in ad libitum controls.				
Comments:						

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 (USEPA 2016a)

[1] 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 x (ln 2 ÷ t½) = 0.17 L/kgbw x (0.693 ÷ 839.5 days) = 0.00014 L/kg bw/day.

[2] 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
Subronic-to-chronic extrapolation denoted as S-to-C

Table 6-A2. RfD Derivation

Identify the critical 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. EPA selected Lau et al 2006 as their critical study (EPA 2016a).

BMD/BMDL values have been generated by authors of some of the key studies. It should be noted that best practices have changed over time and substantial improvements have been made to the BMD software BMD modeling. Therefore, BMD modeling was also conducted by MDH when possible (e.g., data needed was available) and when appropriate (e.g., sufficient number of dose groups). 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. A summary of key studies (e.g., Table 4-8 w/ additional studies selected by MDH) along with estimated average serum concentrations @the NOAEL/LOAEL or BMD/BMDL are presented below

Study (duration)	Effects	Average Serum Concentration (ug/mL)	
		NOAEL/LOAEL	BMDL/BMD[a,b]
DeWitt et al 2008 – Mice Immune toxicity	↓IgM response to SRBC *[≥45% ↑rel liver wt – but no additional hepatic endpts assessed][c]*	38.2/61.9 *[NA/20.2]*	*Authors BMR_{1SD}* *34/53[a]*
Perkins et al 2004 – M Rats Subchronic study	↑liver wt w/hypertrophy ↑liver wt & necrosis	3.0/31.6 31.6/77.4	
Lau et al 2006 – F Mice **Developmental**	**delayed ossification** **accelerated puberty (M)** **↑maternal liver wt** *[but no additional hepatic endpts assessed][c]*	**NA/38**	*Authors BMR_{05}* *33/41[a] pup BW* *(unable to model accel puberty – inverse DR)*
Wolf et al 2007 – F Mice Developmental GD 1-17	↓pup BW	NA/77.9	
Macon et al 2011 – F Mice Developmental GD1-17	↓ mammary gland development	NA/12.4 (qualitative score)	
Butenhoff et al 2002 – Monkeys 26 wk study	↑absol liver wt ↑rel liver wt ↑triglycerides	NA/87	*ATSDR (draft) BMR_{10}* *15.53/22.01* *MDH BMR_{10}* *24.1/33.2 (absol)* *BMR_{1SD}* *29.3/45.9 (trigly)*

[a] MDH estimated average serum concentration that corresponded to the BMD/BMDL administered doses by using the relationship between the average serum concentration and the LOAEL administered dose. See relevant worksheet within Admin dose to Serum Extrapol spreadsheet.
[b] MDH BMD/BMDL modeling reports can be found at \\Data3fb\eh\HRA\COMMON\Guidance - Water\Tox reviews-completed\Final\PFOA\BMD Modeling.
[c] Loveless et al 2008 did assess additional hepatic endpoints in the 29 day male CD-1 mouse study. Serum concentrations were not reported. Increased relative liver wt along with histological changes (e.g., necrosis) were reported at admin dose of 1 mg/kg-d, which is similar to the admin dose LOAEL in DeWitt et al 2008 (0.94 mg/kg-d) and Lau et al 2006 (1 mg/kg-d).

Critical effect(s) and dose: ($LOAEL_{HED}$/BMD_{HED})

At the LDT (1 mg/kg-d, ~ave serum concentration 38 ug/mL) Lau et al 2006 reported increased (≥ 49%) maternal liver weights and in offspring, delayed ossification in proximal phalanges and calvaria (but dose-response was not consistent), trend for decreased pup BW (dose group statis signif at ≥ 3 mg/kg-d (or 77.8 ug/mL); authors calculated $BMDL/BMD_{05}$ of 0.86/1.07 mg/kg-d adm dose), and accelerated preputial separation in males. The later observation (accelerated PPS) is surprising for two reasons – 1) all other developmental parameters are consistent with delayed development and 2) inverse dose-response (greatest effect is observed at the lowest dose level and decreases with increasing dose). Subsequent developmental studies by EPA (in which Lau was a co-author) did not report evaluation of male pups for timing of PPS, therefore, there are no studies in mice which can be used to verify or contradict this effect.

Macon et al and other investigators from this same research laboratory have reported lower qualitative scores for delayed mammary gland development at 0.3 mg/kg-d adm dose (12.4 ug/mL) which is lower than the selected POD (1 mg/kg-d adm in mice, 38 ug/mL in serum). However, evaluations using more quantitative measures of mammary gland development have only reported significant effects at higher dose levels. In addition, lactational challenge study conducted by White et al 2011 did not identify functional impairment. Therefore, this endpoint will not be used as a critical effect.

Point of Departure ($NOAEL_{HED}$, $LOAEL_{HED}$, $BMDL_{HED}$)

EPA's predicted average serum concentration at the LDT LOAEL (1 mg/kg-d) was calculated to be 38 ug/mL (or mg/L)

Human Equivalent Dose Adjustment:

The following equation is used to calculate an HED from the POD serum concentration[2]:
HED (mg/kg-d) = $POD_{ave\ serum\ concen}$ x Clearance.
Where
Clearance = Vol of Distribution (L/kg) x (Ln2/human half-life) = 0.17 L/kg x (0.693/839.5 d) = 0.00014 L/kg-d

HED = 38 mg/L x 0.00014 L/kg-d = 0.0053 mg/kg-d

Uncertainty/Variability Factors:	Interspecies Extrapolation:	3	LOAEL-to-NOAEL:	3	Database:	3
	Intraspecies Variability:	10	Subchronic-to-chronic:		Other:	
	Total[1]:	300				

UF/VF Comments: Interspecies UF of 3 applied to address TD differences, in the absence of chemical information to the contrary the default value of 10 for Intraspecies Variability. With the exception accelerated PPS the effects observed at the LOAEL were mild. An L-to-N UF of 3 was used, along with a DB UF of 3 for the lack of an acceptable 2 generation study. *[Note: the serum concentration corresponding to the RfD below is ~100-fold lower than the LDT LOAEL from Macon et al (0.13 ug/mL vs 12.4 ug/mL].* A DB UF for immunotoxicity concerns was not added at this time.[3]

MDH RfD: 0.0053/300 = 0.000018 mg/kg-d *[corresponding serum concentration 38/300 = 0.13 ug/mL]*

Comments:

[1] Total UF for derivation of a HRL or HBV RfD is ≤ 3000 (RAA could be ≤ 3000 or > 3000)

[2] US EPA 2016 Lifetime Health Advisory Evaluation (USEPA 2016d):

The 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. Olsen et al. (2007) calculated human half-life of 3.8 years in an occupationally exposed cohort. Bartell et al (2010) determined a value of 2.3 years based on the decline in serum levels among members of the general population exposed via drinking water in the area near the DuPont Works plant in Washington, WV. EPA chose to use the half-life from Bartell et al (2010) because it is the most relevant scenario. Thompson et al. (2010) gives a volume of distribution (Vd) of 0.17 L/kg body weight (bw), which is similar to the Vd of 0.198 L/kg determined for monkeys in Butenhoff et al 2004. These two factors (half-life and Vd) are used to determine a clearance of 0.00014 L/kg bw/day using the following equation:

$$CL = Vd \times (\ln 2 \div t_{1/2}) = 0.17 \text{ L/kg bw} \times (0.693 \div 839.5 \text{ days}) = 0.00014 \text{ L/kg bw/day}$$

Where:

Vd = 0.17 L/kg

ln 2 = 0.693

$t_{1/2}$ = 839.5 days (2.3 years x 365 days/year = 839.5 days)

Multiplying the derived average serum concentrations (in µg/mL) for the NOAEL, LOAEL, BMD, or BMDL 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 used in 2007 by MDH were: Vd of 0.2 L/kg instead of 0.17 L/kg and half-life of 1387 days (3.8 yrs) instead of 839.5 days.]*

[3] While 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), the lack of dose response and lack of clear indication of immune system deficits in functional responses to pathogenic challenges in even highly exposed cohorts, hampers quantitative inclusion of these effects reported in epi studies in deriving a reference dose (RfD). MDH will continue to closely monitor the scientific literature regarding immunotoxicity, but based on currently available data it is difficult to justify further increasing the DB UF for PFOA at this time. The study by DeWitt, 2008 demonstrated a NOAEL for immune changes at the critical effect LOAEL, further supporting MDH's decision to not add to the DB UF. The epidemiological literature provides a clear indication that the additivity of PFAS is strongly associated with immunosuppression. 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.

CRITICAL/KEY STUDY SUMMARY

Critical Study(s):

Developmental Gavage Study in CD-1 Pregnant Mice (Lau et al 2006 aci USEPA, 2016a)

Doses & Design: Developmental toxicity study of PFOA was conducted to evaluate the effects of PFOA on prenatal and postnatal development in offspring exposed during pregnancy. Groups averaging 9–45 timed-pregnant CD-1 mice were given 0, 1, 3, 5, 10, 20, and 40 mg/kg PFOA daily by oral gavage on GDs 1–17. Dams were divided into two groups.

Group#1 - dams were sacrificed on GD 18 and underwent maternal and fetal examinations (e.g., maternal liver weight, examination of the gravid uterus for numbers of live and dead fetuses and resorptions). Maternal PFOA serum concentrations were assessed (levels in the fetuses were not examined). Live fetuses were weighed and subjected to external gross necropsy and skeletal and visceral examinations.

Group#2 - an additional dose of PFOA was administered on GD 18. Dams were allowed to give birth on GD 19. The day following parturition was designated as PND 1. Time of parturition, condition of newborns, and number of live offspring were recorded. The number of live pups in each litter and pup body weight were noted for the first 4 days after birth and then at corresponding intervals thereafter. Eye opening was recorded beginning at PND 12. Pups were weaned on PND 23 and separated by gender. The time to sexual maturity was determined by monitoring vaginal opening and preputial separation beginning on PND 24. Two to four pups per gender per litter were randomly selected for observation of postnatal survival, growth, and development. Estrous cyclicity was determined daily by vaginal cytology. After weaning, dams were sacrificed and the contents of the uteri examined for implantation sites. Postnatal survival was calculated based on the number of implantations for each dam.

Effects: *Maternal* - Signs of maternal toxicity were observed following exposure to PFOA during pregnancy. Statistically significant dose-related increases ($p \leq 0.05$) in maternal liver weight were observed at all dose levels (49, 77, 89, 118, 132, & 159% compared to controls). *[MDH Notes: Histological assessment of the liver does not appear to have been conducted].* Dose-related decreases in maternal weight gain during pregnancy were observed beginning at 5 mg/kg/day, with statistical significance ($p \leq 0.05$) seen in the 20- and 40-mg/kg/day dose groups. The number of implantations in treated mice was comparable to control mice. Statistically significant increases ($p \leq 0.05$) in full-

litter resorptions were reported at doses of ≥ 5 mg/kg/day, with complete loss of pregnancies at the highest dose group of 40 mg/kg/day. Slight, but statistically significant, increases ($p \leq 0.05$) in the average time to parturition were observed at 10 and 20 mg/kg/day.
Maternal NOAEL = NA
Maternal LOAEL = 1 mg/kg-d (LDT), based on increased liver weight

Developmental –
Group #1 (fetal examination) - A 20% reduction ($p \leq 0.05$) in live fetal body weight at term was reported at 20 mg/kg/day. A statistically significant increase in prenatal loss was observed in the 20-mg/kg/day dose group. Ossification (number of sites) of the forelimb proximal phalanges was significantly reduced at all doses except 5 mg/kg. Ossification of hindlimb proximal phalanges was significantly reduced at all doses except 3 and 5 mg/kg. Reduced ossification ($p \leq 0.05$) of the calvaria and enlarged fontanel was observed at 1, 3, and 20 mg/kg and at ≥ 10 mg/kg in the supraoccipital bone. Statistically significant increases ($p \leq 0.05$) in minor limb and tail defects were observed in the fetuses at doses ≥ 5 mg/kg/day.

Group#2 (postnatal examination) - Increases ($p \leq 0.05$) in stillbirths and neonatal mortality (or decreases in postnatal survival) were observed at doses ≥ 5 mg/kg/day, with as much as a 30% increase in these effects seen in the 10- and 20-mg/kg/day dose groups. At doses ≥ 3 mg/kg/day, a trend in growth retardation (body weight reductions of 25–30%; $p \leq 0.05$), was observed in the neonates at weaning. Body weights were at control levels by 6 weeks of age for females and by 13 weeks of age for males. A trend for increasing body weight (~6–10% greater than controls) was observed in animals dosed with 5 mg/kg at 13 weeks and in animals dosed with 1 and 3 mg/kg at 48 weeks.

Deficits in early postnatal growth and development also were manifested by significant delays ($p \leq 0.05$) in eye opening at doses ≥ 5 mg/kg/day. Slight delays ($p \leq 0.05$) in vaginal opening and in time to estrous were observed at 20 mg/kg/day in females; in contrast, significant accelerations ($p \leq 0.05$) in sexual maturation were observed in males, with preputial separation occurring 4 days earlier than controls at the 1-mg/kg/day dose and 2–3 days earlier in the 3–10-mg/kg/day dose groups, but the 20-mg/kg/day dose group was only slightly delayed compared to controls.
Developmental NOAEL = NA
Developmental LOAEL = 1 mg/kg-d (LDT), based on delayed ossification and accelerated pubertal development as well as trend for decreased pup body weight

Authors conducted BMD modeling: Values for the benchmark dose (BMD for the maternal and developmental endpoints (BMD_5 and $BMDL_5$) were calculated:

Endpoint	BMD_5 (mg/kg-d)	$BMDL_5$ (mg/kg-d)
Decreased maternal weight gain	6.76	3.58
Increased maternal liver weight at term	0.20	0.17
Neonatal mortality (determined by survival to weaning)	2.84	1.09
Delayed eye opening	2.64	2.10

Alterations in postnatal growth & development and decreased pup body weight at weaning	1.07	0.86
Reduced phalangeal ossification	<1	<1

Co-critical Study(s):

Studies with measured or EPA modelled serum concentrations -

2 Gen Gavage Study in Rats (Butenhoff et al 2004)

2-Generation Gavage Study in Sprague-Dawley Rats (Butenhoff et al 2004a)

Five groups of rats (30 gender/group) were administered PFOA by gavage at doses of 0, 1, 3, 10, and 30 mg/kg/day. At scheduled sacrifices were after completion of the cohabitation period in F0 male rats and on lactation day (LD) 22 in F0 female rats. Rapid elimination in female rats compromises the utility of results. Males F0 - Measured final serum concentration: NA, NA, 51.5, & 45.3 ug/mL. EPA modelled average serum concentrations 45.9, 101.2, 171.1, & 204.4 ug/mL. Effects observed at LDT are considered co-critical as they occur at serum concentrations that are similar to the critical study LOAEL serum concentration (~38 ug/mL): F0 males - increased relative liver weights (21, 47, 61, & 84%, $p<0.01$) as well as increased relative kidney weights (16-17, 22-23, 21-22, 23-27%, $p<0.01$). F1 adult males – decreased body weight at termination (6, 6, 11, & 22%), increased relative liver weights (20, 40, 53, & 76%) and relative kidney weights (11-13, 18-19, 17, & 16-17%). For summary of effects observed at other dose levels see Table 6-A1 above.

Mammary Developmental Gavage Study in Mice (Macon et al 2011)

Cd-1 mice were gavage-dosed with 0, 0.3, 1.0, or 3.0 mg PFOA/kg from GD 1 to GD 17 or with 0, 0.01, 0.1, and 1.0 mg PFOA/kg from GD 10 to GD 17. In the full gestation experiment (Study #1) (GD 1–17), offspring were sacrificed on PNDs 7, 14, 21, 28, 42, 63, and 84, and in the half gestation experiment (Study #2) (GDs 10–17), female offspring were sacrificed on PNDs 1, 4, 7, 14, and 21.

Study #1 (GD1-17) serum concentrations were measured in female offspring on PND7 (earliest time point) 4.98, 11.026, & 20.7 ug/mL & PND14 (peak levels) 4.535, 16.95, & 26.525 ug/mL. EPA modeled ave serum value @0.3 mg/kg-d was 12.4 ug/mL (Table 4-8). Values for other doses not reported. Using the ave serum concen calculated for pregnant CD-1 from Lau et al & Wolf et al the ave serum concen for 1 & 3 mg/kg-d would be 38 & 77.8 ug/mL. Effects observed at the low and mid dose groups are considered co-critical as they occur at or below serum concentrations that are similar to the critical study LOAEL serum concentration (~38 ug/mL): @ lowe dose - increased pup relative liver weight on PND7 (M/F: 26*/19*, 59*/38* , & 97*/76*%, $p<0.05$) and delayed mammary gland development (F pups) @PND14 & 21 (however, developmental scores did not show dose-related trend – e.g., PND21: 1.9, 1.3, & 1.6 vs 3.4 for controls). @ mid dose – increased relative liver weight on PND14 (M/F: 17/26* & 41*/58*%) and delayed mammary gland development (F pups) @PND7 to 84

Study #2 (GD10-17) Gavage @adm dose 0.01, 0.1 & 1 mg/kg-d *[based on Study #1 serum levels likely within co-critical range]* – @lowest dose – statistically significant decrease in qualitative developmental scores @PND21 for mammary gland (2.2, 1.8 & 1.6 vs 3.3 in controls). @mid dose –

statistically significant decrease in number of terminal end buds. @ high dose – statistically significant decrease in quantitative mammary development scores and increased liver weights. For summary of effects observed at other dose levels see Table 6-A1 above.

Immune Drinking Water Study in Mice (DeWitt et al 2008)

Two studies of dose-response were included – groups of 16 female C57BL/6N mice were given 0, 3.75, 7.5, 15, and 30 mg PFOA/kg/day in the drinking water for 15 days during the first experiment. In the second experiment, the doses were 0, 0.94, 1.88, 3.75, and 7.5 mg PFOA/kg/day administered for 15 days in the drinking water. The immunological sensitization and postdose monitoring were identical to that used in the constant dosing versus recovery experiment. Measured final serum concentrations @day 1post-dosing: NA, NA, 74.9, 87.2, 128.1, or 162.6 ug/mL. EPA modelled average serum concentration: 20.2, 38.2, 61.9, 84.4, 111 and 155 ug/mL. Effects observed at the lower two dose groups are considered co-critical as they occur at or below serum concentrations that are similar to the critical study LOAEL serum concentration (~38 ug/mL): @20.2 ug/mL– increased relative liver weight (51-70% one day post treatment & 45-61% 15 days post treatment), however, data was not shown within publication. @61.9 ug/mL– decreased absolute and relative spleen weight post dosing (PD) day 1 (by PD day 15 returned to control levels) (16*, 18, 31*, & 40*%, * $p<0.05$), decreased IgM response to SRBC challenge (7-11%, increasing to 29% @ highest dose), and increased IgG response @ this dose level & 84.4 ug/mL but not higher doses. Author BMD_{1SD} = 53 ug/mL for decreased IgM serum titers. For summary of effects observed at other dose levels see Table 6-A1 above.

13 Week Dietary Study in Rats (Perkins et al 2004)

Male ChR-CD rats (45–55 per group) were administered concentrations of 1, 10, 30, and 100 ppm PFOA for 13 weeks. These doses are equivalent to 0.06, 0.64, 1.94, and 6.50 mg/kg/day. There were two control groups—a nonpair-fed control group and a pair-fed control group for the 100-ppm dose group); both were fed the basal diet. Measured final serum concentrations: 7.1, 41, 70, and 138 ug/mL EPA modelled average serum concentrations: 3.3, 31.6, 76.9, and 149.3 ug/mL. Effects observed at the lower two dose groups are considered co-critical as they occur at or below serum concentrations that are similar to the critical study LOAEL serum concentration (~38 ug/mL): @31.6 ug/mL – increased relative liver weights @wk 4 (13, 45, & 70%) & 13 (4.5, 19, & 56%) w/hepatocellular hypertrophy and increased hepatic palmitoyl CoA oxidase activity @ wk 4 (75*, 200* & 363*#%, *$p<0.05$ vs ad lib controls, #$p<0.05$ pair-fed controls), @wk7 (128,357*, 671*#%), & @13 wk (25, 75#, & 113*#%). Progression of liver toxicity is seen by mild to slight coagulative necrosis at next dose level up (~2.4-fold higher). For summary of effects observed at other dose levels see Table 6-A1 above.

26 Week Oral Capsule Study in Monkeys (Thomford 2001 and Butenhoff et al 2002

Male cynomolgus monkeys (n = 4 or 6 per dose) were administered PFOA by oral capsule containing 0, 3, 10, or 30/20 mg/kg/day for 26 weeks (Butenhoff et al. 2002). Dosing of animals in the 30-mg/kg/day dose group ceased after 12 days and decreased to 20 mg/kg/day when reinstated on day 22 because of low food consumption, decreased body weight, and decreased feces. Measured steady state serum concen (Butenhoff 2004b) were reported to be 81±40, 99±50, & 156±103 ug/mL. EPA modelled average serum concentrations: 87.0, 126.6, and 162.5 ug/mL. The serum concentration at the LDT exceeds the co-critical range. However, the LDT was a LOAEL. BMD modeling identified BMD_{10} for increased absolute liver weight to be 33.2 ug/mL and BMD_{1SD} for triglycerides 45.9 ug/mL. For summary of effects observed at other dose levels see Table 6-A1 above.

Studies without measured or modelled serum concentrations -

2 yr Dietary Study in Rats (Sibinski et al 1987 and Butenhoff et al 2012)
Sprague-Dawley (Crl:CD BR) rats (50 per gender) were fed diets containing 0, 30, and 300 ppm PFOA (0, 1.3, and 14.2 mg/kg/day for males; 0, 1.6, and 16.1 mg/kg/day for females). Groups of 15 additional rats per gender were fed 0 or 300 ppm PFOA and evaluated at the 1-year interim sacrifice. Measured or modelled serum concentrations are not available. However, based on other rat studies effects observed at the LDT would be within the co-critical range: @1.3 mg/kg-d– increased ALT in males (e.g., @12 mons 132* & 217*% vs control levels, *p<0.05), AST (e.g., @ 12 mons 57* & 68*% vs control levels) & ALP (e.g., @ 12 mons 21 & 57*% vs control levels) from 3 to 18 months, but only at 24 mons in high dose grp as well as testicular vascular mineralization (6 & 18*% vs 0% in controls). For summary of effects observed at other dose levels see Table 6-A1 above.

Developmental Gavage (GD0-17 or 18) Study in ICR Mice (Yahia et al 2010)
Pregnant ICR mice (n = 5 per group) were gavage-dosed with 0, 1, 5, and 10 mg PFOA/kg/day from GDs 0–17 or 0–18. The dams dosed from GDs 0–17 were sacrificed on GD 18, and the fetal skeletal morphology was evaluated. Dams dosed from GDs 0–18 were allowed to give birth and their offspring were either processed for pathological examination or observed for 4 days for neonatal mortality. Measured or modelled serum concentrations are not available. However, based on other rat studies effects observed at the LDT would be within the co-critical range: increased relative maternal liver weight (35*, 115*, & 185**%) with hepatic hypertrophy. Increased liver enzyme levels (AST, ALT, ALP) and changes in triglyceride were reported at the next highest dose level indicating a progression of liver toxicity. Increased relative kidney weight (16**, 14.5*, & 27**%) weights. Authors reported that renal cells in outer medullar & proximal tubule were slightly hypertrophic (however, no incidence data or dose-response data were provided) and increased BUN (27.8*, 25.4 & 20.5 vs control 22.6) & phosphorus - - both with no clear dose response. For summary of effects observed at other dose levels see Table 6-A1 above.

Liver Developmental Gavage Study in Mice (Quist et al 2015)
Pregnant CD-1 Mice (N=17-21 dams/dose) were gavage-dosed with 0, 0.01, 0.1, 0.3, or 1 mg/kg-d on GD1-17. On PND35 offspring were placed on HFD with 60% kcal% fat or control diet with 10% kcal% fat (1 pup from 7-10 dams/dose grp). Measured or modelled serum concentrations are not available. However, based on other studies in mice the effects observed at these doses would be within the co-critical range: increased hepatocellular hypertrophy @PND91, lower LDL, HDL and triglyceride levels, and increased relative liver weight.

4 Week Gavage Study in Mice (Yang et al 2009)
21-day-old female BALB/c mice (5 per group) were gavage-dosed with 0, 1, 5, and 10 mg PFOA/kg/day for 5 days per week for 4 weeks to determine the effects of peripubertal PFOA exposure on puberty and mammary gland development. 21-day-old female C57BL/6 mice were also dosed in the same manner and examined the effects of PFOA on mammary gland development and vaginal opening. Measured or modelled serum concentrations are not available. However, based on other studies in mice the effects observed at the LDT would be within the co-critical range: female BALB/c - increased absolute and relative liver weight; decreased absolute and relative uterine weights, and delayed vaginal opening (VO) VO did not occur at 5 or 10 mg/kg-d. C57BL/6 - -increased absolute and relative liver weight and increased absolute and relative uterine weights. For summary of effects observed at other dose levels see Table 6-A1 above.
MDH Notes: effect on uterine wt is in opposing directions and delayed VO at this dose level is not consistent with other studies]

Mammary Gland Development Study in Mice (Tucker et al 2015)
Study of the effects of gestational exposure on mammary gland development as measured at prepubertal time points. Doses of 0, 0.01, 0.1, 0.3, and 1 mg/kg/day were administered to timed pregnant CD-1 and C57Bl/6 mice by gavage on GD 1–17.Serum measured @PND 21, 35 & 56 in CD-1 and PND21 & 61 for C57BL/6 - - but earliest time point was still ~22 days after last exposure. However, based on other studies in mice the effects observed at these doses would be within the co-critical range: CD-1 mice - @>0.01 mg/kg-d decreased qualitative mammary gland develop score @PND35 (2.3, 2.2, 2.3, & 1.9 vs 3.1 in controls) but inconsistent dose-response @PND56 and nonsignificant increase in progesterone levels. @0.1 mg/kg-d decreased qualitative mammary gland score @PND21 (2.3, 2.0 & 1.7 vs 2.9 in controls), and @ 1 mg/kg-d decreased relative liver weight @PND21 (12%) and decreased net BW @PND21 & 35. C57Bl/6 mice @>0.3 mg/kg-d decreased qualitative mammary gland develop score @PND21 (1.8 & 1.8 vs 2.9 in controls) and PND61 (2.1 & 1.7 vs 2.8 in controls). *[MDH Notes: Quantitative scoring not conducted quantitative (rather than qualitative) mammary developmental scores will be relied upon for identification of co-critical effects.]* For summary of effects observed at other dose levels see Table 6-A1 above.

21 Day Drinking Water Studies in Mice (Son et al 2008 and 2009)
2008 study - male ICR mice (N = 10/group) were exposed via drinking water to 0, 0.49, 2.64, 17.63 or 47.21 mg/kg-d for 21 days. Measured or modelled serum concentrations are not available. However, based on other studies in mice the effects observed at the lowest two dose levels would be within the co-critical range: increased relative liver weight (27%) and increased plasma ALT (50%).
2009 study – 4 week old male ICR mice (N=10/group) were exposed via drinking water to equivalent to 0, 0.49, 2.64, 17.63, and 47.21 mg/kg for 21 days to determine if PFOA alters T lymphocyte phenotypes and cytokine expression in mice. Measured or modelled serum concentrations are not available. However, based on other studies in mice the effects observed at the lowest two dose levels would be within the co-critical range: 50% decrease in splenic CD8+ lymphocytes.

For summary of effects observed at other dose levels see Table 6-A1 above.

29 Day Gavage Study in Mice (Loveless et al 2008)
Male CD-1 mice (20/group) were administered 0, 0.3, 1, 10, and 30 mg linear PFOA/kg by oral gavage for 29 days. Measured or modelled serum concentrations are not available. However, based on other studies in mice the effects observed at the lowest two dose levels would be within the co-critical range: @> 0.3 mg/kg-d – increased incidence of microscopic lesion in the liver including mild hepatocellular hypertrophy, increased absolute (25, 84*, 240*, & 230*%, $p<0.05$) and relative (33, 179*, 292*, 317*%) liver weights. @> 1 mg/kg-d – decreased HDL (29*, 39*, & 56*%), moderate-to-severe hypertrophy & individual hepatic cell necrosis (11/20, 20/20, 19/20 vs. 0/19 in controls), liver focal necrosis (3/20, 4/20, 7/19 vs. 0/19 in controls), and decreased absolute (11, 44*, & 56*%) and relative (14*, 35*, & 45*%) spleen weights. For summary of effects observed at other dose levels see Table 6-A1 above.

Table 6-A3. Co-critical Effects Summary

Critical $LOAEL_{HED}$ =	**38 ug/mL serum concentration @LOAEL from Lau et al 2006 based on EPA serum modeling** *[**NOTE**: Not all studies have measured or calculated serum concentrations. When appropriate the oral dose vs EPA 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 ~60 ug/mL (approximately 1.5-fold of the serum benchmark above.]*
Study (source and date):	**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\PFOA\EPA 2016HA PFOA_AdmDoseToSerumExtrap.xlsx.* Effects to be included as co-critical are **bolded**. **Rats –** 1. 13 week Dietary Study in ChR-CD Rats (Perkins et al 2004) @≥31.6 ug/mL [adm dose 0.64 mg/kg-d]: increased **relative liver weight** @wk 4 (13%) and wk 13 (4.5%) accompanied by **hypertrophy and increased hepatic palmitoyl CoA oxidase activity.** Mild to slight coagulative necrosis at next dose level up (~2.4-fold higher) 2. 2-Generation Gavage Study in Sprague-Dawley Rats (Butenhoff et al 2004a) @45.9 ug/mL [adm dose 1 mg/kg-d]: F0 & F1 Males – **increased relative liver (~20%) and kidney (>10%) weights (both F0 and F1 males).** *[MDH Notes: not clear if histological evaluations were conducted however other studies that do include additional liver parameters report more than liver wt changes.]* *Rat studies – estimated serum concentrations* *a.* 29 day Gavage Study in Male CD Rats (Loveless et al 2008) @0.3 & 1 mg/kg-d adm dose *[based on Butenoff et al 2004 serum levels likely <60 ug/mL]* – increased liver wt (not significant until 10 mg/kg-d adm dose), minimum to mild hepatocellular hypertrophy, as well as decreased triglycerides, HDL, and nonHDL cholesterol (however no clear dose response) *[MDH Notes: due to lack of clear dose response reported effects will not be considered as co-critical]* *b.* 2 yr Dietary Study in Crl:CDBR Rats (Sibinski et al 1987, published as Butenhoff et al 2012) @1.3 mg/kg-d adm dose *[based on Butenhoff et al 2004 (assumed at steady state) serum levels likely <60 ug/mL]* – **increased ALT** (132%) **& AST** (57%) in males at 12 months.

	Liver lesions observed at next dose levels up (which was 10-fold higher) **Mice** – 1. Mammary Developmental Gavage Study in CD-1 Mice (Macon et al 2011) Study #1 (GD1-17) Gavage @12.4 & 38 ug/mL [adm dose 0.3 & 1 mg/kg-d] – **increased relative liver wt at PND7 (M/F** 26/19 & 59/38% at 0.3/1) **and 14** (M/F –17/26* at 1vs controls) and delayed mammary development based on qualitative scores (scores, however, did not show a dose-related trend) in offspring exposed in utero. Study #2 (GD10-17) Gavage @adm dose 0.01, 0.1 & 1 mg/kg-d *[based on Study #1 serum levels likely <60 ug/mL]* – decreased qualitative mammary development scores@all doses. **Decreased quantitative mammary gland development scores** only @high dose. **Increased liver wts** also observed @highest dose. *[MDH Notes: histological evaluation of liver does not appear to have been conducted, however, results from other studies report altered hepatic parameters at serum concentrations associated w/adm dose of 1 mg/kg-d].* 2. Immune Drinking Water study in Adult Female C57BL/6N Mice (DeWitt et al 2008) @20.2 ug/mL [0.94 mg/kg-d adm dose] – increased rel liver wt (~50%) *[MDH Notes: data was not shown within publication. Not listed as co-critical but liver already included as Additivity Endpoint.]* @61.9 ug/mL [3.75 mg/kg-d adm dose] – **decreased spleen wt, decreased IgM response to SRBC** [Author BMD_{1SD} = 53 ug/mL for decreased IgM serum titers] *Mice studies – estimated 'average' serum concentrations* a. Developmental Gavage (GD0-17 or 18) Study in ICR Mice (Yahia et al 2010) @1 mg/kg-d adm dose *[based on Lau et al 2006 serum levels would likely be <60 ug/mL]* – **increased maternal relative liver (35%) weight (w/hepatic hypertrophy)** with liver enzyme (increased AST, ALT, ALP) and triglyceride changes at next highest dose level). **Increased relative kidney (16%) weights** (slightly change in renal cells in outer medullar & proximal tubule slightly hypertrophic and increased BUN noted by authors but quantitative results not provided – *therefore these effects will not be listed*) b. Developmental Gavage (GD11-16) Study in CD-1 Mice (Suh et al 2011) @2 mg/kg-d adm dose (LDT) *[based on Lau et al 2006 (GD1-17) serum levels might be <60 ug/mL]* – decreased placental weight, increased incidence of resorption & dead fetuses (post-implantation loss 8.8% vs 3.9% in controls)

	[MDH Notes: too much uncertainty re: serum levels & consistency with other studies – not listed as co-critical] c. Targeted Developmental Gavage (GD1-17) in CD-1 Mice (Quist et al 2015) @0.3 & 1 mg/kg-d adm dose [based on Lau et al 2006 serum levels would likely be <60 ug/mL] – **reported hepatocellular hypertrophy and changes in triglycerides as well as cellular damage and mitochondrial abnormalities in hepatocytes of offspring only exposed *in utero***. d. 4 week (5 d/wk) Gavage Study in BALB/C or C56BL/6 Female Weanling Mice (Yang et al 2009) @1 mg/kg-d adm dose *[based on DeWitt et al 2008 serum levels likely to be <60 ug/mL]* – BALB/C – **increased liver wts w/dose-dependent increase in hepatocellular hypertrophy**, decreased uterine wts & delayed vaginal opening. C57BL/6 – **increased liver wts w/dose-dependent increase in hepatocellular hypertrophy** & increased uterine wts. *[MDH Notes: effect on uterine wt is in opposing directions – will not include as co-critical effect. Delayed VO – not consistent with other studies – will not be included as co-critical]* e. Neurodevelopmental dietary study in C56BL pregnant mice (Onishchenko et al 2011) @0.3 mg/kg-d adm dose – gender specific changes in circadian activity. *[MDH Notes: small group size (6/grp), animals housed 3-4 per cage, not clear if litter effects were controlled for, and only one treatment group evaluated thereby precluding dose-response assessment. Effects will not be identified as co-critical.]* f. Multigenerational Gavage (GD1-17) + Drinking Water Study in CD-1 Mice (White et al 2011) ≥0 mg/kg-d gavage + 5 ppb DW– decreased qualitative mammary gland development scores 1 mg/kg-d gavage adm dose - decreased qualitative mammary gland development scores & increased liver wt. *[MDH Notes: due to combination of gavage & DW exposure & measurement of serum concentrations post-weaning results in great uncertainty regarding serum levels. Effects reported in this study will not be included as co-critical. However, other studies have assessed these effects and will be used to inform identification of co-critical.]* g. Mammary Gland Developmental Gavage (GD1-17) study in CD-1 and C57BL6 Mice (Tucker et al 2015) @0.01, 0.1, 0.3 & 1 mg/kg-d adm dose in CD-1 mice and @0.3 & 1 mg/kg-d adm dose in C57BL6 mice */based on Lau et al 2006 & White et al 2007 the serum levels would likely be ~28, 29, 32 & 42 ug/mL & therefore <60 ug/mL]*– decreased qualitative mammary gland developmental scores in offspring. Quantitative scoring was not conducted.

	[MDH Notes: quantitative (rather than qualitative) mammary developmental scores will be relied upon for identification of co-critical effects.] 1 mg/kg-d adm dose in CD-1 mice – **increased rel liver wts in offspring (12% at PND21)** [MDH Notes: *histological evaluation of liver does not appear to have been conducted, however, results from other studies report altered hepatic parameters at serum concentrations associated w/adm dose of up to 1 mg/kg-d* h. 21 day Immunotox Drinking Water study in 4 wk old Male ICR Mice (Son et al 2009) @0.49 & 2.64 mg/kg-d adm dose *[based on DeWitt et al 2008 serum levels would likely be <60 ug/mL]* – **decreased splenic CD8+ lymphocytes** i. 15 day Immunotox Drinking Water study in PPARαKo & C57BL/6-Tac WT Female Mice (DeWitt et al 2015) Study #2 @1.88 mg/kg-d adm dose *[based on DeWitt et al 2008 serum levels would likely be <60 ug/mL]* – 9-11% decreased antibody response. *[MDH Notes: response is marginal and clear dose response was not observed – effects will not be included as co-critical.]* j. 21 day Drinking Water study in Male ICR Mice (Son et al 2008) @0.49 & 2.64 mg/kg-d adm dose *[based on DeWitt et al 2008 serum levels would likely be < 60 ug/mL]* – **increase rel liver wt and increased plasma ALT** (@2.64 mg/kg-d). k. 29 day Gavage Study in Male CD-1 Mice (Loveless et al 2008) @0.3 & 1 mg/kg-d adm dose *[based on DeWitt et al 2008 & Lau et al 2006 serum levels likely <60 ug/mL]* – **increased incidence of microscopic liver lesions & liver wt, decreased HDL, moderate-to-severe hypertrophy & individual cell necrosis in liver, decreased spleen wts** l. 6 week Testicular toxicity Gavage Study in Male humanized PPARα Mice (Li et al 2011) @1 mg/kg-d adm dose *[based on DeWitt et al 2008 & Lau et al 2006 serum levels are likely <60 ug/mL]* – increased sperm abnormalities and decreased testosterone. Testicular lesions were observed at the next dose level up (5 mg/kg-d adm dose). *[MDH Notes: study does raise questions and supports the need for further study. However, these effects will not be identified as co-critical due to study quality concerns including: group size and only one time point of analysis for evaluating highly variable spermatogenic and hormonal parameters; testes histopath was not quantified or evaluated statistically; absence of motility data (to indicate if baseline data shows acceptable procedures); and lack of reporting & quantification of specific types of sperm abnormalities.]* m. Hormonal latency study of CD-1 mice gestationally exposed (GD1-17) (Hines et al 2009)

	@0.01 – 0.3 mg/kg-d adm dose *[based on Lau et al 2006 serum levels would likely <60 ug/mL]* – increased body weight, serum leptin levels and serum insulin levels at 21-31 weeks of age. No statis signif difference in fat-to-lean ratio at 42 weeks of age.*[MDH Notes: the study design and level of detail in reporting is inadequate to provide sufficiently robust data needed to assess metabolic impacts (e.g., insulin can vary due to fasting status, circadian cycle, age-matching, etc.) therefore these effects will not be identified as co-critical at this time. Further study, replication and validation are needed.]* **Monkeys –** 1. 26 week Oral Capsule Study in Male Cynomolgus Monkeys (Thomford 2001 & Butenhoff et al 2002) @ 87 ug/mL [LDT 3 mg/kg-d adm dose] – increased liver wt, evidence of mitochondrial proliferation in liver, increased triglycerides (statis sign at next dose level up), and decreased tT4 & fT4. ATSDR (draft 2015) **BMD_{10}** for ↑absol and rel **liver wts** 22.01 ug/mL and 53.04 ug/mL. MDH BMD for absol liver wt 33.2 ug/mL and BMD_{1SD} for **triglycerides** 45.9 ug/mL. MDH attempts to model relative liver wt, tT4 or fT4 were unsuccessful. *[MDH Notes: T4 changes were observed at the LDT (serum level 87 ug/mL) which is >2-fold higher than the co-critical benchmark serum level of 39 ug/mL, BMD modeling was unsuccessful. Decreased thyroid hormone levels were also reported in rats (Martin et al 2007) but unfortunately only one high dose level was tested (20 mg/kg-d adm dose). Therefore, at this time there is insufficient data to include changes in T4 as co-critical]*
Co-Critical Effects:	Increased liver weights w/histological changes (e.g., hepatocellular hypertrophy, cell necrosis) changes in triglyceride and cholesterol levels, increased AST, ALT & ALP; increased kidney wt decreased splenic CD8+ lymphocytes, decreased spleen wt & decreased IgM response; and developmental (delayed mammary gland development based on quantitative scoring, hepatic effects following in utero exposure only – liver weights, cellular damage, mitochondrial abnormalities).
Health Endpoints:	Critical Endpoints – Developmental (based on delayed ossification, accelerated preputial separation, & trend for decreased pup body weight), Hepatic (liver) system
	Co-Critical Endpoints – Developmental (mammary gland development, hepatic effects); Hepatic (liver) system; Immune system; and Renal (kidney) system

A. 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:	Yes (Source, in part, EPA 2016a) Thyroid effects: Three large epidemiological studies provide support for an association between PFOA exposure and incidence or prevalence of thyroid disease in female adults or children, but not in males. In addition, associations between PFOA and TSH have also been reported in pregnant females with anti-TPO antibodies. However, no significant associations were found between PFOA and TSH or thyroid hormones (T4 or T3) in people who have not been diagnosed with thyroid disease. Effects of PFOA on thyroid hormones in animals are generally not as well characterized as those of PFOS. Reduced total and free T4 were reported in adult male rats and monkeys at serum levels ≥ 500-fold higher than the serum level corresponding to the RfD. However, these doses were the lowest doses tested within the study and the dose-response relationship of serum total T4 with PFOA exposure has yet to be fully evaluated and the lowest effective dose remains unknown. Other endocrine effects beyond thyroid have not been well-studied, and study results are not entirely consistent. Decreased testosterone and increased estradiol in male rats and mice have been reported, but usually at higher PFOA levels than those which form the basis of the RfD. (See Reproductive Effects for additional information).
Immunologic Effects	Tested:	Yes
	Observed:	Yes (Source, in part, EPA 2016a) Associations between prenatal, childhood, or adult PFOA exposure and risk of infectious diseases (as a marker of immune suppression) have not been consistently seen in epidemiological studies. Although there was some indication of effect modification by gender (i.e., associations seen in female children but not in male children). Three studies have examined associations between maternal and/or child serum PFOA levels and vaccine response (measured by antibody levels) in children and adults. The study in adults was part of the high-exposure community C8 Health Project; a reduced antibody response to one of the three influenza strains tested after receiving the flu vaccine was seen with increasing levels of serum PFOA. The studies in children were conducted in general populations in Norway and in the Faroe Islands. Decreased vaccine response in relation to PFOA levels was seen in these studies, but

		similar results also were seen with correlated perfluorinated chemicals and could not be attributed specifically to PFOA. Several animal studies demonstrate effects on the spleen and thymus weights as well as decreased immune response. These effects were observed at serum concentrations similar to the critical study LOAEL. The Immune system is listed as an Additivity Endpoint based on co-critical effects.
Developmental Effects	Tested:	Yes
	Observed:	Yes (Source, in part, EPA 2016a) There have been numerous human epidemiological studies examining PFOA exposure and developmental effects. Some studies reported an association between PFOA and birth weight. Most studies measured PFOA using maternal blood samples taken in the second or third trimester or in cord blood samples. Studies on the high-exposure C8 community population have not observed associations between PFOA and either birth weight among term births or the risk of low birth weight among all (singleton) births. In contrast, several analyses of general populations indicate a negative association between PFOA levels and birth weight, while others did not attain statistical significance. A meta-analysis of many of these studies found a mean birth weight reduction of 19 g (95% CI: -30, -9) per each 1-unit (ng/mL) increase in maternal or cord serum PFOA levels. However, when low GFR was accounted for in PBPK simulations, the association reported between PFOA and birth weight is less than that found in their meta-analysis of the epidemiology data and shows that, in individuals with low GFR, there are increased levels of serum PFOA and lower birth weights. This suggests that a portion of the association between PFOA and birth weight could be confounded by low maternal GFR under conditions such as preeclampsia and pregnancy-induced hypertension. Two epidemiological studies examined development of puberty in females in relation to prenatal exposure to PFOA as measured through maternal or cord blood samples in follow-up of pregnancy cohorts, however, the results of these two studies are conflicting, with no association (or a possible indication of an earlier menarche seen with higher PFOA) in one study and a later menarche seen with higher PFOA in the other study. Among the animal studies, decreased postnatal growth leading to developmental delays (e.g., lower body weight, delayed eye opening, delayed vaginal opening, and accelerated preputial separation) has been observed. These effects form the basis of the RfD and were observed at serum concentrations ~300-fold higher than the serum concentration corresponding to the RfD.

		Qualitative scoring assessment found delayed mammary gland development of female offspring exposed *in utero* at serum levels just slightly higher than the serum concentration corresponding to the RfD. However, MDH had concerns regarding the inherent variability in qualitative scoring. The use of quantitative measures of average length of mammary gland ducts and number of terminal end buds in female pups were also assessed in one study and identified statistically significant delays at higher dose levels. These effects have been included as co-critical effects. An additional study evaluated the correlation between mammary duct branching patterns and the ability to support pup growth through lactation. No significant impacts were found. Doses resulting in serum concentrations >700-fold higher than the serum concentration corresponding to the RfD resulted in decreased neonatal survival.
Reproductive Effects	Tested: Observed:	Yes Yes (Source, in part, EPA 2016a) A series of studies in the high-exposure C8 Health Project study population have reported associations between PFOA exposure and pregnancy-induced hypertension or preeclampsia. Limited data suggest a correlation between higher PFOA levels in females and decreases in fecundity and fertility, however, reverse causality has been suggested since birth and lactation are elimination routes. No clear effects of PFOA on male fertility endpoints have been identified. Among the animal studies, there was no effect of PFOA on reproductive or fertility parameters in female rats. However, it should be noted that female rats have very high elimination rate compared to male rats or other species. Increased full litter resorptions and increased stillbirths were observed in pregnant mice exposed at serum concentrations >700-fold higher than the serum concentration corresponding to the RfD. No evidence of altered testicular and sperm structure or function was reported in adult male rats exposed to doses producing serum concentrations ≥350-fold higher than the serum concentration corresponding to the RfD. One study has reported increased sperm abnormalities and decreased testosterone at dose levels similar to the critical study LOAEL, however, MDH has concerns regarding the quality of this study and other studies have reported these effects only at higher doses.
Neurotoxicity Effects	Tested: Observed:	Yes (limited) Yes (Source, in part, EPA 2016a)

		The data pertaining to neurotoxicity (including neurodevelopmental effects) of PFOA are limited, but do not indicate the presence of associations between PFOA and a variety of outcomes. Epidemiological studies have found no association between maternal serum PFOA concentrations and fine motor skills, gross motor skills, and cognitive abilities of children aged 6 and 18 months or between behavioral or coordination problems in children aged 7 years and prenatal PFOA exposure. Epidemiology studies of children derived from the NHANES and C8 populations found a weak statistical association between serum PFOA with parental reports of ADHD (Hoffman et al. 2010; Stein et al. 2013). Information from animal studies is also quite limited. The offspring of mice fed PFOA throughout gestation had detectable levels of PFOA in their brains at birth. Locomotor activity, anxiety-related or depression-like behavior, or muscle strength were not altered. Circadian activity tests revealed gender-related differences in exploratory behavior patterns. In the social group setting, the PFOA-exposed males were more active and PFOA-exposed females were less active than their respective controls. The results of an *in vitro* study of hippocampal synaptic transmission and neurite growth in the presence of 50 and 100 µmol PFOA increased spontaneous synaptic current and had an equivocal impact on neurite growth. These data suggest a need for additional studies of potential neurological effects of PFOA.

Other Studies/Effects/Considerations

Tan et al (2013) aci EPA 2016a

Study was designed to determine if dietary fat content could be an important variable influencing the impact of PFOA on serum lipids. Groups of seven or eight 4-month-old male C57BL/6N mice were given either a liquid regular fat diet (RFD) or a high-fat diet (HFD), with or without PFOA, for 3 weeks. The RFD provided 12% and the HFD provided 35% of their calories from fat. The fats were primarily monounsaturated (olive oil) or polyunsaturated (safflower and corn oil). PFOA was added to both diets for 3 weeks at a level that maintained a dose of 5 mg/kg/day to the mice. The PFOA treated groups were fed ad libitum, and the control groups were given the amount consumed by the PFOA-treated groups the previous day.

The fat content of the diets alone resulted in significant differences in body weight and subcutaneous white adipose tissue, but not in liver weight. The addition of PFOA to the RFD resulted in significant increases in body weight, liver weight, ALT, ALP, and plasma free fatty acids, but not in AST or bilirubin. The addition of PFOA to both the RFD and HFD resulted in decreases in the mass of both epididymal and subcutaneous white fat deposits. The HFD alone did not result in definitive alterations in liver histopathology. When PFOA was added to the RFD, indications of hepatocyte hypertrophy, necrosis, and inflammatory cell infiltration were observed. The liver damage in the animals being fed the HFD with PFOA was increased more than in the RFD-PFOA animals, as indicated by higher levels of necrosis and inflammation accompanied, in this case, by lipid droplet accumulation and significantly increased liver triglycerides, but not liver cholesterol or free fatty acids. In the epididymal adipose tissues, adipocyte size was increased in the HFD control compared to the RFD control but decreased with the addition of PFOA compared to both the RFD and HFD controls. Inflammatory cell infiltration was observed in the epididymal adipose tissues when PFOA was added to the HFD but not the RFD. No data for the subcutaneous white fat tissues was provided.

The authors evaluated the hepatic expression of 84 genes involved in the regulation of fatty acid metabolism using RT2 Profiler PCR Arrays. HFD and/or PFOA altered the expression of 33 genes (> 1.5 fold). PFOA alone upregulated 13 genes (>1.5) and downregulated 4 (>1.5) genes with fatty acid and triglyceride catabolism. Eight fatty acid transport-related genes were upregulated by PFOA and one was downregulated. The study demonstrates the importance of the fat content of the diet as a modulator of the effects of PFOA on the liver in animals. Damage to the liver tissues was intensified in the presence of the HFD

Wolf et al 2008a aci EPA 2016a

To characterize hepatic effects wild-type 129S1/SvlmJ mice (n = 7–8 per group) and PPARα-null mice (129S4/SvJae-PPARαtm1Gonz/J, n = 6–8 per group) were gavage-dosed with 0, 1, 3, or 10 mg PFOA/kg or 50 mg Wyeth 14,643 (a PPARα agonist) and wild-type CD-1 (n = 7–8 per group) with 0, 1, and 10 mg PFOA/kg for 7 days. The mice were sacrificed 24 hours following the last dosing. Blood was collected for serum, and the livers were removed and weighed. Liver sections were stained with hematoxylin and eosin for examination by light microscopy and with uranyl acetate for transmission electron microscopy. Liver sections were also processed for immunohistochemistry of PCNA. Hepatocyte hypertrophy and vacuolation, observed in both strains of wild-type mice, were assigned a score from 0 to 4 based on severity, with 0 being no lesions observed and 4 being panlobular hypertrophy with cytoplasmic vacuolation. Hepatic lesions in PPARα-null were assigned a score (0–4) based on cytoplasmic vacuolation as no hypertrophy was observed.

Compared to control values, the absolute and relative liver weights, lesion score, and labeling index were significantly increased ($p<0.05$) in a dose-dependent manner in both strains of wildtype mice exposed to PFOA and also were significantly increased ($p<0.05$) in the wild-type 129S1/SvlmJ mice exposed to Wyeth 14,643. The absolute and relative liver weights and lesion score were significantly increased ($p\leq0.05$) in a dose-dependent manner in all PFOA-exposed PPARα-null mice. The labeling index was significantly increased ($p<0.05$) in PPARα-null mice exposed to 10 mg PFOA/kg. Absolute and relative liver weights, lesion score, and labeling index of PPARα-null mice exposed to Wyeth 14,643 were no different from control values. (see Table 3-15 from EPA 2016a below)

Table 3-15. Hepatic Effects in PFOA-Treated Mice

Group	Liver Weight (g)	Relative Liver Weight (%)	Lesion Score	Labeling Index
Wild-type CD-1 Mice				
Control	1.53 ± 0.14	4.5 ± 0.4	0.3 ± 0.5	0.6 ± 0.4
1 mg/kg/day PFOA	2.26 ± 0.24*	6.5 ± 0.5*	2.1 ± 0.9	0.7 ± 0.5
10 mg/kg/day PFOA	3.48 ± 0.54*	10.5 ± 0.8*	3.0 ± 0*	7.7 ± 3.0*
Wild-type 129S1/SvlmJ Mice				
Control	0.87 ± 0.08	3.3 ± 0.4	0.3 ± 0.5	0.3 ± 0.2
1 mg/kg/day PFOA	1.22 ± 0.22*	1.6 ± 0.2*	2.0 ± 0.0*	0.7 ± 0.6
3 mg/kg/day PFOA	1.70 ± 0.12*	6.4 ± 0.4*	2.0 ± 0.0*	1.0 ± 0.4
10 mg/kg/day PFOA	2.20 ± 0.23*	8.3 ± 0.2*	4.0 ± 0.0*	2.4 ± 0.9*
50 mg/kg/day Wyeth 14,643	1.5 ± 0.13*	5.6 ± 0.1*	3.3 ± 0.5*	2.1 ± 1.2*
PPARα-null Mice				
Control	0.92 ± 0.08	3.4 ± 0.4	1.1 ± 0.4	0.2 ± 0.2
1 mg/kg/day PFOA	1.2 ± 0.14*	4.5 ± 0.2*	1.9 ± 0.6*	0.6 ± 0.4
3 mg/kg/day PFOA	1.46 ± 0.21*	5. 8 ± 0.3*	3.0 ± 0.0*	0.6 ± 0.3
10 mg/kg/day PFOA	2.8 ± 0.18*	9.4 ± 0.6*	4.0 ± 0.0*	7.7 ± 3.0*
50 mg/kg/day Wyeth 14,643	1.07 ± 0.24	3.9 ± 0.5	1.4 ± 0.5	0.6 ± 0.5

Source: Wolf et al. 2008a

Note: * Statistically different from control, $p < 0.05$.

Ultrastructure evaluations were done on liver sections from wild-type 129S1/SvlmJ mice and PPARα-null mice, but not from CD-1 mice. There were the expected differences in the characteristics of hepatocytes from the control wild-type mice when compared to both the PFOA-treated and Wyeth 14,643 wild-type mice. In the PPARα-null mice, the responses of the control and Wyeth 14,643-dosed animals were similar, but the response of the PFOA-dosed animals differed. (see Table 3-16 from EPA 2016a below)

Table 3-16. Mouse Hepatocyte Ultrastructure After PFOA or Wythe 14,643 Treatment

	Characteristics				
Mouse/Treatment	**Glycogen**	**Golgi/ Rough ER**	**Mitochondria**	**Peroxisomes**	**Lipid-like Vacuoles**
Wild-type/Control	Prominent	Prominent	Numerous	Few	Rare
Wild-type/PFOA (10 mg/kg)	Negative	Nominal/ scarce ER	Numerous	Numerous	Scattered
Wild-type/Wyeth	Negative	Nominal/ scarce ER	Numerous	Numerous	Scattered
PPARα-null/Control	Prominent	Prominent	Numerous	Absent	Scattered
PPARα-null/PFOA (10 mg/kg)	Limited	Limited	Not reported	Not reported	Numerous[a]
PPARα-null/Wyeth	Prominent	Prominent	Numerous	Absent	Scattered

Source: Wolf et al. 2008a

Note: [a] Described as electron-dense, nonmembrane-bound spaces morphologically consistent with lipids ranging from the size of mitochondria to the size of nuclei. The vacuoles were believed to be an accumulation of PFOA.

It is apparent that PFOA and Wyeth 14,643 behaved similarly in the wild-type strains but differently in the PPARα-null mice. The hepatocytes of PFOA-dosed PPARα-null mice exhibited lower glycogen content, Golgi bodies, and associated rough ER than both the control and Wyeth 14,643 PPARα-null mice. In addition, the PFOA-dosed PPARα-null mice had numerous large nonmembrane-bound lipid-like vacuoles throughout the cytoplasm. At the high dose (10 mg/kg/day), there was an increase in the labeling index that was not observed with Wyeth 14,643. The authors concluded that the large lipid-like vacuoles in the hepatocytes of PFOA-dosed PPARα-null mice were likely accumulations of PFOA. Under the conditions of this study, the LOAEL was 1 mg/kg/day based on increased absolute and relative liver weight and hepatic morphology changes; no NOAEL was established.

Nakamura et al 2009 aci EPA 2016a –

The functional difference in PFOA response between mice and humans was investigated using a humanized PPARα transgenic mouse strain (hPPARα). Humanized PPARα mice express a high level of human PPARα protein in the liver. Male 8-week-old wildtype (mPPARα) mice, PPARα-null mice, and hPPARα mice were gavage-dosed with 0, 0.1, and 0.3 mg/kg/day PFOA (n = 4–6 per group) for 2 weeks and sacrificed 18–20 hours following the last dose. Blood was collected and analyzed for triglyceride and cholesterol concentrations, and ALT measurements. Livers were collected and analyzed for triglyceride and cholesterol concentrations, plus histopathological changes. (see Table 3-17 from EPA 2016a below).

Table 3-17. Relative Response of hPPARα, mPPARα, and PPARα-null Mice to PFOA

Parameter	hPPARα	mPPARα	PPARα-null
Liver weight	ND	↑ compared to control (0.3 mg/kg/day)	↓ compared to control (0.1 mg/kg/day)
Liver/body weight ratio	ND	↑ compared to control (0.3 mg/kg/day)	ND
Hepatocyte hypertrophy	Mild (0.3 mg/kg/day)	Mild (0.3 mg/kg/day)	ND
ALT	ND	ND	ND
Plasma cholesterol	↑ compared to mPPARα (all doses)	ND	ND
Liver cholesterol	↓ compared to PPARα-null (0.1, 0.3 mg/kg/day), mPPARα (0.3 mg/kg/day)	↑ compared to control (0.3 mg/kg/day)	ND
Plasma triglyceride	ND	ND	ND
Liver triglyceride	↓ compared to PPARα-null (0.3 mg/kg/day)	↓ compared to PPARα-null (0.1, 0.3 mg/kg/day; ↑ compared to control (0.3 mg/kg/day)	↑ compared to mPPARα (all doses)

Source: Nakamura et al. 2009
Notes:
hPPARα: transgenic mice (that express a high level of human PPARα protein in the liver); mPPARα: wild-type mice.
↑ = significant increase ($p < 0.05$).
↓ = significant decrease ($p < 0.05$)
ND = no differences.

The hPPARα mice differed from the wild-type mice in that their plasma cholesterol was significantly increased and their liver cholesterol and triglycerides significantly decreased at the highest dose. In addition, the increases in absolute and relative liver weights were less than those observed in the wild-type mice. The PPARα-null mice differed from the wild-type in that liver triglycerides were significantly increased.

Under the conditions of the study, the NOAEL/LOAEL for mPPARα mice was 0.1/0.3 mg/kg/day of PFOA based on increased liver weight and increased liver triglyceride and cholesterol concentrations. The NOAEL for PPARα-null mice was 0.3 mg/kg/day (HDT) because the changes in absolute liver weight were not dose-related and the increase in relative liver weight was not significantly different from the control. The NOAEL for hPPARα mice was also 0.3 mg/kg/day of PFOA. However, a nonsignificant but dose-related increase was observed in plasma cholesterol.

Li et al 2011 aci EPA 2016a –
The involvement of mouse and human PPARα in PFOA-induced testicular toxicity was investigated. Wild-type, PPARα-null, and humanized PPARα male 129/Sv mice were given PFOA daily by gavage at doses of 0, 1, and 5 mg/kg/day for 6 weeks. Body weight and testis weight were not affected by treatment in any group. Absolute and relative weights of the epididymis and seminal vesicle plus prostate gland were decreased only in high-dose wild-type mice compared to the wild-type controls. No effects on sperm count and motility were seen in any group. Sperm abnormalities were significantly increased in both treated groups of wild-type and humanized PPARα mice, but not in the PPARα-null mice. Plasma testosterone levels were slightly decreased in low-dose wild-type mice, and significantly decreased in high-dose wildtype and low- and high-dose humanized PPARα mice compared to the control groups. Testosterone levels were slightly reduced in a dose-related manner in the PPARα-null mice, but statistical significance was not attained.

mRNA levels for several genes associated with testicular cholesterol synthesis, transport, and testosterone biosynthesis were examined. Levels HMG-CoA synthase, HMG-CoA reductase, and aromatase were not changed after treatment in any group. Expression of 65teroidogenic acute regulatory protein (which transports cholesterol into mitochondria) was inhibited in wild-type mice at the high dose and in humanized PPARα mice at both doses;

peripheral benzodiazepine receptor level was decreased only in high-dose humanized PPARα mice; cytochrome P450 sidechain cleavage enzyme was decreased in both groups of wild-type mice; cytochrome P450 17α-hydroxylase/C17-20 lyase was inhibited at the high dose in both wild-type and humanized PPARα mice; and 3β-hydroxysteroid dehydrogenase was decreased in both treated groups of humanized PPARα mice. Decreased expression of 17β-hydroxysteroid dehydrogenase was the only change found in treated PPARα-null mice. In the mitochondria, carnitine palmitoyltransferase (CPT) was decreased in both groups of wild-type and high-dose humanized PPARα mice, and SOD levels were reduced in all treated wild-type and humanized PPARα mice. Histopathological lesions of the testes, including abnormal seminiferous tubules, lack of germ cells, or necrotic cells, were observed in high-dose wild-type and humanized PPARα mice. No morphological changes were observed in the testes from PFOA treatment in PPARα-null mice. The 1-mg/kg/day dose was the author's LOAEL for significant ($p<0.05$) sperm abnormalities, decreased testosterone, and several biochemical alterations in the PPARα and hPPARα mice, but not in the PPARα-null mice. There were dose related decreases in testosterone in the PPARα-null mice, but they did not achieve statistical significance.
MDH Notes this study might indicate need for further study, however, study quality concern regarding effects reported in hPPARα: Group sizes too small for adequate sperm evaluations or testosterone evaluations, motility data was not reported , types of sperm abnormalities (by head, tail, mid-section, etc.) were not reported, testosterone only evaluated at one time-pt, no clear dose-response for T from low to high, accessory sex organs are a sensitive indicator of low T but no effects on combined prostate & seminal vesicle wt. were observed, testes histopath was not quantified by incidence or severity and not statistically evaluated.

Abbott et al 2007 aci EPA 2016a
Male and female 129S1/SvImJ and PPARα-null mice were used in studies to determine if PFOA-induced developmental toxicity was mediated by PPARα. Pregnant 129S1/SvImJ wild-type and PPARα-null mice were orally dosed from GD 1–17 with 0, 0.1, 0.3, 0.6, 1, 3, 5, 10, and 20 mg PFOA/kg/day. Heterozygous (HET) litters also were produced by mating wild-type and PPARα-null males with wild-type and PPARα-null dams to determine if genetic background affected survival. The HET litters were sacrificed on PND 15.

There was no effect of treatment on maternal weight or maternal weight gain (excluding those with full-litter resorptions), number of implants, or pup weight at birth. Wild-type dams exposed to ≥0.6 mg/kg/day and PPARα-null dams exposed to ≥5 mg/kg/day had a significantly greater percentage of litter loss compared to their respective controls. At ≥5 mg/kg/day in wild-type dams and 20 mg/kg/day in PPARα-null dams, 100% litter loss occurred. Relative liver weight was significantly increased in wild-type adult females dosed with ≥1 mg/kg/day and in PPARα-null adult females dosed with ≥3 mg/kg/day. Body weight in wild-type offspring born of dams dosed with 1.0 mg/kg/day was significantly reduced ($p<0.05$) compared to control offspring body weight gain on PND 9, 10, and 22 (males) and PND 7–10 and PND 22 (females). No differences were observed between PPARα-null offspring body weight and control offspring body weight. Survival of pups from birth to weaning was significantly reduced ($p<0.05$) in wild-type litters exposed to ≥0.6 mg/kg/day, but was not affected in PPARα-null litters. Survival was significantly decreased ($p<0.05$) for wild-type and HET pups born to wild-type dams dosed with 1 mg/kg/day and for HET pups born to PPARα-null dams dosed with 3 mg/kg. Offspring born of wild-type dams showed a dose-related trend for delayed eye opening compared to control offspring (significantly delayed at 1 mg/kg/day, $p<0.05$), but no difference in day of eye opening was observed in the offspring born of PPARα-null dams. At weaning, relative liver weight was significantly increased ($p<0.05$) in wild-type offspring gestationally exposed to ≥0.1 mg/kg/day and in PPARα-null offspring gestationally exposed to 3 mg/kg/day.

The authors concluded that survival of PPARα-null pups and deaths of HET pups born to PPARα-null dams indicates that expression of PPARα is required for PFOA-induced postnatal lethality; however, early prenatal lethality was independent of PPARα. Delayed eye opening and reduced postnatal weight gain appeared to be mediated by PPARα, but other mechanisms might also contribute.

Albrecht et al 2013 aci EPA 2016a –
To further evaluate the developmental effects potentially mediated by PPARα, groups of female wild-type, PPARα-null, and PPARα-humanized mice were given 0 and 3 mg PFOA/kg on GDs 1–17 by oral gavage. Females were either sacrificed on GD 18 (n = 5–8 per group) or allowed to give birth and then sacrificed, along with their litters (n = 8–14), on PND 20.

Evaluation on GD 18 showed no effects of PFOA administration on maternal body weight, body weight gain, gravid uterine weight, number of implantations per dam, or number of resorptions per litter in dams of any genotype. For animals allowed to litter, the average day of parturition was slightly later in PFOA-treated humanized mice than in the controls. Body weight of dams during lactation, the number of pups born per litter, pup body weight during lactation, and the onset of pup eye opening were similar between treated and control groups for all genotypes. Offspring survival during PNDs 1–5 was significantly reduced in the wild-type PFOA-treated group, but not in the other genotypes.

Maternal liver weight was significantly increased in the treated groups of all genotypes on GD 18 and in wild-type animals on PND 20. Maternal liver weight was not affected on PND 20 in the PPARα-null or PPARα-humanized mice. On GD 18, maternal liver samples from treated groups showed increased expression of Acox1 in wild-type mice and Cyp4a10 in wild-type and humanized mice. Expression of Cyp2b10 and Cyp3a11 were also increased in all three genotypes. On PND 20, maternal liver samples from treated groups showed increased expression of Acox1 in wild-type mice; expression of Cyp2b10 was unchanged in all groups; and expression of Cyp3a11 was increased in all three genotypes.

Microscopic evaluation of the maternal liver showed centrilobular hepatocellular hypertrophy in all PFOA-treated groups on GD 18 and PND 20, with decreased incidence and severity by PND 20. On GD 18, the liver lesions were graded as mild in the wild-type mice, minimal-to-mild in the humanized mice, and minimal in the null mice. The morphological features of the liver lesions differed slightly between genotypes.

Relative fetal liver weight on GD 18 was significantly increased in fetuses from treated wild-type and humanized dams. On PND 20, relative liver weight was increased only in pups from treated wild-type dams. For fetuses on GD 18, liver samples from treated groups showed increased expression of Acox1 and Cyp4a10 in wild-type and humanized mice. Expression of Cyp2b10 was unchanged following maternal PFOA administration in all three genotypes, while expression of Cyp3a11 was increased in humanized fetal liver. On PND 20, pup liver samples from treated dams showed increased expression of Acox1 and Cyp4a10 in wild-type mice; expression of Cyp2b10 was increased in all genotypes; and expression of Cyp3a11 was increased following maternal PFOA administration in wild-type and humanized pups. Thus, expression of PPARα target genes that modulate lipid metabolism was increased in both wild-type and humanized mice coincident with increased liver weight and microscopic lesions; however, the neonatal mortality was observed only in wild-type offspring.

Hormone Disruption (EPA 2016a – Section 3.3.3)

Thyroid:
Martin et al. (2007) administered 20 mg PFOA/kg to adult male Sprague-Dawley rats (n = 4 or 5) for 1, 3, or 5 days by oral gavage and determined the impact of PFOA on hormone levels. Blood was collected via cardiac puncture and the serum was analyzed for cholesterol, testosterone, FT4 and total T4, and total T3. RNA extracted from the livers was used for gene expression profiling, genomic signatures, and pathway analyses to determine a mechanism of toxicity. Following a 1-day, 3-day, and 5-day dose, a significant decrease ($p<0.05$) was observed in serum cholesterol (~↓45-72%), total T4 (~↓83%), FT4 (~↓80%), and total T3 (~↓25-48%). Serum testosterone was significantly decreased ($p<0.05$, ~↓70%) following a 3-day and 5-day PFOA dose. PFOA treatment was matched to hepatotoxicity-related genomic signatures, as well as signatures for hepatocellular hypertrophy, hypocholesterolemia, hypolipidemia, and peroxisome proliferation. PPARα nuclear regulated genes were induced by PFOA treatment. Genes associated

with the thyroid hormone release and synthesis pathway including Dio3, which catalyzes the inactivation of T3, and Dio1, which deiodinates prohormone T4 to bioactivate T3, were affected by PFOA. Treatment with PFOA resulted in significantly upregulated expression of Dio3 and downregulated expression of Dio1 ($p<0.05$). Expression of HMG-CoA reductase (involved in cholesterol biosynthesis) was significantly upregulated and cholesterol biosynthesis was downregulated in a manner consistent with PPARγ agonists.

Reproductive Hormones:
Cook et al. (1992) gavage-dosed male CD rats (n = 15 per group) for 14 days with 0, 1, 10, 25, and 50 mg PFOA/kg/day to examine the possibility that an endocrine related mechanism might explain Leydig cell adenomas observed in rats. A separate control group was pair-fed to the 50-mg/kg/day group. Blood and testicular interstitial fluid were collected at necropsy for hormone analysis including testosterone, estradiol, and LH. A separate group of rats was dosed with 0 and 50 mg PFOA/kg/day for 14 days and challenged with 100 Ius of human chorionic gonadotropin (hCG) or 2 mg naloxone/kg 1 hour prior to necropsy to induce testosterone concentrations. Blood was collected and analyzed for testosterone and LH. Serum from rats challenged with 100 Ius hCG also was analyzed for P, 17α-hydroxyprogesterone, and androstenedione.

The relative liver weight at 10, 25, and 50 mg PFOA/kg/day was significantly increased ($p<0.05$). The accessory sex organ unit relative weight was significantly decreased ($p<0.05$) at 25 and 50 mg PFOA/kg/day compared to those weights in control rats. The relative weights of the liver, accessory sex organ unit, and ventral prostate were significantly decreased at the highest dose compared to the pair-fed control.

Serum estradiol was significantly increased at ≥10 mg PFOA/kg compared to the control. No differences were observed in testosterone and LH between the treated rats and control. In the challenge experiment, serum testosterone was significantly decreased ($p<0.05$) by treatment with 50 mg PFOA/kg after challenge with 100 Ius hCG. No differences in testosterone concentration were observed in the naloxone-challenged rats, and no differences in LH were observed after either challenge. In the hCG-challenged rats, androstenedione was significantly reduced at 50 mg PFOA/kg, but no differences in concentrations were observed in P or 17 α-hydroxyprogesterone between control and treated rats. The authors suggested that the observed decreased serum testosterone levels could be due to decreased conversion of 17 α-hydroxyprogesterone to androstenedione as a result of increased serum estradiol levels. The LOAEL was 10 mg/kg based on increased liver weight and increased serum estradiol levels, and the NOAEL was 1 mg/kg.

Biegel et al. (1995) gavaged male CD rats were gavage-dosed for 14 days with 0, 0 pair-fed, or 25 mg PFOA/kg and necropsied on day 15. Blood and testicular interstitial fluid were collected for hormone analysis. Liver samples were collected for analysis of peroxisomal β-oxidation and microsomal aromatase activities. Serum estradiol was significantly increased ($p<0.05$) by 25 mg PFOA/kg when compared to the ad libitum and pair-fed control rats. Testicular interstitial fluid testosterone concentration was significantly decreased ($p<0.05$) and microsomal aromatase activity, and peroxisomal β-oxidation activity were significantly increased ($p<0.05$) in PFOA-treated rats compared to the pair-fed control rats.

Hines et al. (2009) examined the roles that exposure to PFOA and ovarian hormones might play in animals exposed during gestation compared to during their adult years. Timed-pregnant CD-1 mice were gavage-dosed in two blocks on GDs 1–17, but not thereafter. Block 1 animals were dosed with 0, 1, 3, and 5 mg PFOA/kg, and block 2 animals were dosed with 0, 0.01, 0.1, 0.3, 1, and 5 mg PFOA/kg/day. At birth, pups were pooled within each block and dose group and randomly redistributed among the dams (10 pups per litter). Offspring were weaned at 3 weeks, and a subset of females from each dose group (0, 0.01, 0.1, 0.3, 1, and 5 mg PFOA/kg/day) was OVX at weaning or the day after weaning. All animals were observed until they reached 18 months of age.

Body weight of offspring born to dams exposed to 5 mg PFOA/kg was significantly decreased ($p<0.05$) on PND 1 and through 18 months of age compared to control offspring body weight. At weaning, the body weight of offspring born to dams exposed to 1 mg PFOA/kg/day was significantly decreased ($p<0.05$) compared to control offspring body

weight. A significant increase ($p<0.05$) in body weight, due to more rapid weight gain after week 10, compared to intact control body weight, was observed in intact mice exposed to 0.01–0.3 mg PFOA/kg/day .

Glucose tolerance testing showed no statistically significant differences in baseline glucose or response to glucose challenge at 15–16 weeks or at 17 months. At 21 and 31 weeks of age, a significant increase in serum leptin and insulin levels was observed in intact mice exposed to 0.01 and 0.1 mg PFOA/kg/day. No statistically significant difference was observed between the fat-to-lean ratio of intact control and intact gestationally exposed animals at 42 weeks of age. No significant difference was observed in food consumption between intact control and intact gestationally exposed animals at 42 weeks of age. Serum estradiol levels were not different between intact control and intact gestationally exposed animals at 18 months. Exposure to PFOA as an adult did not result in body weight differences among the groups at 18 months of age. The body weight of intact mice gestationally exposed to 1 mg PFOA/kg/day was significantly increased ($p<0.05$) compared to adult mice exposed to 1 mg PFOA/kg/day. No other differences in body weight among the groups were observed.

The authors concluded that developmental exposure to low doses and high doses of PFOA resulted in different phenotypes in mice. At low doses, increased weight, increased serum insulin, and increased serum leptin were observed in adult mice. At high doses the animals displayed decreased weight in early and late life, decreased white fat, increased brown fat, and decreased spleen weight. Under the conditions of the study, the developmental LOAEL was 0.01 mg PFOA/kg based on increased weight gain and increased serum insulin and leptin levels. No developmental NOAEL was established.
MDH Notes - the study design and level of detail in reporting is inadequate to provide sufficiently robust data needed to assess metabolic impacts (e.g., insulin vary due to fasting status, circadian cycle, age, etc.) therefore these effects will not be identified as co-critical at this time. Further study, replication and validation are needed.].

B. Duration Specific Health-based Water Criteria Derivation

Exposure Decision Tree from (U.S. Environmental Protection Agency (EPA) 2000) used as basis for RSC selection.

Relative Source Contribution (RSC)		
Henry's Law Constant (atm m3/mol)	9.08×10^{-2}	EpiSuite
What is the volatility[1]?		
Is there documentation to justify the use of an RSC other than the defaults?[2]	If yes, explain	

[1] Nonvolatile (<3 x 1E-7 atm m3/mol); Low (3 x 1E-7 to 1E-5 atm m3/mol); Moderate (1E-5 to 1E-3 atm m3/mol) or High (>1E-3 atm m3/mol)
[2] Non-volatile/low volatility/moderate volatility – 0.5 for acute/short-term, 0.2 for subchronic/chronic
High volatility – 0.2 for acute/short-term/subchronic/chronic

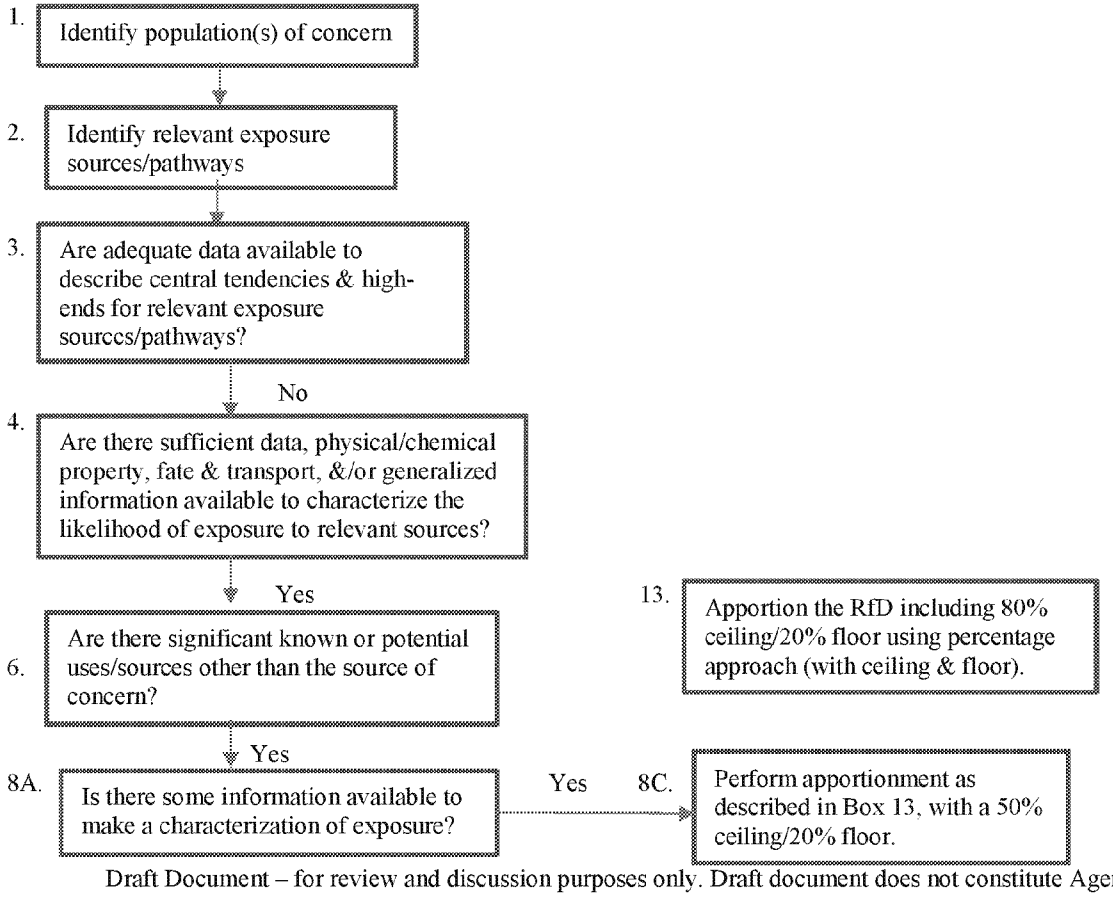
RSC evaluation from EPA (USEPA 2016b) (See Section 8.6 for more information):
Findings from studies on populations in the United States, Canada, and Western Europe support the conclusion that diet is the major contributor to total PFOA exposure, typically with drinking water and/or dust as important additional exposure routes, especially for sensitive subpopulations. EPA used an RSC of 0.2 and the 90th percentile intake rate for lactating women (0.054 L/kg-d) to calculate a lifetime HA for PFOA of 0.07 µg/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 ingestion of water, 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 PFOA, the RSC concept needed to be applied in a framework recognizing the long elimination half-life, 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.

In order to examine the relative impact of non-water exposures, MDH reviewed the source studies reported in Egeghy and Lorber (Egeghy PP and M Lorber 2011). The sparseness of media-specific data results in highly uncertain estimates of intake rates. The framework proposed by Egeghy and Lorber also included use of serum concentrations reported in the 2003-2004 NHANES biomonitoring effort to estimate intakes. MDH decided to use the most recent NHANES biomonitoring data (2013-2014) and East Metro new resident biomonitoring data (2014) in a similar fashion to estimate current upper-end non-water exposures.

MDH utilizes 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 (MDH 2008). 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 PFOA 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 38 µg/mL. The serum concentration associated with the resulting RfD, which incorporated a total UF of 300, is 0.13 µg/mL (or 130 µg/L). Background (i.e., exposure from non-water ingestion routes of exposure) 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 PWS and volunteer participants had blood levels measured at three time points: 2008, 2010 and 2014:
2008 – 14.9 ug/L geo mean (CI 12.9 – 17.3); 95th percentile 60 ug/L (range 1.6 – 117)
2010 – 11.2 ug/L geo mean (CI 9.7 – 13.1); 95th percentile 48.7 ug/L (range 0.94 – 110.5)
2014 – 5.5 ug/L geo mean (CI 4.6 – 6.4); 95th percentile 26 ug/L (range <LOD – 47)

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 may be representative of non-water exposures: 1.8 geo mean ug/L (CI 1.6-2.0); 95th percentile 5 ug/L (range 0.17-8.1). These levels are very similar to the 2013-14 NHANES data for the general public.

General population (NHANES) biomonitoring data demonstrate that serum levels have been decreasing over time (CDC 2017). The 2013-14 data provide the most recent data regarding 'background' serum levels in the US general population.

Year	Geometric Mean (ug/L) (95% CI)	95th Percentile (ug/L) (95% CI)
1999 – 2000	5.21 (4.72-5.74)	11.9 (10.9-13.5)
2003-2004	3.95 (3.65-4.27)	9.80 (7.40-14.1)
2005-2006	3.92 (3.48-4.42)	11.3 (8.80-14.5)
2007-2008	4.12 (4.01-4.24)	9.60 (8.90-10.1)
2009-2010	3.07 (2.81-3.36)	7.50 (6.20-9.70)
2011-2012	2.08 (1.95-2.22)	5.68 (5.02-6.49)
2013-2014	1.94 (1.76-2.14)	5.57 (4.60-6.27)

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

- (Schecter 2012) sampled children in Dallas, Texas between August and November 2009. Reported median and maximum PFOA serum concentrations were: 2 and 9.6 ug/L, respectively, in children less than three years of age. Reported median and maximum PFOA serum concentrations were: 3.1 and 11.1 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 95th percentile PFOA serum concentrations were: 4.46 and 7.4 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 90th percentile PFOA serum concentrations were: 4.2 and 7.9 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 2013-14 NHANES. Eighty percent of the serum concentration associated with the RfD would be 104 ug/L (130 ug/L x 0.8). Subtracting the 95th percentile values, as a high-end estimate of background, non-water exposures, from the 2013-14 NHANES (5.57 ug/L) produces a residual serum concentration of roughly 98 ug/L, or approximately 75% of the serum concentration at the RfD (130 ug/L). This calculation can be used qualitatively, along with the ceiling proscribed in Box 8C of the Decision Tree to select 50% as the RSC for water ingestion.

A.1. Develop Non-Cancer Guidance Value

The most appropriate dose metric for PFOA is serum concentration. PFOA is a bioaccumulative chemical, with a half-life of 2 – 3 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 bottle-fed or breast-fed consume a much greater volume of liquid on a per body weight basis than older children and adults. In addition, PFOA crosses the placenta and is transferred to breastmilk. Empirical data from the published literature indicates that breastfeeding can result in significant exposures, result 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.

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

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.

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

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 (MDH 2017b)

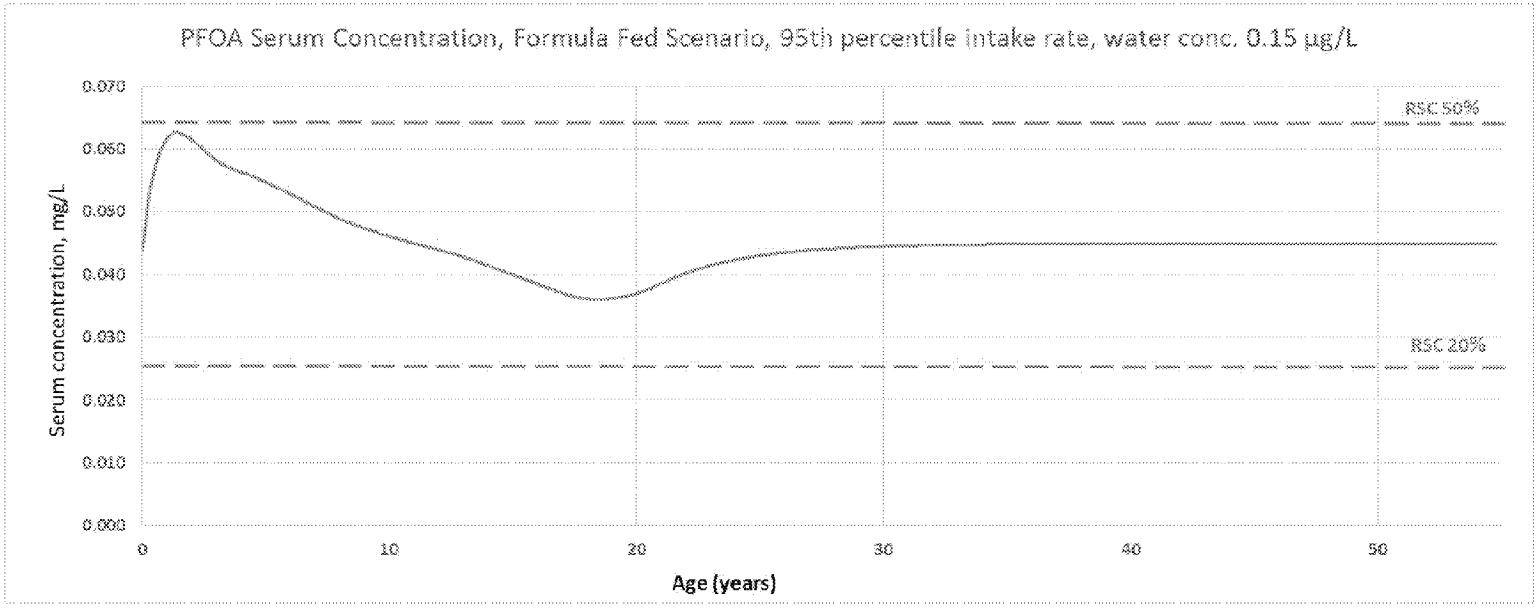
Model Parameter	Value(s)
Half-life (days)	840 days
Volume of distribution (Vd)	0.17 L/kg
Vd Age Adjustment Factor (Vd AF)	Range from 2.1 @age 1-30 days to 1.2 @age 5 – 10 years. Value of 1 used for ages >10 years.
Clearance Rate (CR)	0.17 L/kg x (Ln 2/840 days) = 0.00014 L/kg-d
Placental transfer factor	87% (% of maternal serum level)
Breastmilk transfer factor	5.2% (% of maternal serum level)
Water Intake (L/kg-d)	95th 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 95th 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 bottle-fed Infant

The water concentration that keeps the serum concentration attributable to drinking water (solid line below in Figure 1) below an RSC of 50% (0.13 x 0.5 = 0.065 mg/L) throughout life is 0.15 µg/L.

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



Scenario #2 - Breast-fed Infant

While a water concentration of 0.15 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 0.15 µg/L in water. Under scenario #2 infant PFOA serum levels exceed the serum concentration at the reference dose for over 4 years and the 50% RSC threshold for over 9 years. See Figure 2.

Figure 2. Serum concentration for an exclusively breast-fed for 1 year, followed by water ingestion, based on upper/95th percentile ingestion rates and an RSC of 50% at a water concentration of 0.15 µg/L.

In order to maintain serum concentrations below an RSC threshold of 50% (0.13 x 0.5 = 0.065 mg/L) for infants exclusively breast-fed for one year the water concentration must be lowered to 0.035 µg/L. See Figure 3.

Figure 3. Serum concentrations for an exclusively breast-fed for 1 year, followed by water ingestion, based on upper/95th percentile ingestion rate and an RSC of 50% at a water concentration of 0.035 µg/L.

Even a small incremental increase in the water concentration (0.036 µg/L) raises the serum concentration above the 50 percent threshold for approximately one month. Given the health endpoints of concern include developmental concerns, the acceptable water concentration was set at 0.035 µg/L and not rounded to one significant digit.

*** 7. Cancer Effects ***

7-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 $(mg/kg-d)^{-1}$**	**Reference (note limitations in comment filed)***
2 year Dietary Study – Crl:CDBR Rats 50/sex/dose Dietary levels 0, 30 or 300 ppm *Sexually mature Fs have very short half-life therefore, the results of cancer bioassay, except at sufficiently high doses, in female rats is of limited utility in assessing carcinogenic potential.*	M/F 0/0, 1.3/1.6, or 14.2/16.1 mg/kg-d Add'l grp of 15/sex for 0 & 300ppm evaluated @1yr interim sac	**See Table 6-A above for discussion of non-neoplastic findings.** *Neoplastic findings: [control, 30, & 300 ppm]* Males: Liver hepatocellular carcinoma 6, 2 & 10%; Leydig cell adenomas 0, 4 & 14*%, *p<0.05 *[4% was indicated to be within historical controls by authors & EPA 2016]*; Thyroid C-cell adenoma 0, 4 & 9% Females: Mammary gland fibroadenoma 22, 42 & 48*% *[all considered to be within the norm for background variation. Re-evaluation found no statis signif difference for fibroadenoma, adenocarcinoma, total benign neoplasms, or total malignant neoplasms]*			Sibinski et al 1987 published as (Butenhoff 2012) and aci EPA 2016a
2 yr Mechanistic dietary study – Crl:CD BR **Male** Rats (156/grp) (follow-up to study above) 0 or 300 ppm	0 or 13.6 mg/kg-d Interim sac conducted every 3 months up to 21 months	*Neoplastic findings:* Liver adenomas - 1% in pair-fed controls, 3% in *ad libitum* controls and 13%, in trt animals Leydig cell adenomas – 3% in pair-fed controls, 0% in *ad libitum* controls, and 11% in trt animals. [Notc: ↑ incidence of Leydig cell hyperplasia (46% vs 14% in controls was observed)]			Biegel et al 2001 aci EPA 2016a

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

A. Oral Cancer-related Study Summaries:

Human Carcinogenicity Data:

EPA 2016a – Section 3.4.2 Synthesis and Evaluation of Carcinogenic Effects.

Evidence of carcinogenic effects of PFOA in epidemiology studies is based primarily on studies of kidney and testicular cancer. These cancers have relatively high survival rates (e.g., 2005–2011 5-year survival rates 73% and 95%, respectively, for kidney and testicular cancer based on NCI Surveillance, Epidemiology and End Results data). Thus studies that examine cancer incidence are particularly useful for these types of cancer. The high-exposure community studies also have the advantage, for testicular cancer, of including the age period of greatest risk, as the median age at diagnosis is 33 years. The two occupational cohorts in Minnesota and West Virginia (most recently updated in Raleigh et al. 2014 and Steenland and Woskie 2012) do not support an increased risk of these cancers, but each of these is limited by a small number of observed cases (six kidney cancer deaths, 16 incident kidney cancer cases, and five incident testicular cancer cases in Raleigh et al. 2014; and 12 kidney cancer deaths and 1 testicular cancer death in Steenland and Woskie 2012). Two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrollment 0.024 µg/mL) and kidney and testicular cancers (Barry et al. 2013; Vieira et al. 2013); there is some overlap in the cases included in these studies. No associations were found in the general population between mean serum PFOA levels up to 0.0866 µg/mL and colorectal, breast, prostate, bladder, and liver cancer (Bonefeld-Jørgensen et al. 2014; Eriksen et al. 2009; Hardell et al. 2014; Innes et al. 2014); none of these studies examined kidney or testicular cancer.

Animal Carcinogenicity Data:

EPA 2016a – Section 3.4.2 Synthesis and Evaluation of Carcinogenic Effects.

Two animal carcinogenicity studies indicate that PFOA exposure can lead to liver adenomas (Biegel et al. 2001), Leydig cell adenomas (Biegel et al. 2001; Butenhoff et al. 2012), and PACTs (Biegel et al. 2001) in male Sprague-Dawley rats. Liver adenomas were observed in the Biegel et al. study (2001) at an incidence of 10/76 (13%) at 20 mg/kg/day. The incidence in the control group was 2/80 (3%). Although no liver adenomas were observed in Butenhoff et al. (2012), carcinomas were identified in the male controls, males in the low-dose group (2 mg/kg/day), and male and female rats in the high-dose group (20 mg/kg/day). The differences from control were not significant in either study, but the carcinoma incidence among the Butenhoff et al. (2012) high-dose males (10/50) was similar to that for the adenomas in the Biegel et al. study (2001) (10/76). Liver lesions were identified in the males and females at the 1- and 2-year sacrifices (Butenhoff et al. 2012). An increased incidence of diffuse hepatomegalocytosis and hepatocellular necrosis occurred at 20 mg/kg/day. At the 2-year sacrifice, hepatic cystoid degeneration (characterized by areas of multilocular microcysts in the liver parenchyma) was observed in 8, 14, and 56% in males of the control, 2-, and 20-mg/kg/day dose groups, respectively. Hyperplastic nodules in male livers were increased in the high-dose group (6% versus 0% in control rats).

Filgo et al. (2015) examined the livers of three strains of mice exposed only during gestation/lactation for tumors when they were sacrificed at 18 months. Liver tumors were found in each dose group, but tumor types varied and the data did not display any evidence of dose response. The animals were survivors from two different projects and the number per dose group was small. Thus, the data are not adequate for determining whether PFOA is a carcinogen in mice. *[Study authors noted that this study was NOT designed to evaluate carcinogenesis – but was a result of a previous study that reported liver tumors in PPARα-deficient mice – so this study was considered an initial mechanistic study to confirm that PFOA can mediate hepatotoxic effects via non-PPARα pathways.]*

'Conclusions' re: liver tumors: Overall, the tumor response observed in the available studies was not strong and did not demonstrate a dose-related response. Butenhoff et al. (2012) and Biegel et al. (2001) studies suggest that PFOA is not a potent hepatic carcinogen based on the low tumor incidence and finding of hyperplastic nodules. [**MDH Notes**: available data are quite limited. Only species examined has been rats and it is clear that female rats quickly excrete PFOA unlike humans or other animals.]

Testicular Leydig cell tumors (LCTS) were identified in both the Butenhoff et al. (2012) and Biegel et al. (2001) studies. The tumor incidence was 0/50 (0%), 2/50 (4%), and 7/50 (14%) for the control, 2.0-, and 20-mg/kg/day dose groups, respectively (Butenhoff et al. 2012). The Biegel et al. study (2001) included one dose group (20 mg/kg/day); the tumor incidence was 8/76 (11%) compared to 0/80 (0%) in the control group. LCT incidence at 20 mg/kg/day was comparable between the two studies (11 and 14%).

'Conclusions' re: LCTs: The induction of LCTs by PFOA could be attributed to a hormonal mechanism whereby PFOA either inhibits testosterone biosynthesis and/or lowers testosterone by increasing its conversion to estradiol through increased aromatase activity in the liver. Both of these mechanisms appear to be mediated by PPARα. Several of the available PFOA studies support an impact of PFOA on decreased testosterone production. Studies conducted by Cook and colleagues (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996) found that adult male rats administered PFOA by gavage for 14 days had decreased serum testosterone and increased serum estradiol levels (Cook et al. 1992). These endocrine changes correlated with its potency to induce LCTs (Biegel et al. 2001).

Data are not currently sufficient to demonstrate that the other key steps in the postulated MOA are present in PFOA-treated animals following exposures that lead to tumor formation. Studies are needed to demonstrate the increase of GnRH and LH in concert with the changes in aromatase and estradiol. There was also no indication of increased Leydig cell proliferation at the doses that caused adenomas in the Biegel et al. study (2001). Thus, additional research is needed to determine if the hormone testosterone estradiol imbalance is a key factor in development of LCTs as a result of PFOA exposure.

Pancreatic acinar cell tumors (PACTs) were only observed in the Biegel et al. study (2001). The incidence was 8/76 (11%; 7 adenoma, 1 carcinoma) at 20 mg/kg/day while none were observed in the control animals. Although no PACTs were observed by Butenhoff et al. (2012), pancreatic acinar hyperplasia was observed at 2 and 20 mg/kg/day at incidences of 2/34 (6%) and 1/43 (2%), respectively, which lacked dose response. Reexamination of the pancreatic lesions in Butenhoff et al. (2012) and Biegel et al. (2001) resulted in the conclusion that 20 mg/kg/day increased the incidence of proliferative acinar cell lesions in both studies. Some lesions in the Biegel et al. study (2001) had progressed to adenomas.

'Conclusions' re: PACTs: Two hypothetical MOAs have been proposed: 1) A change in the bile acid flow or composition that leads to cholestasis, thereby causing an increase in CCK activating a feedback loop resulting in proliferation of the secretory pancreatic acinar cells. CCK is a peptide hormone that stimulates the digestion of fat and protein, causes the increased production of hepatic bile, and stimulates contraction of the gall bladder. An HFD, trypsin inhibition, and changes in bile composition are proposed initiators for this sequence of events.; and 2) Increased levels of testosterone support the growth of acinar cell preneoplastic foci, leading to the development of carcinomas. There is minimal information on the relationship of PFOA exposure to either of the proposed MOAs. [However, EPA notes: PFOA appears to <u>suppress</u> testosterone production through the induction of aromatase and to increase the estradiol. Therefore, the second proposed MOA for PACTs does not appear to apply to PFOA.]

The initial findings from the Butenhoff et al. study (2012) were equivocal for mammary fibroadenomas in female rats. However, a reexamination of the tissues by a PWG found no statistically significant differences in the incidence of fibroadenomas or other neoplasms of the mammary gland between control and treated animals (Hardisty et al. 2010). The PWG used the diagnostic criteria and nomenclature of the Society of Toxicological Pathologists for the reexamination. Under those criteria, there was an increase in the number of tumors documented in the control group, especially fibroadenomas originally classified as lobular hyperplasia. The reclassification led to a loss of significance when the tumors in the treated animals were compared to tumors in the control animals.

Ovarian tubular hyperplasia and adenomas also were observed in female rats (Butenhoff et al. 2012). Mann and Frame (2004) reexamined the ovarian lesions using an updated nomenclature system, which resulted in some of the hyperplastic lesions being reclassified. The ovarian lesions originally described as tubular hyperplasia or tubular adenomas were regarded as gonadal stromal hyperplasia and/or adenomas. After the reclassification, there were no statistically significant increases in hyperplasia (total number), adenomas, or hyperplasia/adenoma combined in treated groups compared to controls.

Genotoxicity Data:
EPA 2016a Section 3.3.1 Summary:
PFOA has been tested for genotoxicity in a variety of *in vivo* and *in vitro* assays. The data from the *in vitro* studies are summarized in the table below –

Table 3-32. Genotoxicity of PFOA *In Vitro*

Test System	End-point	With Activation	Without Activation	Reference
$C_3H10T_{½}$ mouse embryo fibroblasts	Cell Transformation	NA	-	Garry and Nelson 1981
C_3H $10T_{½}$ mouse embryo fibroblasts	Cytotoxicity	NA	-	Garry and Nelson 1981
S. typhimurium TA1537	Gene Mutation	-	+ (not reproducible)	Lawlor 1995, 1996
E. coli	Gene Mutation	-	-	Lawlor 1995, 1996
CHO cells	Chromosomal Aberrations	+, +	+, -	Murli 1996b, 1996c
CHO cells	Polyploidy	+, +	+, -	Murli 1996b, 1996c
Human lymphocytes	Chromosomal Aberrations	-	-	Murli 1996c; NOTOX 2000
K-1 CHO cells	Gene Mutation	-	-	Sadhu 2002
S. typhimurum TA98, TA100, TA102, TA104	Gene Mutation	-	-	Freire et al. 2008

Note: NA= not applicable.

PFOA was tested in a cell transformation and cytotoxicity assay conducted in C3H10T½ mouse embryo fibroblasts. The cell transformation was determined as both colony transformation and foci transformation. There was no evidence of transformation at any of the dose levels tested in

either the colony or foci assay methods. PFOA was tested twice for its ability to induce mutation in the Salmonella – E. coli/mammalian-microsome reverse mutation assay. The tests were performed both with and without metabolic activation. A single positive response seen in S. typhimurium TA1537 when tested without metabolic activation was not reproducible. PFOA did not induce mutation in either S. typhimurium or E. coli when tested either with or without metabolic activation. PFOA did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations. Sadhu (2002) reported that PFOA did not induce gene mutation when tested with or without metabolic activation in the K-1 line of CHO cells in culture. Murli (1996b, 1996c) tested PFOA twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, PFOA induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, PFOA induced significant increases in chromosomal aberrations and in polyploidy (Murli 1996b). The effects were observed only at toxic concentrations (EFSA 2008). PFOA did not display mutagenic activity with or without metabolic activation in S. typhimurium strains TA98, TA100, TA102, or TA104 (Freire et al. 2008).

In vitro data summarized above in Table 3-32 above suggest that PFOA is not a mutagen. A single positive result in S. typhimurium was not reproducible by the same authors and was not replicated in other studies. Potential chromosomal effects were found in CHO cells at toxic concentrations, but not in human lymphocytes.

PFOA was tested twice in the *in vivo* mouse micronucleus assay. PFOA did not induce any significant increases in micronuclei and was considered negative under the conditions of this assay (Murli 1995, 1996d). G. Zhao et al. (2010) used AL cells to determine the mutagenicity of PFOA to mammalian cells. AL cells are a human-hamster hybrid containing CHO-K1 chromosomes and a single copy of human chromosome 11. The significance of human chromosome 11 is that it encodes for expression of the human cell surface protein CD59. At 100 and 200 µmol PFOA, AL cell viability was significantly decreased after incubation for 1, 4, 8, and 16 days. CD59 mutation frequencies were increased in AL cells after a 16-day incubation with 200 µmol PFOA. There was no increase in mutations in mitochondria-deficient AL cells after incubation with 100 or 200 µmol PFOA.

C. Critical Cancer Study Information:

Cancer Classification (source & date):	Under the EPA 2005 cancer guidelines, the evidence for the carcinogenicity of PFOA is considered *suggestive* because only one species has been evaluated for lifetime exposures and the tumor responses occurred primarily in males*. (EPA 2016a) **Note: unlike male rats, female rats rapidly excrete PFOA*
Slope Factor Source, Date of Development:	EPA 2016 (NJ has also derived a cancer slope factor – see description below)
Slope Factor Study Quality:	Two studies exist – one (Butenhoff et al 2012) has two treatment groups and was conducted on both male and female rats. However, female rats rapidly excrete PFOA. Cancer bioassay data is not available in other species (e.g., mice). A follow-up study (Biegel et al 2001) was conducted

only in male rats and utilized only one treatment group (equal to the highest dose grp in Butenhoff et al 2012).

Describe the Basis for the Toxicity Value:

The increase in hepatocellular tumors did not show a direct relationship to dose in male rats and was not significantly elevated in either males or females at the high dose when compared to controls. There was a dose-related significant increase in LCTs in male rats in the Butenhoff et al. study (2012), which was confirmed by the high dose in the single-dose mechanistic study by Biegel et al. (2001). The PACT tumors, only detected in the single dose Biegel et al. study (2001), do not support quantification. Therefore, dose-response data are only available for the LCTs from one study, Butenhoff et al 2012. Two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrollment of 0.024 µg/mL) and kidney and testicular cancers (Barry et al. 2013; Vieira et al. 2013). Therefore, the data on LCTs from Butenhoff et al. (2012) were modeled to provide a perspective on the magnitude of the potential cancer risk as it compares with the level of protection provided by the RfD.

The dose-response for the LCTs from Butenhoff et al. (2012) was modeled using EPA's Benchmark Dose Software (BMDS) Version 2.3.1. The multistage cancer model predicted the dose at which a 4% increase in tumor incidence would occur. The 4% was chosen as the low-end of the observed response range within the Butenhoff et al. (2012) results. Both the first and second degree polynomials gave identical goodness-of-fit criteria (p value and Akaike's Information Criterion [AIC]).

Results from EPA's modeling are shown below:

Table 4-11. Multistage Cancer Model Dose Prediction Results for a 4% Increase in LCT Incidence

	BMD (mg/kg/day)	BMDL (mg/kg/day)
First Degree Polynomial Fit	3.51	1.99
Second Degree Polynomial Fit	3.51	1.99
AIC = 62.6936	P = 0.2245	

Source: Butenhoff et al. (2012)

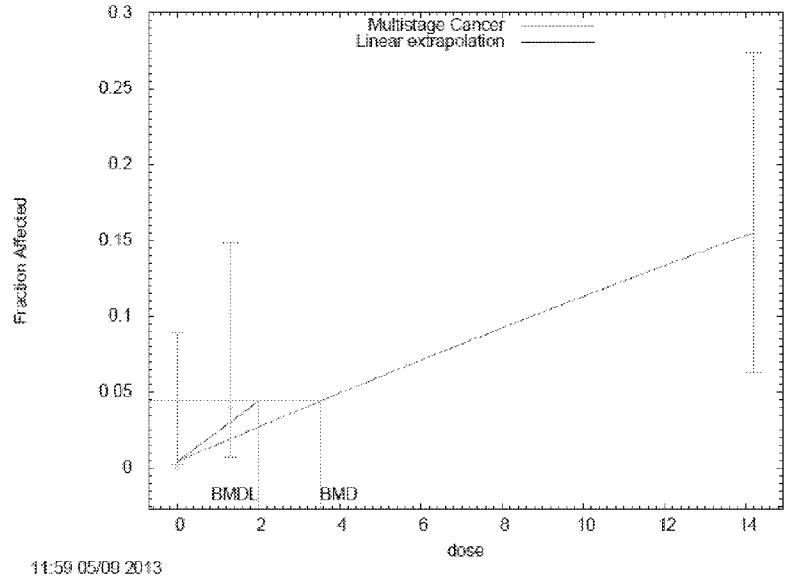


Figure 4-1. BMD Model Results for LCTs (Butenhoff et al. 2012)

The CSF for PFOS is derived from the BMDL04 of 1.99 mg/kg/day after converting the animal BMDL to a HED using body weights to the ¾ power. The HED is calculated as follows:

$$HED = \text{Animal BMDL} \times (\text{animal body weight})^{1/4} \div (\text{human body weight})^{1/4}$$

$$HED = 1.99 \text{ mg/kg/day} \times [(0.523 \text{ kg})^{1/4} \div (70 \text{ kg})^{1/4}] = 1.99 \text{ mg/kg/day} \times 0.29 = 0.58 \text{ mg/kg/day}$$

Where:

1.99 mg/kg/day = $BMDL_{04}$ for LCTs
0.29 = DAF

The CSF is calculated from the BMDL04 HED as follows

$$CSF = \text{response} \div BMDL_{04} \text{ HED}$$

$$\mathbf{CSF = 0.04 \div 0.58 \text{ mg/kg/day} = 0.07 \text{ (mg/kg/day)}^{-1}}$$

The CSF should not be used at doses > 0.58 mg/kg/day, the HED corresponding to the POD for the 4% incidence of LCTs following lifetime exposure to PFOA. The observed dose-response

relationships do not continue linearly above this level, and the fitted dose-response models better characterize the dose-response for the higher exposures.

[MDH Notes: given what is known about the TK of PFOA utilization of the BW scaling default approach in inappropriate and would result in underestimating the associated cancer risk. NJ has also derived a cancer slope factor based on increased incidence of LCTs. NJ CSF is 0.021 per mg/k-d admin dose in rats. See below.]

Since serum concentrations were not available NJ conducted BMD modeling using administered dose. The Gamma and Log-logistic models gave acceptable and similar results so the values were averaged: $BMDL/BMD_{05}$ = 2.36/4.23 mg/kg-d. For a BMR of 5% this corresponds to a cancer potency slope of 0.021 per mg/kg-d adm dose. To convert the administered dose in rats to a HED NJ utilized the the TK (half-life) differences between male rats and humans: 840 days/7 days = 120. Using this value the CSF in rats corresponds to a CSF in humans of 2.52 per mg/kg-d (0.021 per mg/kg-d x (840 days/7 days))]

NOTE: MDH conducted BMD modeling using administered dose. Results:
Using 4% (same as EPA) – $BMDL/BMD_{04}$ = 1.99/3.51 mg/kg-d
Using 5% (same as NJ) – $BMDL/BMD_{05}$ = 2.50/4.41 mg/kg-d
Using 10% - $BMDL/BMD_{10}$ = 5.15/9.06 mg/kg-d
Resulting CSF = 0.02 per mg/kg-d adm dose in rats.

The default of BW scaling is not appropriate for calculating HEDs. If the oral dose to serum concentration relationship calculated by EPA for similar admin dose levels based on Perkins et al (2004) is used to estimate corresponding serum concentrations the BMDL values of 1.99 and 2.5 mg/kg-d would correspond to serum concentrations of 80.09 and 99.73 ug/mL. Using 0.00014 Cl these serum concentrations would correspond to an HED of ~0.011 and 0.014 mg/kg-d. The resulting CSF would be 3.6 per mg/kg-d, which is similar in magnitude to NJ-based value (HED calculated using half-life differences) of 2.5 per mg/kg-d but significantly higher than EPA's BW-scaling based value of 0.07 per mg/kg-d.

*****MDH conducted BMD modeling and derived a CSF based on LCT for comparison purposes only. MDH does not feel that the existing database is sufficient to support a quantitative cancer assessment (see below for additional rationale)*****

Supporting Study Description: Basis for EPA cancer classification - The findings for cancer in humans provide support for an association between PFOA and kidney and testicular cancers; however, the number of independent studies examining each of these is limited.

The two studies conducted in laboratory animals, both in rats, support a positive finding for the ability of PFOA to be tumorigenic in one or more organs of male, but not female, rats. *[MDH Notes: female rats, unlike male rats and other species, rapidly excretes PFOA.]* There are no carcinogenicity data from a second animal species. There are some data that provide support for the hypothesis that the PPARα agonism MOA is wholly or partially linked to each of the observed tumor types. The data support a PPARα MOA for the liver tumors and thus are indicative of lack of relevance to humans. PPARα activation also could play a role in the other tumor types observed, but more data to support intermediate steps in the proposed MOAs are needed. The mutagenicity data on PFOA are largely negative, although there is some evidence for clastogenicity in the presence of microsomal activation and at cytotoxic concentrations. Given the chemical and physical properties of PFOA—including the fact that it is not metabolized, binds to cellular proteins, and carries a net negative electrostatic surface charge—the clastogenic effects are likely the result of an indirect mechanism. PFOA has the potential to interfere with the process of DNA replication because of its protein binding properties and the fact that histone proteins, spermine and spermidine, carry a net positive surface charge.

Despite the limitations in the data for the LCTs and PACTs, under the U.S. EPA Guidelines for Carcinogen Risk Assessment (USEPA 2005a) there is *suggestive evidence of carcinogenic potential* of PFOA in humans.

MDH Notes: the existing database for assessing the carcinogenic potential of PFOA is insufficient for quantitative assessment:

- Only two dose levels were assessed
- The TK of female rats is unique and therefore the existing database provides limited data for 1 sex (males) in one species (rats).
- No MOA(s) have been identified, however, PFOA is not genotoxic and a hormonal mechanism has been suggested as a potential MOA. This MOA would likely have a threshold response. In addition, the dose response from for LCT, the only response that can sufficiently be related to exposure, is nonlinear in shape. The response at 30 ppm is within historical control levels. In addition, the response observed at the highest dose level (300 ppm) is limited in magnitude (14%).
- Relevance of LCT response in rats to humans. There are several physiological differences between rats and humans that indicate rats would be significantly more sensitive to Leydig cell tumorigenesis (Cook 1999) (Steinbach 2015).

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)?
EPA 2016a: The modes of toxicological/carcinogenic action of PFOA are not clearly understood. However, available data suggest that the induction of tumors is likely due to nongenotoxic mechanism involving membrane receptor activation, perturbations of the endocrine system, and/or the process of DNA replication and cell division. PFOA lacks the ability to react with and modify DNA, although its electrostatic properties would permit interaction with chromosomal histone proteins with a net positive surface charge.

3. Is there evidence that the mode of action is not relevant to humans?
Some. PPARα has been suggested as a possible MOA for liver tumors. This MOA has been show to not lead to tumor formation (other liver effects may still occur) in humans.

4. Is there evidence of life-stage sensitivity?
No carcinogenic potency evaluations regarding early-life stages.

5. Are there structure-activity correlations available?
No

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

E. Develop a Cancer Guidance Value

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

*****Calculated for comparison purposes only*****

SF*	Cancer Guideline [ug/L]
3.6	0.029

*Enter in Slope Factor

Rounded to 0.03 ug/L ***Calculated for comparison purposes only***
Comments: Within the EPA Health Effects Document (page 4-20) it states: *"Under the EPA 2005 cancer guidelines, the evidence for the carcinogenicity of PFOA is considered suggestive because only one species has been evaluated for lifetime exposures and the tumor responses occurred primarily in males. Dose-response data are only available for the LCTs in one study. However, two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrolment of 0.024 µg/mL) and kidney and testicular cancers (Barry et al. 2013; Vieira et al. 2013). Therefore, the data on LCTs from Butenhoff et al. (2012) were modeled to provide a perspective on the magnitude of the potential cancer risk as it compares with the level of protection provided by the RfD."* This language is consistent with our derivation of a 'for comparison purposes only' values.

(Minnesota Department of Health (MDH) 2017)

Resources Consulted During 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 nvestigations in Australia." from http://www.health.nsw.gov.au/environment/factsheets/Documents/pfas-interim-health-values-ahppc.pdf.

Butenhoff, J., G Costa, C Elcombe, D Farrar, K Hansen, H Iwai, R Jung, G Kennedy Jr, P Lieder, G Olsen, P Thomford. (2002). "Toxicity of Ammonium Perfluorooctanoate in Male Cynomolgus Monkeys after Oral Dosing for 6 Months." Toxicological Sciences **69**: 244-257.

Butenhoff, J., GL Kennedy JR, PM Kinderliter, PH Lieder, R Jung, KJ Hansen, GS Gorman, PE Noker, PJ Thomford. (2004b). "Pharmacokinetics of Perfluorooctanoate in Cynomolgus Monkeys." Toxicological Sciences **82**: 394-406.

Butenhoff, J., GL Kennedy Jr., SR Frame, JC O'Connor, RG York. (2004a). "The reproductive toxicology of ammonium perfluorooctanoic (APFO) in the rat." Toxicology **196**: 95-116.

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.

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.

Cook, J., GR Klinefelter, JF Hardisty, RM Sharpe, PMD Foster. (1999). "Rodent Leydig Cell Tumorigenesis: A Review of the Physiology, Pathology, Mechanisms, and Relevance to Humans." Critical Reviews in Toxicology **29**: 169-261.

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.

DeWitt, J., CB Copeland, MJ Strynar, RW Luebke, (2008). "Perfluorooctanoic Acid-Induced Immunomodulation in Adult C57BL/6J or C57BL/6N Female Mice." Environmental Health Perspectives **116**(5): 644-650.

DeWitt, J., WC Williams, NJ Creech, RW Luebke, (2015). "Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPARa and T- and B-cell targeting." Journal of Immunotoxicology **13**(1): 38-45.

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.

Filgo, A., EM Quist, MJ Hoenerfoff, AE Brix, GE Kissling, SE Fenton. (2015). "Perfluorooctanoic Acid (PFOA)–induced Liver Lesions in Two Strains of Mice Following Developmental Exposures: PPARa Is Not Required." Toxicologic Pathology **43**: 558-568.

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). "Perfluorooctanoic Acid (PFOA) in Drinking Water. Public Consulation Draft Document. ." from http://healthycanadians.gc.ca/health-system-systeme-sante/consultations/acide-perfluorooctanoic-acid/document-eng.php.

Lau, C., JR Thibodeaux, RG Hanson, MG Narotsky, JM Rogers, AB Lindstrom, MJ Strynar. (2006). "Effects of Perfluorooctanoic Acid Exposure during Pregnancy in the Mouse." Toxicological Sciences **90**(2): 510-518.

Loveless, S., D Hoban, G Sykes, SR Frame, NE Everds. (2008). "Evaluation of the Immune System in Rats and Mice Administered Linear Ammonium Perfluorooctanoate." Toxicological Sciences **105**(1): 86-96.

Macon, M., LR Villanueva, K Tatum-Gibbs, RD Zehr, MJ Strynar, JP Stanko, SS White, L Helfant, SE Fenton. (2011). "Prenatal Perfluorooctanoic Acid Exposure in Cd-1 Mice: Low-Dose Developmental Effects and Internal Dosimetry." Toxicological Sciences **122**(1): 134-145.

Maine Center for Disease Control & Prevention. (2014). Maximum Exposure Guideline for Perfluorooctanoic Acid in Drinking Water CAS Registry Number (Free Acid): 335-67-1.
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 (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 (2017b). (Minnesota Department of Health) Background Document: Toxicokinetic Model for PFOS and PFOA and Its Use in the Derivation of Human Health-based Water Guidance Values. .

Michigan Department of Environmental Quality (2011). Human & Wildlife Toxicity Summary. Perfluorooctanoic acid (PFOA).

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.

New Jersey Department of Environmental Protection. (2007). Memorandum: Guidance for PFOA in Drinking Water at Pennsgrove Water Supply Company.

New Jersey Drinking Water Quality Institute. (2017). Health-based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA).

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

Perkins, R., JL Butenhoff, GL Kennedy, MJ Palazzolo, (2004). "13-Week Dietary Toxicity Study of Ammonium Perfluorooctanoate (APFO) in Male Rats." Drug and Chemical Toxicology **27**(4): 361-378.

Post, G., PD Cohn, KR Cooper, (2012). "Review: Perfluorooctanoic acid(PFOA), an emerging drinking water contaminant: A critical review of recent literature." Environmental Research **116**: 93-117.

Quist, E., AJ Filgo, CA Cummings, GE Kissling, MJ Hoenerhoff, SE Fenton. (2015). "Hepatic Mitochondrial Alteration in CD-1 Mice Associated with Prenatal Exposures to Low Doses of Perfluorooctanoic Acid (PFOA)." Toxicologic Pathology **43**: 546-557.

Schecter, 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.

Steinbach, T., RR Maronpot, JF Hardisty, (2015). Human Relevance of Rodent Leydig Cell Tumors. Hamilton & Hardy's Industrial Toxicology, Sixth Edition. M. B. RD Harbison, GT Johnson,, John Wiley & Sons, Inc.

Suh, C., NK Cho, CK Lee, CH Lee, DH Kim, JH Kim, BC Son, JT Lee, (2011). "Perfluorooctanoic acid-induced inhibition of placental prolactin-family hormone and fetal growth retardation in mice." Molecular and Cellular Endocrinology **337**: 7-15.

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.

Tucker, D., MB Macon, MJ Strynar, S Dagnino, E Andersen, SE Fenton, (2015). "The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6mice following perinatal perfluorooctanoic acid (PFOA) exposure." Reproductive Toxicology **54**: 26-36.

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 (EPA). 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.

USEPA. (2016a). "US Environmental Protection Agency - Office of Water. Health Effects Support Document for Perfluorooctanoic Acid (PFOA)." Retrieved May 19, 2016, from https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final-plain.pdf.

USEPA. (2016b). "US Environmental Protection Agency - Office of Water. Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA)." Retrieved May 19, 2016, from https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_health_advisory_final-plain.pdf.

USEPA. (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.

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.

White, S., JP Stanko, K Kato, AM Calafat, EP Hines, SE Fenton, (2011). "Gestational and Chronic Low-Dose PFOA Exposures and Mammary Gland Growth and Differentiation in Three Generations of CD-1 Mice." Environmental Health Perspectives **119**(8): 1070-1076.

Wolf, C., SE Fenton, JE Schmid, AM Calafat, Z Kuklenyik, XA Bryant, J Thibodeaux, KP Das, SS White, CS Lau, BD Abbott, (2007). "Developmental Toxicity of Perfluorooctanoic Acid in the CD-1 Mouse after Cross-Foster and Restricted Gestational Exposures." Toxicological Sciences **95**(2): 462-473.

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

Yahia, D., MA El-Nasser, M Abedel-Latif, C Tsukuba, M Yoshida, I Sato, S Tsuda, (2010). "Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction." The Journal of Toxicological Sciencs **35**: 527-533.

Yang, Q., Y Xie, AM Eriksson, BD Nelson, JW DePierre, (2001). "Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluoroctanoic acid in mice." Biochemical Pharmacology **62**: 1133-1140.