Oyebode A. Taiwo
Corporate Medical Director

3M Corporate Occupational Medicine

3M Center, Building 0220-06-W-08
St. Paul, MN 55144-1000 USA
Office: 651 736 2350
Mobile: 651 285 2983
Fax: 651 733 9066
Email: oataiwo@mmm.com

January 31, 2020

Washington State Department of Health
Office of Drinking Water

To Whom It May Concern:

The 3M Company (3M) appreciates the opportunity to review and provide comments on the Washington State Board of Health (Board) revisions to the Group A public water supplies rule (chapter 246-290 WAC). The revisions propose to set State Action Levels (SALs) for Perfluorbutane Sulfonic Acid (PFBS), Perfluorhexane Sulfonic Acid (PFHxS), Perfluorononanoic Acid (PFNA), Perfluorooctanoic Acid (PFOA), and Perfluorooctane Sulfonic Acid (PFOS). DOH authority for this rulemaking stems in large part from RCW 70.142.010, which authorizes the Board to establish standards for chemical contaminants in drinking water, and RCW 43.20.050(2)(a), which authorizes the Board to adopt rules for group A public water systems. Taken together these two rules require the Board to consider the best available scientific information in establishing the rules necessary to assure safe and reliable public drinking water and to protect public health.

As a science-based company, 3M has significant concerns with the proposed SALs as they do not reflect the best available science regarding these substances. These SALs are overly conservative, technically flawed, and are selectively based on other agency actions, which also relied on flawed studies. In addition, these proposed SALs are premature as the process and criteria for adopting SALs is under review and has not yet been finalized.

3M requests that DOH take the time to select the appropriate criteria to adopt SALs and revise the proposed SALs accordingly to account for the best available scientific information. The following comments are primarily on the document DOH relied on to establish the proposed SALs, the Washington Department of Health (DOH) Draft Recommended State Action Levels for PFAS in Drinking Water: Approach, Methods and Supporting Information (November 2019) (SAL Technical Document). 3M's detailed comments on this document are included as Attachment A.

I. DOH Should Not Adopt SALs Prior to Adopting the Selection Criteria

DOH proposes to establish both the process and criteria to set SALs for unregulated contaminants at the same time it proposes to establish the SALs for PFBS, PFHxS, PFNA, PFOA, and PFOS. Taking regulatory action on the proposed SALs prior to finalizing the criteria for decision making is arbitrary and capricious. DOH must base its decision to propose SALs on final criteria that has been vetted and well considered, not a draft subject to review and

modification. In making the SAL proposal prior to finalizing the SAL criteria for decision making, DOH is not able to consider the relevant factors since those factors have not been fully established.

II. The Administrative Procedures Act Requires a Cost-Benefit Analysis

We understand this proposed rule is a "significant legislative rule" according to the Washington Administrative Procedures Act (APA) such that a preliminary cost-benefit analysis must be made available upon issuance of a notice of proposed rulemaking. See RCW 34.05.328(5); RCW 34.05.320. That cost-benefit analysis must "[d]etermine that the probable benefits of the rule are greater than its probable costs, taking into account both the qualitative and quantitative benefits and costs and the specific directives of the statute being implemented." RCW 34.05.328(1)(d). The APA also requires that alternative versions of the rule be considered and that "the rule being adopted is the least burdensome alternative for those required to comply with it that will achieve the general goals and specific objectives" of the statute the rule implements. RCW 34.05.328(1)(e).

The Department of Health has not made available for public review any evaluation of alternative versions of the rule, nor has it provided a cost-benefit analysis. Failing to comply with these APA provisions makes evaluation of the appropriateness of the proposed rulemaking impossible. 3M looks forward to reviewing such analyses during the formal public comment period.

III. The SALs are Based on Assumptions About Health Effects Not Supported by the Scientific Literature

The SAL Technical Document recognizes several times that the information on PFAS, referencing the broad class of per- and polyfluoroalkyl substances, does not substantiate a causal relationship to human health effects. As an example, the document notes that the "health effects of PFAS are still under study" and there is "limited ability to measure all PFAS contaminants in water or to assess their effect on health." (SAL Technical Document at 9-10). ATSDR has also acknowledged this in its Draft Toxicological Profile for PFAS, stating that "cause and effect relationships have not been established for any of the effects, and the effects have not been consistently found in all studies." (ATSDR 2018 at 635-636). The vast body of scientific evidence does not show that PFAS cause any adverse health effects in humans at current exposure levels, or even at the historically higher levels found in blood prior to the U.S. phase out of PFAS and PFOA.

Several European studies have also supported this lack of causal connection to public health effects. A review of PFAS studies on exposed populations commissioned by the Australian Expert Health Panel concluded there is mostly limited or no evidence for any link with human disease (Expert Health Panel 2018). The report further stated that "After considering all the evidence, the Panel's advice to the Minister on this public health issue is that the evidence does not support any specific health or disease screening or other health interventions for highly exposed groups in Australia, except for research purposes." Drinking water standards and guidance levels for PFOA and PFOS set by German, Dutch, Canadian,

Swedish and Australian environmental authorities have arrived at different toxicity values and drinking water guidance levels for the same chemicals. Nonetheless, all of these drinking water guidance values are orders of magnitude higher than the SALs DOH proposes.

IV. DOH Proposes SALs that are Overly Conservative and Not Based on the Best Available Science

Overall, the proposed SALs are not derived using the best available science. There are many deficiencies and unnecessarily conservative and scientifically flawed assumptions associated with these proposed SALs. DOH must use the most accurate data in determining the accurate SAL for any PFAS chemicals. Agencies may not make decisions based on inaccurate data. An agency is required to "examine the relevant data and articulate a satisfactory explanation for its action including a rational connection between the facts found and the choice made." *Motor Vehicle Mfrs. Ass'n v. State Farm Mut. Auto. Ins. Co.*, 463 U.S. 29, 43, 103 S.Ct. 2856, 77 L.Ed.2d 443 (1983). An agency "must not act cursorily in considering the facts and circumstances surrounding its actions." *Puget Sound Harvesters Ass'n*, 157 Wash. App. 935, 951, 239 P.3d 1140 (2010). DOH must consider the deficiencies of the information it relies on, as further described herein and in the attachments.

a. The SAL for PFOA is based on a flawed ATSDR Report

The DOH proposed SAL for PFOA is based on the 2018 ATSDR Draft Toxicological Profile for PFAS. 3M has already provided comments to ATSDR that highlight the deficiencies in that document. For example, the two studies selected by ATSDR – Onishchenko et al. (2011) and Kodkela et al. (2016) - lacked fundamental scientific rigor (e.g., using a single dose study without any dose-response, small sample size with only six pregnant dams; no details on the reproductive nor the developmental hallmarks, litter bias, non-standard testing methods, no internal serum PFOA dosimetry data). Detailed comments on the deficiency of the ATSDR Report are included as Attachment B. Given these flaws and those described in Attachment B, the proposed ATSDR MRLs were not derived using best available science and do not provide adequate support for the DOH proposal.

b. The SAL for PFOS is based on a flawed study

The DOH relies on analysis by the Minnesota Department of Health (MDH) to set its proposed SAL for PFOS, but the MDH analysis relies on a flawed study, as there was a technical omission by Dong et al. (2011) that critically impacts the point of departure (POD). DOH should not accept the no observed adverse effect level (NOAEL) as the POD since the Dong et al. (2011) study related to an incomplete dataset presented in the manuscripts (see Attachment A). Had MDH used the complete dataset and performed the recommended benchmark dose modeling, the resulting $BMDL_{1SD}$ would become 3.0 mg/L, which is 21% higher than the existing NOAEL. Likewise, the resulting RfD and drinking water guidance value for PFOS would be 21% higher (which will yield 3.9 ng/kg-d as RfD and 18 ug/L as the water guidance value). Furthermore, DOH should acknowledge that because of the numerous technical deficiencies in the Dong et al. study, it does not provide any robust or compelling scientific evidence to support the claim that PFOS is associated with immune suppression in mice.

c. The SALs for PFHxS and PFBS do not reflect best available science

In determining the SALs for PFHxS and PFBS, DOH relied on the serum T4 measurement to determine thyroid impacts. However, this measurement alone does not fully represent overall thyroid function. Thyroid histology and/or serum TSH (the primary diagnostic indicator for serum thyroid hormone status) should be included in any determination of thyroid status in laboratory studies when feasible. The available rodent studies do not lead to a conclusion that the collective data supports a hazard for a thyroid effect with either PFHxS or PFBS.

In addition, with respect to PFBS, the developmental outcomes reported from the non-GLP short-term gestation exposure in mice (Feng et al. 2017) were vastly different than those reported from the full GLP two-generation study in rats by Lieder et al. (2009). The discrepancies from the short-term study need to be carefully evaluated prior to any meaningful risk assessment for humans.

V. DOH should determine SALs individually for PFAS

DOH requests comment on whether SALs for additional PFAS chemicals should be developed individually or as a group. Even a review of these five proposed SALs highlight that PFAS chemicals are all different. The proposed SALs for these five are based on a varying combination of studies and a group approach would not achieve the same result. In addition, when the selection criteria is finalized, it should be applied individually to PFAS chemicals to determine whether a SAL should be proposed and any proposed SAL should be based on scientific support individualized to that chemical.

3M appreciates the opportunity to provide comments on the proposed rule. Thank you for your consideration.

Regards,

Oyebode A. Taiwo, MD, MPH

Attachment A

3M DETAILED TECHNICAL COMMENTS

I. DRINKING WATER INGESTION RATE (page 21) AND DOH MODIFICATION OF SEVERAL PARAMETERS OF THE MDH MODEL

The Minnesota Department of Health (MDH) model (Goeden et al. 2019) relied on by DOH has never been validated with external data. It is unclear how well the model actually describes real-life situations. In addition, the model ignores correlations among inputs (e.g., between body weight and intake rate).

3M agrees with DOH that assuming exclusive breastfeeding duration through 1 year, as done by the MDH model (Goeden et al. 2019), does not represent a "reasonable maximum exposure", especially since this goes against the recommendation by American Academy of Pediatrics that solid foods should begin to be introduced to the infant at 6 months. We also agree with DOH, as is done with EPA, that a 90th percentile of water intake ingestion is more appropriate for chronic intake of water by adults, than using the 95th percentile ingestion rate from the US EPA handbook, as was done in MDH model.

II. TOXICOLOGY

a. PFOA

For PFOA, DOH deferred to the provisional assessment by ATSDR for the critical study selection, which were Onishchenko et al. (2011) and Koskela et al. (2016), its companion study. The critical effects chosen were neurobehavioral activities and skeletal alteration in offspring in mice. These critical effects were not supported by the available animal data (described in detail below) and 3M respectfully disagrees with the resulting PFOA SAL recommended by DOH. There are major technical concerns associated with these two published studies with respect to their use in any human risk assessment. They include:

1. <u>A single dose experiment cannot address (any) dose-response relationship.</u>

Albeit published five years apart, these two publications actually originated from one single study. From the same pregnant dams treated with a single dietary PFOA dose during gestation, the pups evaluated by Onishchenko et al. (2011) were litter-mates of the pups evaluated by Koskela et al. (2016). As such, it was really one study and the corresponding outcomes (from both studies) should be consolidated when discussed. In essence, there was only one PFOA dose group used in these two studies and it is impossible to interpret the experimental data reported by these authors in terms of any dose-response. Others, including the Minnesota Department of Health, echoed the same opinion in their public comments to ATSDR (MDH 2018). Considering the inherent variations in biological responses in any animal study, the

nature of a single-dose study simply does not allow any specific evaluation of any dose-and-effect responses or biological plausibility inference.

2. An uncertainty factor of 10 (LOAEL-to-NOAEL extrapolation) was not scientifically justified.

Given that there was only one PFOA dose group used, the study design did not follow the fundamental practice of toxicology testing such as evaluation of a dose-response relationship. Given the lack of any dose-response, it is scientifically impossible to establish a realistic NOAEL and/or LOAEL for the data reported. Therefore, an uncertainty factor of 10 was not scientifically justified. This opinion was also echoed by the Minnesota Department of Health in their comments to ATSDR.

In addition to the flawed experimental designs, there are major technical concerns associated with these two studies which preclude meaningful scientific interpretation of the results. These include limited sample size, lack of reproduction and developmental outcome information, pup litter selection bias, questionable dietary preparation, inadequate timing for behavior assessments, non-standard behavior assessment procedures, and absence of background data for bone morphology and bone density (see Attachment B, 3M's comments to ATSDR, for further details). Overall, the studies by Onishchenko et al. (2011) and Koskela et al. (2016) lack the scientific rigor to properly address the selected developmental endpoints and they should not be used for any human risk assessment.

b. PFOS toxicology

1. There is a technical omission by Dong et al. (2011) as it relates to incomplete data presented in the manuscript.

In reviewing the data from Dong et al. (2011), 3M made a request to Dr. Dong (the corresponding author for the study) for the actual numerical source data for IL-4 in order to conduct benchmark dose modeling. The IL-4 data (which was the critical endpoints chosen by MDH for its PFOS derivation) were only provided as a bar graph as Figure 1b in the paper by Dong et al.(2011) and it is excerpted below for the purpose of illustration.

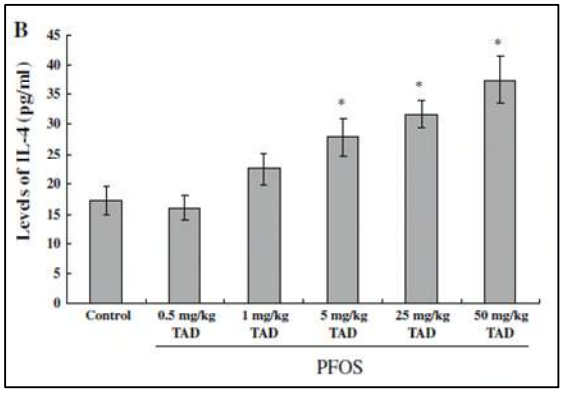


Figure 1B from Dong et al. 2011 (Arch Toxicol 85 1235-1244)

Dr. Dong graciously provided 3M with the IL-4 numerical data (as mean ± SEM), which are shown below. However, upon receiving the data for the six dose groups that were published in the paper (control, 0.5, 1, 5, 25, and 50 mg/kg TAD), there was an extra dose group (125 mg/kg TAD) that Dr. Dong provided to 3M but was not part of the published paper. When asked about the inconsistency of the dataset, Dr. Dong disclosed to 3M that one of the journal reviewers requested not to include the highest dose group data in the publication, which Dr. Dong agreed not to do. This extra dose group (125 mg/kg TAD) is provided in the table below.

Numerical Data for Serum PFOS and IL-4 Values

Total Administered Dose (TAD), mg/kg	Serum [PFOS], ug/mL		Serum [IL-4], pg/mL*	
	Mean	SEM	Mean	SEM
0 (Control)	0.05	0.01	17.25	2.32
0.5	1.07	0.11	16.04	2.07
1	2.36	0.47	22.53	2.58
5	10.75	0.82	27.89	3.11
25	22.64	2.29	31.67	2.25
50	51.71	3.81	37.42	3.94
125*	98.43*	6.70*	43.98	4.13

** Numerical data provided to 3M by Dr. G. Dong, personal communication (June 2019)*

MDH derived an RfD for PFOS from this study with the published data (e.g., six dose group). MDH used the serum PFOS concentration of 2.36 mg/L (the NOAEL from 1 mg/kg TAD dose groups) as the POD and calculated an RfD of 3 ng/kg-d. It was unclear why MDH did not perform a benchmark dose modeling for this study, as recommended by federal and state agencies such as USEPA and MDH itself. Had MDH used the complete dataset including the 125 mg/kg TAD dose group and performed a benchmark dose modeling (BMDS, Version 3.1), the resulting $BMDL_{1SD}$ would become 3.0 mg/L, which is 21% higher than the existing NOAEL. Furthermore, even with the same uncertainty factor allocation, the RfD and drinking water guidance value for PFOS would be 21% higher (which will yield 3.9 ng/kg-d as RfD and 18 ug/L as the water guidance value).

Given that DOH accepted the methodology by which MDH derived the RfD for PFOS, 3M suggests DOH contact Dr. Dong directly to confirm these above data. In addition, it is imperative for DOH to recognize that the USEPA benchmark dose modeling input requires standard deviation (SD), not standard error of mean (SEM). The values provided in the chart above are SEM and need to be converted to SD if one chooses to conduct benchmark dose modeling.

2. Evidence of immune suppression was not supported by Dong et al. (2011) data.

From a fundamental immunology perspective, there were several important technical aspects that Dong et al. (2011) did not address, and their study also lacked overall scientific validity to support the conclusion that PFOS causes immune suppression. Specifically:

- In a standard immunology study, it is imperative to assess the total number of immune cell populations among primary immune organs. Dong et al. did not measure any immune cell numbers nor did they look at / report blood lymphocyte counts, which is part of the standard CBC panel parameters. Furthermore, Dong et al. did not provide any histological evidence for thymus, spleen, or bone marrow. These are important technical omissions if immunosuppression is assumed by the authors.
- The standard clinical marker for antibody titers to vaccination is secondary IgG antibody isotype, not primary IgM. Dong et al. reported the PFOS dose-dependent reductions in serum IgM with statistical significance at higher dose groups; however, there were no statistically significantly decreases in serum IgG or IgE. In addition, the use of the SRBC-induced antibody response to measure antigen-induced antibody response is very crude and non-specific to T cell activation. Furthermore, the memory response was not properly evaluated in this study; they only challenged the animals with SRBC (antigen) once, which was insufficient to determine a memory response.
- While Dong et al. reported alterations in cytokine releases upon PFOS treatment, however, the data were solely based on splenocyte and they did not evaluate other key immune organs (such as thymus and serum) to illustrate that the responses are consistent in other primary immune organs. By way of similar scientific rationale, Dong et al. should have looked at immunoglobulin profiles in thymus and spleen as well.

For a known immunosuppressing agent, one would expect several hallmark events to occur across major key organs. Responses such as decreased IgM and IgG in spleen, thymus, and serum; and decreased cytokine levels and altered histology in these organs are often indicative of the suppressive immune responses. As discussed above, the study by Dong et al. did not provide any robust or compelling scientific evidence to support the claim that PFOS is associated with immune suppression in mice.

c. PFHxS toxicology

For PFHxS, the DOH selected the NTP 28-day repeated oral dose study in rats as the critical study. The critical effect selected was decreased serum free thyroxine (T4) levels. As described in detail below, this thyroid endpoint was not fully evaluated with the available accompanying data and 3M respectfully disagrees with the resulting PFHxS drinking water health-based value (HBV) recommended by DOH.

1. Serum free T4 alone does not fully represent the overall thyroid function.

The NTP 28-day rat study reported decreased total T4, total T3, and free T4 in serum at the end of 28 days dosing with PFHxS, however, these three endpoints alone did not provide adequate (clinical) evidence to suggest that thyroid was being affected. Specifically, thyroid histology should be included in any determination of thyroid status in rodents when terminal sacrifice is part of the study protocol because "in the rodent, thyroid gland histopathology is a more sensitive indicator of thyroid status than T3 or T4 serum hormone values." (Jahnke et al. 2004). In addition, if thyroid histology is not available, serum TSH should be used as the primary diagnostic indicator for serum thyroid hormone status (Oppenheimer et al. 1995).

2. Thyroid histology and serum TSH were normal in the NTP 28-day study.

The DOH does not explicitly recognize that thyroid histology is considered the "gold standard" for determining thyroid status, nor did it recognize that serum TSH is the primary diagnostic indicator for serum thyroid hormone status. In the NTP 28-day study, thyroid histology and serum TSH were normal. This observation is important because these studies showed a lack of dose-response in either thyroid histology and/or serum TSH with PFHxS treatment, which further suggest that thyroid was not being affected.

3. The DOH failed to recognize the critical negative bias measurement issue associated with high serum PFHxS levels.

The DOH did not sufficiently recognize the sensitivity of the assays used to measure serum thyroid hormones to the presence of compounds that can interfere and compete with thyroxine for protein bindings. In such situations, this interference can negatively bias the free T4 results when conventional analog methods are used. This is in fact the case with PFHxS and other PFAS such as perfluorobutanoate and perfluorooctane sulfonate (Chang et al. 2007; Weiss et al. 2009; Butenhoff et al. 2012a). Therefore, the workaround is to measure free T4 by *equilibrium dialysis*-based methods. This was not done in the NTP 28-day study, which was acknowledged by NTP in its report for this technical omission.

Therefore, given that there were normal TSH levels (primary diagnostic indicator for thyroid hormone status) and normal thyroid histology in these same rats (where decreased serum total T4, total T3, and free T4 were reported as measured by analog method only), collectively, these data strongly suggested that overall thyroid hormone status in these rats was normal. Based on the criteria for overall evidence to support a hazard based on animal data, these data do not lead to a conclusion that the collective thyroid data supports a hazard for a thyroid effect.

d. PFBS toxicology

For PFBS, the DOH deferred to the provisional toxicity assessment by USEPA for the critical study selection, which was a mouse developmental study by Feng et al. (2017). The

critical effect selected was decreased serum total thyroxine (T4) levels in newborn mice. As described in detail below, this thyroid endpoint was not fully evaluated with the available accompanying data and 3M respectfully disagrees with the resulting PFBS drinking water SAL recommended by DOH. 3M's key technical comments include:

1. Serum total T4 levels primarily are the biologically inactive T4 and it does not represent the overall thyroid function.

In this gestation exposure study in mice with PFBS, Feng et al. (2017) reported decreased total T4, decreased total T3 (triiodothyronine), and normal TSH in serum at birth for female pups. However, decreased total T4 and T3 alone did not provide adequate (clinical) evidence to suggest that thyroid was being affected. Serum total T4 and total T3 measurements are measurements of largely (> 99.5%) inactive thyroid hormones and they alone do not represent functional aspects of the thyroid (Oppenheimer et al. 1995). As stated earlier, thyroid histology should be included in any determination of thyroid status in rodents when terminal sacrifice is part of the study protocol because "in the rodent, thyroid gland histopathology is a more sensitive indicator of thyroid status than T3 or T4 serum hormone values" (Jahnke et al. 2004). In addition, if thyroid histology is not available, serum TSH should be used as the primary diagnostic indicator for serum thyroid hormone status (Oppenheimer et al. 1995).

2. Serum TSH is normal.

DOH does not explicitly recognize that the serum TSH is the primary diagnostic indicator for serum thyroid hormone status. Again, in the study by Feng et al. (2017), total T4 and total T3 alone did not provide adequate (clinical) evidence to suggest that thyroid was being affected, especially when TSH, the primary diagnostic indicator for thyroid hormone status was normal.

3. Feng et al. (2017) did not provide adequate information to allow a full interpretation of thyroid status.

Albeit terminal necropsies were performed in this study, it was unclear why there were no thyroid histology data reported for either dams or offspring. In addition, on the thyroid-related parameters, there were no TRH mRNA or serum FT4 measured in offspring even though it was done for dams.

4. The observations from Feng et al. (2017) study need to be validated.

There was a total of eight individual serum hormones measured and reported by Feng et al. (2017) based on the blood samples collected from the newborn mice; and each of the hormones was measured using the commercial ELISA kits obtained from USCN Life Science Inc., as described in the paper. According to the manufacturer's information (see https://www.cloud-clone.us), each ELISA kit requires 50 uL of serum sample volume. Given that a newborn mouse pup is quite small in size (approximately 1 gram), it is not clear how Feng et al. was able to measure all the hormones with such a limited blood volume. To better understand this, 3M consulted with Charles River Laboratories who concluded that, if they were to repeat the Feng et al. study, at least 75 dams per dose group would have been needed to

achieve the blood sample volume required for the specified hormone measurements. Feng et al. only had 30 dams per dose group.

5. <u>The discrepancies between mouse and rat developmental data need to be addressed.</u>

The developmental endpoints from the short-term gestation exposure study in mice by Feng et al. (2017) were vastly different than the outcomes from the full 2-generation study in rats by Lieder et al. (2009). These differences need to be properly assessed before a scientific conclusion can be made. Key observations included:

- Effects reported by Feng et al. lacked dose-responses; the effects from 200 mg/kg-d were usually similar in magnitude to 500 mg/kg-d.
- The study design and PFBS dosing regimen by Lieder et al. (2-generation in rats) was more rigorous than Feng et al. (gestational only in mice) in terms of treatment duration, doses, as well as direct treatments to developing fetuses and pups during sensitive life stages, see Table 4 below for comparison.

			Lieder et al. 2009	Feng et al. 2017
Species			Sprague Dawley rats	ICR mice
Test guideline			OECD 416 / OPPTS 870.3800 (2-gen)	None
GLP			Yes	No
Daily K^+PFBS treatments (direct gavage)	Daily doses		30, 100, 300, 1000	50, 200, 500
	P-generation	Pre-mating, males	Yes, 70 days	No
		Pre-mating, females	Yes, 70 days	No
		Gestation, dams	Yes	Yes
		Lactation, dams	Yes	No
	F1-generation pups (before mating)	Weaning and on	Yes, ≥ 70 days	No

- It was not clear why Feng et al. did not include male offspring in their evaluation.
- The female mouse offspring in the Feng et al. study were not directly dosed with K^+PFBS, however, the reported myriad of adverse developmental outcomes occurred in these female mouse pups (e.g., reduced body weight and changes in reproductive organ morphology). In contrast, female rat offspring (from Lieder et al. 2009) were not only exposed to PFBS during gestation and lactation, they were also directly dosed with PFBS (at higher dose levels than the Feng et al. study) after weaning and into their adulthood. There were no developmental effects noted in the female rat pups in Lieder et al. study.
- Regarding the alterations in ovary and uterus-related data, as reported by Feng et al., there were several technical details not provided by the study authors which precluded a meaningful interpretation of the data. They include:
    - Evaluation was reported for female pups at PND 60 only, not on PND 30 and not for dams (who were directly dosed with PFBS).

- "Impaired" development reported by Feng et al. was based on decreased surface area (on microscopic slides) and limited morphological measurements. Surface area can be also attributed from different sectioning location (of the tissue). Feng et al. did not address how this was controlled among different animals. In addition, Feng et al. only provided relative organ-to-body weight data. There were no absolute organ weight data for the readers to interpret. Organ-to-brain weight data were not presented either.
- Feng et al. did not take body weight into consideration when interpreting estrous cycle data which is unfortunate because they are related (Bermejo-Alvarez et al. 2012).
- In Feng et al. (2017), albeit there were changes in female reproductive organ morphology, functional aspects of reproduction appeared not to be affected according to study authors (i.e., maternal body weight, maternal body weight-gain, and various pregnancy outcomes).

6. <u>The DOH should use $BMD_{0.4SD}$, not $BMDL_{20}$, to determine POD if T4 is continued to be used as the critical endpoint.</u>

In EPA's draft assessment for PFBS, a benchmark response (BMR) of 20% relative deviation (i.e., dose that results in a 20% reduction of mean T4) was used to derive a $BMDL_{20}$ value. 3M respectfully disagrees with the selection of T4 as well as a $BMDL_{20}$ value based on the assumption of a continuous dataset, which, in itself was inconsistent with EPA's past practices with many other compounds.

A better alternative analysis for consideration requires a different dose-response model and a definition of the BMR using standard deviation (SD). This is fully explained by 3M and Mr. Bruce Allen who is a biostatistician and consultant to both EPA and 3M. 3M's entire written comments to EPA, which included Mr. Allen's detailed explanation as an appendix, are attached in this report (see Appendix I). According to Mr. Allen, the POD estimate would yield a $BMDL_{0.4SD}$ value of 8.3 mg/kg-d, which is approximately two-fold higher than the current POD (4.2 mg/kg-d). Correspondingly, the PFBS HBV should be raised by a factor of two to 840 ng/L (420 ng/L x 2 = 840 ng/L). We strongly recommend to the state of Washington to thoroughly understand the reasoning behind Mr. Allen's recommendation.

III. Epidemiology:

<u>Page 27 (PFOA and kidney cancer)</u>

The DOH stated that PFOA exposure was positively associated with kidney and testicular cancer in a large epidemiological study (C8 Health Project). However, the DOH failed to cite several studies that conflict with such an association with kidney cancer in occupational and toxicological studies. These other studies had too few testicular cancers to allow a full evaluation.

For kidney cancer, DOH cites two studies from the C8 Science Panel (Barry et al. 2013; Viera et al. 2013). However, these two studies overlapped each other as far as case ascertainment and in the Barry et al. study, there was, in fact, not a statistically significant trend for kidney cancer ($p > 0.1$). Not mentioned by DOH is another C8 Science Panel study by Steenland and Woskie (2012) that was a DuPont worker cohort mortality study that examined for evidence of an association with kidney cancer and estimated PFOA exposure; however, they only examined kidney cancer mortality – not incidence. At this DuPont plant, PFOA (ammonium salt) was used as a processing aid in the polymerization of tetrafluoroethyene (TFE) to make polytetrafluoroethylene (PTFE). TFE is a known renal carcinogen in rats. Steenland and Woskie chose not to consider the potential confounding exposure of tetrafluoroethylene (TFE) at this DuPont West Virginia plant due to the explosive nature of TFE. DOH should appreciate the fact that the lower explosion limit for TFE is 110,000 ppm (ACGIH, 2001; Olsen, 2015). The 8-hour time weighted average for worker exposure to TFE is 2 ppm. Thus, TFE exposure would have occurred at the DuPont plant but considerably below the lower explosion limit. Therefore, the potential confounding effect of TFE exposure was not considered by Steenland and Woskie (2012). Furthermore, DOH should be aware of Consonni et al. (2013), who concluded they could not "disentangle' the association between TFE and PFOA in their multiple plant cohort mortality study of TFE exposures (which included the DuPont West Virginia plant). The Barry et al. (2013) study did not find an association with kidney cancer incidence in a subset of DuPont workers from the C8 Science Panel community worker cohort study. Furthermore, DOH did not cite the other major cancer incidence study that involved PFOA manufacturing workers at a 3M plant in Cottage Grove, Minnesota (Raleigh et al. 2014). PFOA manufactured at this plant was sold to the DuPont plant. Raleigh et al. studied both kidney cancer mortality and incidence at the 3M Cottage Grove PFOA manufacturing plant that had a near absence of exposure to TFE (unlike the DuPont worker population). Raleigh et al. did not find an increase in kidney cancer incidence with increasing categorical exposures of PFOA. The Raleigh et al. study reported hazard ratios (HR) of 1.07, 1.07, 0.98, and 0.73 for increased quartiles of PFOA exposure at this plant compared to a reference group of a similar sized nearby 3M worker population that was non-occupationally exposed to PFOA (HR reference = 1.0). DOH should also note that an excess of kidney cancer has <u>not</u> been reported in three 2-year bioassays of PFOA exposure in rats (Biegel et al., 2001; Butenhoff et al., 2012; NTP 2019).

<u>Page 32 (PFOA and fetal growth)</u>

On page 32, DOH cites a systematic review of fetal growth by Johnson et al (2014). DOH indicates this review concluded there was a sufficient evidence that PFOA reduced fetal growth in humans through a meta-analysis of 9 epidemiological studies. The systematic review by Johnson et al. (2014) also concluded there was insufficient evidence that lower glomerular filtration rate (GFR) could explain some of the associations between low birth weight in humans and higher serum PFOA observed in epidemiological studies. However, subsequent to this publication by Johnson et al. (2014) and their colleagues [Lam et al. (2014), and Vesterinen (2014)], research by Morken et al. (2014) and the PBPK model/Monte Carlo simulation models developed by Verner et al. (2015) indicated there was, indeed, an association between GFR and fetal growth as well as the confounding of GFR that occurs in the association between fetal growth and measured PFOA concentrations. Verner et al. concluded such confounding could be upwards of 50 percent. More importantly, this association between fetal growth and maternal

measurement of PFOA was seen only in the second and third trimesters in the simulation models by Verner et al, not the first trimester, likely because the effect of GFR would be subsequent of plasma volume expansion that occurs in the first trimester.

Recognizing these findings by Verner et al., Steenland et al. (2018) subsequently conducted a meta-analysis of 24 epidemiologic studies – 15 more than done by Johnson et al. (2014). They stratified their results as to whether the maternal PFOA concentration was measured in the first or the combined second and third trimesters. Steenland et al. reported with first trimester measurements of maternal PFOA, there was a non-statistically significant -3.3 gram (95% CI -9.6, 3.0) reduction in birthweight per ng/mL PFOA. When PFOA was measured second/third trimester, there was a statistically significant -17.8 gram reduction (95 CI -25.0, -10.6) in birthweight per ng/mL PFOA. Steenland et al. (2018) concluded "restriction to studies with blood sampling conducted early in pregnancy or shortly before conception showed little or no association such that these results are consistent with confounding and /or reverse causation being responsible for the inverse association seen in studies with low background exposure levels and blood sampling conducted later in pregnancy, when confounding and/or reverse causality are likely to be more important."

Subsequent to the Steenland et al. (2018) meta-analysis, other studies have been, and will continue to be published regarding associations about fetal growth and the timing of measurements of PFAS, including those studies by Buck et al. (2018), Buck Louis et al. (2018), Manzano-Salgado et al. (2017), Marks et al. (2019), Meng et al. (2018), Shoaff et al. (2018), and Starling et al. (2017). Thus, DOH needs to acknowledge the association reported between fetal growth (few gram reduction) per ng/mL PFOA is likely not causal but rather consistent with confounding and/or reverse causation via GFR.

Page 42 (PFOS and pubertal development in children)

DOH cites several studies with inconsistent results pertaining to pubertal development in children. Not cited by DOH was an important analysis by Wu et al. (2015) who developed a Monte Carlo physiologically-based pharmacokinetic model of PFAS to simulate plasma PFAS levels in a hypothetical female population aged 2 to 20 years old. Physiological parameters as well as timing of growth spurts and menarche were incorporated in the model. Simulated data pertaining to the PFAS level and delayed menarche were compared to the epidemiological association reported in the literature. The delay of menarche in days per natural log increase in PFAS concentrations in the simulated data were about one third as large as the observed values. The authors concluded that the relationship between PFAS and age at menarche was at least partly explained by pharmacokinetics rather than a toxic effect.

REFERENCES

ACGIH (2001). Documentation of the Threshold Limit Values and Biological Exposure Indices.

ATSDR (2018). Toxicological profile for perfluoroalkyls (draft). https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf.

Biegel, L. B., M. E. Hurtt, S. R. Frame, J. C. O'Connor and J. C. Cook (2001). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicol Sci 60(1): 44-55

Buck, C. O., Eliot, M. N., Kelsey, K. T., Calafat, A. M., Chen, A., Ehrlich, S., Lanphear, B. P., and Braun, J. M. (2018). Prenatal exposure to perfluoroalkyl substances and adipocytokines: the HOME Study. Pediatr Res.

Buck Louis, G. M., Zhai, S., Smarr, M. M., Grewal, J., Zhang, C., Grantz, K. L., Hinkle, S. N., Sundaram, R., Lee, S., Honda, M., Oh, J., and Kannan, K. (2018). Endocrine disruptors and neonatal anthropometry, NICHD Fetal Growth Studies - Singletons." Environ Int 119: 515-526.

Butenhoff, J. L., J. A. Bjork, S. C. Chang, D. J. Ehresman, G. A. Parker, K. Das, C. Lau, P. H. Lieder, F. M. van Otterdijk and K. B. Wallace (2012a). Toxicological evaluation of ammonium perfluorobutyrate in rats: Twenty-eight-day and ninety-day oral gavage studies. Reprod Toxicol 33(4): 513-530.

Butenhoff, J. L., G. L. Kennedy, Jr., S. C. Chang and G. W. Olsen (2012b). Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. Toxicology 298(1-3): 1-13.

Chang, S. C., J. R. Thibodeaux, M. L. Eastvold, D. J. Ehresman, J. A. Bjork, J. W. Froehlich, C. S. Lau, R. J. Singh, K. B. Wallace and J. L. Butenhoff (2007). Negative bias from analog methods used in the analysis of free thyroxine in rat serum containing perfluorooctanesulfonate (PFOS). Toxicology 234(1-2): 21-33.

Consonni, D., K. Straif, J. M. Symons, J. A. Tomenson, L. G. van Amelsvoort, A. Sleeuwenhoek, J. W. Cherrie, P. Bonetti, I. Colombo, D. G. Farrar and P. A. Bertazzi (2013). Cancer risk among tetrafluoroethylene synthesis and polymerization workers. Am J Epidemiol 178(3): 350-358.

Dong, G-H., M-M. Liu, D. Wang, L. Zheng, Z-F. Liang, Y-H. Hin. (2011). Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. Arch Toxicol 85:1235-1244.

Expert Health Panel for Per and Poly-Fluoroalkyl Substances (2018). Report to the Australian Minister of Health.

Goeden, H.M., C.W. Greene, J.A. Jacobus. (2019). A transgenerational toxicokinetic model and its use in derivation of Minnesota PFOA water guidance. J Expos Sci Environ Epidemiol 29:183-195.

IARC (2017). "IARC monographs on the evaluation of carcinogenic risks to humans. Some chemicals used as solvents and polymer manufacture." Vol. 110. IARC Press, Lyon. http://monographs.iarc.fr/ENG/Monographs/vol110/mono110.pdf.

Jahnke, G. D., N. Y. Choksi, J. A. Moore and M. D. Shelby (2004). Thyroid toxicants: assessing reproductive health effects. Environ Health Perspect 112(3): 363-368.

Johnson, P. I., Sutton, P., Atchley, D. S., Koustas, E., Lam, J., Sen, S., Robinson, K. A., Axelrad, D. A., and Woodruff, T. J. (2014). The Navigation Guide-Evidence-Based medicine meets

environmental health: systematic review of human evidence for PFOA effects on fetal growth. Environ Health Perspect 122(10): 1028-1039.
Koskela, A.H., M.A. Finnilä, M. Korkalinen, S. Spulber, J. Koponen, J. Häkansson, J. Tuukkanen, M. Vilukesela. (2018). Eeffects of developmental exposure to perflorooctanoic acid (PFOA on long bone morphology and bone cell differentiation. Toxicol Appl Pharmacol 301:14-21.
Lam, J., Koustas, E., Sutton, P., Johnson, P. I., Atchley, D. S., Sen, S., Robinson, K. A., Axelrad, D. A., and Woodruff, T. J. (2014). The Navigation Guide - evidence-based medicine meets environmental health: integration of animal and human evidence for PFOA effects on fetal growth. Environ Health Perspect 122(10): 1040-1051.
Lieder, P. H., R. G. York, D. C. Hakes, S. C. Chang and J. L. Butenhoff (2009). A two-generation oral gavage reproduction study with potassium perfluorobutanesulfonate (K+PFBS) in Sprague Dawley rats. Toxicology 259(1-2): 33-45.
Manzano-Salgado, C. B., Casas, M., Lopez-Espinosa, M. J., Ballester, F., Iniguez, C., Martinez, D., Costa, O., Santa-Marina, L., Pereda-Pereda, E., Schettgen, T., Sunyer, J., and Vrijheid, M. (2017). Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort. Environ Int 108: 278-284.
Marks, K. J., Cutler, A. J., Jeddy, Z., Northstone, K., Kato, K., and Hartman, T. J. (2019). Maternal serum concentrations of perfluoroalkyl substances and birth size in British boys. Int J Hyg Environ Health.
Meng, Q., Inoue, K., Ritz, B., Olsen, J., and Liew, Z. (2018). Prenatal exposure to perfluoroalkyl substances and birth outcomes; An updated analysis from the Danish National Birth Cohort. Int J Environ Res Public Health 15(9).
Morken, N. H., Travlos, G. S., Wilson, R. E., Eggesbo, M., and Longnecker, M. P. (2014). Maternal glomerular filtration rate in pregnancy and fetal size. PLoS One 9(7): e101897.
NTP Technical Report on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CAS No. 335-67-1) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats Technical Report 598 (2019). https://ntp.niehs.nih.gov/ntp/about_ntp/trpanel/2019/december/tr598draft.pdf
Olsen, G., W. PFAS biomonitoinr in higher exposed populations. (2015). (In) Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. (ed) DeWitt, J.C. Humana Press:New York
Onishchenko, N., C. Fischer, W. Norhamidah, W. Ibrahim, S. Negri, S. Spulber, D. Cottica, S. Ceccatelli. (2011). Prenatal exposure to PFOS or PFOA alters motor function in mice in a sex-related manner. Neurotox Res 19:452-461.
Oppenheimer, J. H., A. L. Schwartz and K. A. Strait (1995). An integrated view of thyroid hormone actions in vivo. Molecular Endocrinology: Basic Concepts and Clinical Correlations. B. D. Weintraub. New York, Raven Press, Ltd.: 249-265.
Raleigh, K. K., B. H. Alexander, G. W. Olsen, G. Ramachandran, S. Z. Morey, T. R. Church, P. W. Logan, L. L. Scott and E. M. Allen (2014). Mortality and cancer incidence in ammonium perfluorooctanoate production workers. Occup Environ Med 71(7): 500-506.
Shoaff, J., Papandonatos, G. D., Calafat, A. M., Chen, A., Lanphear, B. P., Ehrlich, S., Kelsey, K. T., and Braun, J. M. (2018). Prenatal Exposure to Perfluoroalkyl Substances: Infant Birth Weight and Early Life Growth. Environ Epidemiol 2(2).
Starling, A. P., Adgate, J. L., Hamman, R. F., Kechris, K., Calafat, A. M., Ye, X., and Dabelea, D. (2017). Perfluoroalkyl substances during pregnancy and offspring weight and adiposity at

birth: Examining mediation by maternal fasting glucose in the Healthy Start Study. Environ Health Perspect 125(6): 067016.
Steenland, K., V. Barry and D. Savitz (2018). Serum perfluorooctanoic acid and birthweight: An updated meta-analysis with bias analysis. Epidemiology 29(6): 765-776.
Steenland, K. and S. Woskie (2012). "Cohort mortality study of workers exposed to perfluorooctanoic acid." Am J Epidemiol 176(10): 909-917.
Verner, M. A., A. E. Loccisano, N. H. Morken, M. Yoon, H. Wu, R. McDougall, M. Maisonet, M. Marcus, R. Kishi, C. Miyashita, M. H. Chen, W. S. Hsieh, M. E. Andersen, H. J. Clewell and M. P. Longnecker (2015). Associations of perfluoroalkyl substances (PFAS) with lower birth weight: An evaluation of potential confounding by glomerular filtration rate using a hysiologically based pharmacokinetic model (PBPK). Environ Health Perspect 123(12): 1317-1324.
Vesterinen, H. M., Johnson, P. I., Atchley, D. S., Sutton, P., Lam, J., Zlatnik, M. G., Sen, S., and Woodruff, T. J. (2015). Fetal growth and maternal glomerular filtration rate: a systematic review. J Matern Fetal Neonatal Med 28(18): 2176-2181.
Vieira, V. M., Hoffman, K., Shin, H. M., Weinberg, J. M., Webster, T. F., and Fletcher, T. (2013). Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. Environ Health Perspect 121(3): 318-323.
Weiss, J. M., P. L. Andersson, M. H. Lamoree, P. E. Leonards, S. P. van Leeuwen and T. Hamers (2009). Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicol Sci 109(2): 206-216.
Wu, H., M. Yoon, M.-A. Verner, J. Xue, M. Luo, M. E. Andersen, M. P. Longnecker and H. J. Clewell III (2015). Can the observed association between serum perfluoroalkyl substances and delayed menarche be explained on the basis of puberty-related changes in physiology and pharmacokinetics? Environ Int 82: 61-68.

Appendix I:

3M's written comments to USEPA on its draft toxicity value for PFBS, January 2019

Oyebode A. Taiwo
Corporate Medical Director

3M Corporate Occupational Medicine

3M Center, Building 0220-06-W-08
St. Paul, MN 55144-1000
651 736 2350 Office
651 285 2983 Cell
651 733 9066 Fax
oataiwo@mmm.com

Docket HQ- OW-2018-0614
US Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington, D.C. 20460

Re: Request for Public Review and Comment: Draft Human Health Toxicity Assessment for Perfluorobutane Sulfonic Acid (PFBS) and Related Compound Potassium Perfluorobutane Sulfonate; Docket ID No. EPA-HQ-OW-2018-0614

To Whom It May Concern:

The 3M Company (3M) appreciates this opportunity to provide comments to the U.S. Environmental Protection Agency (EPA) regarding its Draft Human Health Toxicity Assessment for Perfluorobutane Sulfonic Acid (PFBS) and Related Compound Potassium Perfluorobutane Sulfonate. As a science-based company, 3M encourages EPA to use the best available science when assessing PFBS and other chemicals. As our comments reflect, 3M has substantial experience and expertise regarding PFBS, informed in part by the fact that 3M scientists are authors or contributors to many of the studies referenced by the EPA.

The following offers 3M's thoughts responsive to EPA's request for comments regarding PFBS. Please let us know if you have any questions.

Regards,

Oyebode A. Taiwo, MD, MPH

Executive Summary

The 3M Company (3M) appreciates the opportunity to review and comment on EPA's Draft Human Health Toxicity Values for Perfluorobutane Sulfonic Acid and Related Compound Potassium Perfluorobutane Sulfonate (Draft PFBS Document). As authors or a sponsor of many of the human epidemiology and toxicology studies discussed in the Draft PFBS Document, we offer these detailed comments to assist with EPA's effort.

3M Summary Comment No. 1 - PFBS Exposure to the General Population is Minimal

Ever since 2007-2008 including the 2013-2014 biomonitoring cycle, CDC National Health and Nutrition Examination Survey (NHANES) has determined that the 95th-percentile is at the limit of detection (0.1 ng/mL) for Perfluorobutane sulfonate (PFBS). This has led CDC NHANES to not include the measurement of PFBS in its latest biomonitoring cycle (2015 – 2016). The Draft PFBS Document cites the CDC NHANES findings through 2014, but omits the CDC NHANES decision not to include the measurement of PFBS in its latest biomonitoring cycle. This decision by CDC NHANES strongly suggests that the US general population has minimal exposure to PFBS based on CDC NHANES analytical methods. Therefore, EPA should have included this important point about the lack of human exposure based on NHANES data in the Draft PFBS Document.

3M Summary Comment No. 2 - The Data Does Not Support a PFBS Thyroid Effects Hazard

3M disagrees with EPA's conclusion that "evidence in animals for thyroid effects *supports a hazard."* Given the available data that have been evaluated, there is sufficient uncertainty to conclude that PFBS cannot be categorized as "supports a hazard" for thyroid effects.

Thyroid histology should be included in any determination of thyroid status in rodents when terminal sacrifice is part of the study protocol because "in the rodent, thyroid gland histopathology is a more sensitive indicator of thyroid status than T3 or T4 serum hormone values." (see NTP-sponsored Thyroid Toxicant Workshop on chemical-induced thyroid dysfunction in experimental animals and its relevance to humans on reproductive and developmental effects: Jahnke et al. 2004, Environ Health Perspect 112 363-368). The Draft PFBS Document does not explicitly recognize that thyroid histology is considered the "gold standard" for determining thyroid status; nor did it recognize that serum TSH is the primary diagnostic indicator for serum thyroid hormone status (Oppenheimer et al 1995 Mol Endo Bas Conc Clin Corr 249-268). Three of the five thyroid studies cited by the Draft PFBS Document assessed and reported thyroid histology. Thyroid histology was normal in each of these studies when performed. Two of the five thyroid studies cited by the Draft PFBS Document assessed and reported serum TSH values. Serum TSH values were normal without dose-response in each of these studies when performed.

The Draft PFBS Document also does not sufficiently recognize the sensitivity of the assays used to measure serum thyroid hormones to the presence of compounds that can interfere and compete with thyroxine for protein bindings. In such situations, this interference can negatively bias the free T4 results when conventional analog methods are used. This is in fact the case with PFBS and other PFAS such as perfluorobutanoate and perfluorooctane sulfonate

(Chang et al. 2007 Toxicology 234 21-33; Weiss et al. 2009 Toxicol Sci 109 206-216; Butenhoff et al. 2012 Reprod Toxicol 33 513-530). Therefore, the workaround is to measure free T4 by *equilibrium dialysis*-based methods. This was not done in the thyroid assessment studies relied upon by the Draft PFBS Document, nor did any of the peer reviewers or EPA mentioned this very important issue with PFBS. Furthermore, total T4 is an assay that represents primarily biologically inactive T4. Thus, the total T4 and the analog free T4 do not provide sufficient or definite answers as to thyroid effects. Because of the resulting questionable confidence in the analog assays, thyroid histology should be used as the gold standard to determine whether there was a thyroid effect. The thyroid histology was normal as reported in the NTP study, as well as in both 28-day (3M 2001) and 90-day studies (Lieder et al. 2009a). Although terminal sacrifices were done, no thyroid histology was reported by Feng et al. (2017).

Based on the criteria for overall evidence integration judgments to support a hazard based on animal data (Table 3, page 16 of the EPA Draft PFBS Document), the summarized information (see below, Table 1 and Table 2) from these five studies does not lead to a conclusion that the collective thyroid data "supports a hazard" for a thyroid effect.

Table 1

			3M 2001	**Lieder et al. 2009a; York 2003**	**Lieder et al. 2009b; York 2003**	**Feng et al. 2017**	**NTP, 2011; 2018**
			28-day	90-day	2-generation	Developmental screening	28-day
Thyroid weight			Normal	Not performed	Not performed	Not reported	Normal
Thyroid histology			Normal	Normal	Not performed	Not reported	Normal
Serum thyroid hormones	Biologically active	TSH	Not performed	Not performed	Not performed	Normal for F1 pups on PND1	Normal
		Free T4 by equilibrium dialysis (gold standard)	Not performed	Not performed	Not performed	Not performed	Not reported[a]
		Free T4 by analog	Not performed	Not performed	Not performed	Not performed	Reported (decreased with dose-response)
	Biologically inactive	Total T4 by analog	Not performed	Not performed	Not performed	Reported (decreased, but questionable dose-response)	Reported (decreased with dose-response)
Evidence of compromised thyroid morphology and compensatory feedback response (between TSH and free T4 by equilibrium dialysis)			No	No	No	No	No

[a] Highly unlikely done given analytical complexity

Table 2	
EPA's criteria for "supports a hazard" (Table 3 on Page 16 of the EPA Draft PFBS Document)	**3M's response**
The evidence for effects is consistent or largely consistent in at least one high- or medium-confidence experiment.[a] Although notable uncertainties across studies might remain, any inconsistent evidence or remaining uncertainties are insufficient to discount the cause for concern from the positive experiments. In the strongest scenarios, the set of experiments provide evidence supporting a causal association across independent laboratories or species. In other scenarios, including evidence for an effect in a single study, the experiment(s) demonstrate additional support for causality such as coherent effects across multiple related endpoints; an unusual magnitude of effect, rarity, age at onset, or severity; a strong dose-response relationship; and/or consistent observations across exposure scenarios (e.g., route, timing, or duration), sexes, or animal strains.	There was no evidence of compromised thyroid morphology and compensatory feedback response (measurement of TSH in conjunction with measurement of free T4 by equilibrium dialysis). It is a scientific weakness to offer any interpretation of the results from these studies given the known negative bias associated with PFAS in analog free T4 measurements. Thus, the lack of measurements of free T4 by equilibrium dialysis by these studies is more than just "notable uncertainties across (the) studies… insufficient to discount the cause for concern from the positive experiments" as so stated in the EPA Draft PFBS Document (see left). The gold standard for measuring thyroid effects is histological evaluation of the thyroid gland. Thyroid histology was normal when all such evaluations were reported. The above summarized information from these five studies does not lead to a conclusion that the collective thyroid data "supports a hazard" for thyroid effects.

3M Summary Comment No. 3 - Concerns with EPA's Model Selection for Thyroid Effects

In addition to 3M's concern that the five thyroid studies evaluated by EPA do not "support a hazard" for a thyroid effect, there are technical concerns with EPA's model selection process for thyroid effect. EPA considered model selection based on model fit (e.g., AIC) and model prediction (e.g., $BMDL_{20}$). 3M retained an independent modeling expert (Bruce Allen) to review EPA's model selection process for thyroid effect. Mr. Allen concluded that EPA should not have used model prediction as a measure for the evaluation of the model fit (see Mr. Allen's report attached in Appendix A). As Mr. Allen wrote in his comments, "The predictions are what get selected, not the basis for that selection process."

Provided below are four important findings from Mr. Allen's review on EPA's model selection process.

Finding 1 – EPA's Model Selection Approach was Inappropriate: On page F-4 the EPA wrote, "Among all models providing adequate fit, the BMDL from the model with the lowest Akaike's information criterion (AIC) was selected as a potential POD when BMDL values were sufficiently close (within threefold)." Mr. Allen clearly demonstrated (see page 2 of Appendix A) the error of this logic in that a model prediction (an estimate of BMD or BMDL) "has no bearing on how well the model(s) fit the data . . . Predictions are what get selected;

they are not the basis for that selection process." Of the models considered by EPA (see Table F-2, page F5, Draft PFBS Document), the EPA selected the Exp-M4. This is not the best fitted model from an AIC perspective. Nonetheless, EPA selected it because the BMDL response was 3-fold lower. According to Mr. Allen, using a prediction as a selection measure of model fit "makes no sense." He indicated that instead, the Exp-M2 model would be the better fitting model because of the lower AIC value. He further stated that the EPA would be better served by using a weighted average of the BMDLs from each model with weights for that average equal to exp(-AIC).

Finding 2 - The BMD_{1SD} Results Should Have Been Used to Determine Points of Departure (POD) Based on the T4 endpoint: Regardless of the model choice (see Finding 1), the EPA used a BMR of 20% relative deviation (i.e., dose that results in a 20% reduction of mean T4) to derive a $BMDL_{20}$ value. The selection of a $BMDL_{20}$ value using continuous data is inconsistent with EPA's past practices with many other compounds. More importantly, it is especially inconsistent with the use of a POD based on a $BMDL_{10}$ from the dichotomous data modeled by EPA from the Lieder et al. study related to papillary tubular/ductal epithelium hyperplasia in female rats. The latter POD is based on an extra risk of 10%. The former is based on the magnitude of mean T4 change. Thus, according to Mr. Allen (see pages 3-4, Appendix A) the EPA should calculate the change in the mean T4 that will give the target 10% extra risk of low T4 in terms of the standard deviation (1.1*SD) if 1% of the unexposed population has a low T4 as was assumed by EPA in this particular analysis (see Crump et al. 1995, Risk Analysis 15:79-89). The BMD_{1SD} model better reflects this for continuous data by incorporating a conservative rounding down from 1.1SD to 1SD. Taking into account Findings 1 and 2, Mr. Allen suggests the EPA should have considered the POD (23.4 mg/kg-d) for a $BMDL_{1SD}$ from the Exp-M2 model (ignoring any model-averaging process).

Finding 3 - EPA Presents Only Weak Support for a $BMDL_{20}$ as a Biologically Based Benchmark Response Level. The EPA assessment relied primarily on 3 studies in support of the $BMDL_{20}$ estimates based on their written material (see page 55-56, Draft PFBS Document): a) a study with a 25% decrease in maternal T4 during second trimester; b) thyroid insufficiency in women below the 10^{th} percentile; and c) decreases in mean T4 of 10 – 17 percent that have elicited neurodevelopmental toxicity in rats. Using these examples, Mr. Allen concludes it is not possible to make consistent probabilistic statements without taking SD into consideration. Merely only examining relative deviations from the mean is not sufficient.

Finding 4 – A Better Alternative Analysis is Available: Mr. Allen suggested a better alternative analysis for EPA's consideration that involved a different dose-response model and a definition of the BMR using a SD approach. This involved the biological point discussed in Finding 3 (thyroid insufficiency in pregnant women defined as having T4 levels

below the 10th percentile for the study population). Assuming this background rate and specifying the BMD to be the dose that gives a 10% extra risk above the background (consistent with the analysis of a dichotomous endpoint), Crump (Risk Analysis 1995;15:79-89) indicated that the SD multiplier for the BMR should be approximately 0.4 (if the mean T4 changes by 0.4*SD, then the extra risk associated with that change will be 10%). Mr. Allen showed the results of different $BMD_{0.4SD}$ models in Appendix A. The Exp-M2 model provided the best model fit (AIC = -5.34, page 10 of Appendix A) for POD estimation. It had a $BMDL_{0.4SD}$ value of 8.3 mg/kg-d (page 11 in Appendix A). Using the same 10% extra risk above the background, Mr. Allen showed the Exp-M4 model had a higher AIC (-3.85) indicating not as good model fit with a $BMDL_{0.4SD}$ of 2.58 mg/kg/d (pages 10 – 11 in Appendix A). Although this prediction is more than 3-fold lower than that predicted with Mr. Allen's Exp-M2 model, again, as discussed in Finding 1, BMDL predictions should not be used as a basis for assessing model fit nor for performing model selection. (Note: The $BMDL_{0.4SD}$ value under Exp-M2 model is approximately twice the $BMDL_{20}$ value.)

3M Summary Comment No. 4 – If the Best Fit Model Proposed by Mr. Allen is Used, the Candidate Chronic RfD Based on Thyroid Effects Would be Higher Than That Proposed by EPA

As noted above, Mr. Allen evaluated the results of different $BMD_{0.4SD}$ models (Appendix A, pp. 8-11) and concluded that the Exp-M2 model provided the best model fit (AIC = -5.34) for POD estimation. It resulted in a $BMDL_{0.4SD}$ value of 8.3 mg/kg-d. Using this value as the POD for RfD calculation instead of 4.2 mg/kg-d used by EPA, while retaining the existing composite uncertainty factor (UFC) of 300 for the thyroid effect used by EPA, results in a candidate chronic RfD for PFBS of 0.03 mg/kg-d instead of the candidate chronic RfD of 0.01 mg/kg-day proposed by EPA.

Candidate Chronic RfD for K^+PFBS (Thyroid) = $BMDL_{0.4SD}$ (HED) ÷ UFC
= 8.3 mg/kg-day ÷ 300
= 0.028 mg/kg-day
= 3×10^{-2} mg/kg-day

3M Summary Comment No. 5 – Uncertainty Factors Used by EPA for Kidney Effects-based RfD Should be Reduced

The evidence of renal hyperplasia (based on the study by Lieder et al.) could support EPA's definition of a hazard, however, the EPA Draft PFBS Document is <u>incorrect</u> in its assessment of UF_S allocations. The Feng et al. (2017) study was deemed to be a developmental study by the Draft PFBS Document given that it was a gestation exposure study. The combined UF_D (10) x UF_S (1) allocated to the study by Feng et al. is 10, including the absence of chronic study exposure duration as part of the UF_D allocation.

Unlike the study by Feng et al., Lieder et al. (2009b) was a 2-generation study with direct K^+PFBS dosing regiments that spanned from pre-mating, mating, gestation, lactation, and post-weaning. It is scientifically unclear why the Lieder et al. study (2009b) was not considered a developmental study by the EPA. The combined UF_D (3) x UF_S (10) allocated to the study by

Lieder et al. is 30. The UF_S of 10 is "applied to account for less than chronic-duration exposure because the POD comes from a subchronic duration study."

EPA has allocated an additional factor of 3 for the study by Lieder et al. (2009b) that lacks support. At the very maximum, the combined UF_D x UF_S value should be 10 (or lower) for the Lieder et al. study; which should be the same as the combined UF_D x UF_S value of 10 used for the Feng et al. study.

3M Summary Comment No. 6 – 3M agrees with EPA Use of the Dichotomous-Hill Model

3M agrees with EPA that the Dichotomous-Hill model meets the criteria of the best-fit model for papillary tubular/ductal hyperplasia in P0 female rats. For this model, the $BMDL_{10}$ was 11.4888 mg/kg-day. This is also the expert opinion verbally expressed to 3M by Mr. Allen.

3M Summary Comment No. 7 – The Candidate Chronic RfD for PFBS Should be 0.04 mg/kg-d

Taking together 3M Summary Comments 6 and 7, using existing BMD_{10} value of 11.488 mg/kg-d and proposed composite UF of 300 for the renal hyperplasia, the proposed candidate chronic RfD for PFBS would then equal the following:

Candidate Chronic RfD for K^+PFBS (Kidney) = $BMDL_{10}$ (HED) ÷ UFC
= 11.5 mg/kg-day ÷ 300
= 0.038 mg/kg-day
= 4×10^{-2} mg/kg-day

This results in a candidate chronic RfD for PFBS of 0.04 mg/kg-d instead of the candidate chronic RfD of 0.01 mg/kg-day proposed by EPA.

3M Summary Comment No. 8 – EPA needs to inform the public why EPA selected the 2-generation study in rats (Lieder et al. 2009b) as the critical study rather than the 90-day study in rats (Lieder et al. 2009a) that the peer reviewers were charged to assess as to whether it (Lieder et al. 2009a) is scientifically justified and defensible to be the critical study.

By reviewing EPA's Response to Peer Review Comments on the Draft Human Toxicity Value for PFBS, it became apparent that EPA asked the peer reviewers to assess whether the 90-day study in rats by Lieder et al. (2009a) was scientifically justified and defensible to be the critical study. All the reviewers agreed with EPA's choice of using the 90-day study in rats by Lieder et al. (2009a) as one of the critical studies.

Yet in the current Draft PFBS Document, it selected the 2-generation study in rats (Lieder et al. 2009b) as the critical study rather than the 90-day study in rats (Lieder et al. 2009a) that the peer reviewers were charged to assess. Therefore, there is a discordance and ultimately a lack of explanation between the publicly released Draft PFBS Document and the draft document that was given to the peer reviewers. EPA needs to explain its rationale for making the switch between the two studies (Lieder et al., 2009a; 2009b) because the EPA peer review panel never provided their professional opinion on Lieder et al. 2009b and the uncertainty factors allocated for this particular study.

Detailed Comments

<u>Page ix of the EPA Draft PFBS Document:</u> "Of the examined outcomes, only asthma, serum cholesterol, and high-density lipoprotein levels were found to exhibit a statistically significant positive association with PFBS exposure."

<u>3M comments:</u>

This statement is inaccurate. No epidemiology study has reported a significant association between high-density lipoprotein (HDL) levels and PFBS exposure. The single "low-confidence" study (Zeng et al., 2015) cited by the EPA, reported a non-significant increase in HDL cholesterol (β = 5.78, 95% CI: -2.09-13.65) mg/dL increase per unit increase in PFBS. As such, the EPA should remove "high-density lipoprotein" from their statement.

Further, the EPA's statement could be misinterpreted that an association exists between these health outcomes and PFBS exposure in humans. The EPA clearly states in the Draft PFBS Document that the evidence in humans is "equivocal" for asthma (page 46; Table 7, page 53) and for lipid or lipoprotein homeostasis (Table 7, page 52). The EPA further states that "the association between asthma and PFBS exposure was observed in a single study with concern regarding the potential for residual confounding" (page 53) and that the association between total cholesterol and PFBS exposure was observed in a "low-confidence" cross-sectional study with "concern for potential reverse causality" (page 52). Accordingly, the EPA should clearly communicate that the overall evidence for an association between PFBS exposure and these health outcomes is equivocal in humans.

<u>Page x of the EPA Draft PFBS Document</u>: "The available rat and mouse studies support identification of thyroid, developmental, and kidney endpoints as potential health effects following repeated exposures in utero and/or during adulthood."

<u>3M comments:</u>

The EPA should revise this statement to be more specific for the following reasons:

- The available rat studies by 3M (28-day, 90-day, and 2-generation) did not identify thyroid as potential health effects with exposure to K^+PFBS (identified as 3M, 2001; Lieder et al. 2009a; Lieder et al. 2009b in the EPA Draft PFBS Document).
- The NTP 28-day rat study (identified as NTP 2018 in the EPA Draft PFBS Document) reported decreased total T4, total T3, and free T4 in serum at the end of 28 days dosing, however, these three endpoints <u>alone</u> did not provide adequate (clinical) evidence to suggest that thyroid was being affected (see 3M Summary Comment No. 2 above). Given that there were normal TSH levels (primary diagnostic indicator for thyroid hormone status) and normal thyroid histology in these same rats (where decreased serum total T4, total T3, and free T4 were reported as measured by analog method only), this suggested that overall

thyroid hormone status in these rats was normal. The following studies support this position:

  - PFBS at higher concentrations, similar to its eight-carbon congener PFOS, is likely capable of displacing T4 from binding proteins (Chang et al. 2007 Toxicology 234 21-33; Weiss et al. 2009 Toxicol Sci 109 206-216).
  - With increased hepatic hypertrophy reported in the rats from the NTP study (due to activation of peroxisome proliferation, reported by NTP as increased acetyl CoA activities), it also suggested that there was enhanced hepatic metabolism, which is commonly observed in rodents upon peroxisome proliferation (Corton et al. 2014 Crit Rev Toxicol 44 1-49). As a result, the increased hepatic metabolism would result in enhanced excretion of displaced thyroid hormones, which likely explain why there were alterations in total T4 and total T3.
  - Total T4 and total T3 measurements are measurements of largely (> 99.5%) inactive thyroid hormones and they alone do not represent functional aspects of the thyroid (Oppenheimer et al 1995 Mol Endo Bas Conc Clin Corr 249-268).
  - Although not specified, it is likely that NTP used an analog assay to measure free T4 and that binding displacement (by PFBS) likely contributed to a negative bias in the measurement (of free T4). The bias is commonly observed with compounds that can compete with thyroxine for protein binding and it can be avoided when an equilibrium dialysis-based free T4 method is used (Ekins 1983 Lancet 322 402-403).

- Like the NTP 28-day study, the mouse developmental study (identified as Feng et al. 2017 in the EPA Draft PFBS Document) reported decreased total T4, decreased total T3, and normal TSH in serum at birth for female pups. Again, total T4 and total T3 <u>alone</u> did not provide adequate (clinical) evidence to suggest that thyroid was being affected, especially when TSH, the primary diagnostic indicator for thyroid hormone status was normal. Feng et al. did not provide the following information to allow a full interpretation of thyroid status:
  - Albeit the pups were necropsied, no thyroid histology was reported.
  - There were no TRH mRNA or serum FT4 measured in offspring (these were done for dams).
- Study by Feng et al. (2017) did not identify kidney effects as potential health effect with exposure to K^+PFBS.

<u>Page 3 of the EPA Draft PFBS Document:</u> "PFBS has been reported in serum of humans in the general population. In American Red Cross samples collected in 2015, 8.4% had a quantifiable serum PFBS concentration; the majority of samples were below the lower limit of quantitation (.2 nanograms per milliliter [ng/mL]) (Olsen et al., 2017). The National Health and Nutrition Examination Survey (NHANES) 2013-2014 data reported the 95th percentile for PFBS at or below the level of detection (0.1 ng/mL)."

3M Comments:

Regarding the measurement of PFBS in American Red Cross adult blood donors (Olsen et al. 2017) and the National Health and Nutrition Examination Survey (NHANES), not only was PFBS not reported above the level of detection in the CDC NHANES 2013-2014 sampling analysis, the CDC NHANES has recently released preliminary data for their 2015-2016 environmental biomonitoring assessment that indicates they chose not to even analyze for PFBS in 2015-2016. See https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/PFAS_I.htm

Although CDC NHANES does not explicitly state this in the above website that they did not analyze for PFBS in 2015-2016, it is clear from reading this website that only the following PFASs (and their LLOD) were analyzed based on the 2015-2016 NHANES codebook. This table is copied from the above website. There is no mention of PFBS in the table below.

Table 3

(from https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/PFAS_I.htm)

The lower limit of detection (LLOD, in ng/mL) for each PFAS:

Variable Name	SAS Label	LLOD
LBXPFDE	Perfluorodecanoic acid (PFDeA) (ng/mL)	0.10
LBXPFHS	Perfluorohexane sulfonic acid (PFHxS) (ng/mL)	0.10
LBXMPAH	2-(N-methylperfluoroctanesulfonamido)acetic acid (Me-PFOSA-AcOH) (ng/mL)	0.10
LBXPFNA	Perfluorononanoic acid (PFNA) (ng/mL)	0.10
LBXPFUA	Perfluoroundecanoic acid (PFUA) (ng/mL)	0.10
LBXPFDO	Perfluorododecanoic acid (PFDoA) (ng/mL)	0.10
LBXNFOA	n-perfluorooctanoic acid (n-PFOA) (ng/mL)	0.10
LBXBFOA	Branch perfluorooctanoic acid isomers (Sb-PFOA) (ng/mL)	0.10
LBXNFOS	n-perfluorooctane sulfonic acid (n-PFOS) (ng/mL)	0.10
LBXMFOS	Perfluoromethylheptane sulfonic acid isomers (Sm-PFOS) (ng/mL)	0.10

Page 7, section 1.3.5.2 of the EPA Draft PFBS Document: "For rats receiving an oral dose, terminal serum K+PFBS elimination half-lives were significantly different ($p \leq 0.05$) for males (t1/2 = 4.68 ± 0.43 hours) versus females (t1/2 = 7.42 ± 0.79 hours). Thus, the half-life

estimates of Olsen et al. (2009) (4–7.5 hours) are roughly twice those estimated by Chengelis et al. (2009) based on urine data (2.4 and 3.1 hours)"

3M comments:

- $T_{1/2}$ values cited in the EPA Draft PFBS Document for Olsen et al. (2009) rat data were based on oral gavage dosing; while $T_{1/2}$ values cited in the Draft PFBS Document for Chengelis et al. (2009) rat data were based on IV dosing.
- For comparison purpose, Olsen et al. (2009) also derived a terminal half-life for rats after IV dosing, and they were 4.51 ± 2.22 and 3.96 ± 0.21 hours, respectively, in male and female rats.
- The difference could also be due to the fact that a non-compartmental model was used to calculate the kinetic parameters in Chengelis et al (2009) while a two-compartment model was used in Olsen et al. (2009).

Page 7, section 1.3.5.3 of the EPA Draft PFBS Document: "The study of Chengelis et al. (2009) indicated that, under conditions of equivalent exposure, the areas under the serum concentration-time curves (AUCs) were lower and the elimination half-lives were shorter for PFHxA than those for PFBS in both S-D rats and cynomolgus macaques. In the monkeys, for instance, PFHxA was cleared more rapidly and resulted in a lower AUC value (approximately an order of magnitude lower) with a shorter terminal half-life (2.4–5.3 hours, data not shown in the study) than PFBS at an equivalent dose (i.v. dose at 10 mg/kg)."

3M comments:

PFHxA is a 6-carbon perfluoroalkyl carboxylate. PFBS is a 4-carbon perfluoroalkyl sulfonate. Accordingly, the relevance of this statement to PFBS is unclear.

Page 26, section 4.1.2 of the EPA Draft PFBS Document: "Statistically significant dose-dependent decreases in total T3, total T4, and free T4 were also reported after exposure in male and female rats to K^+PFBS for 28 days at all doses tested (≥ 62.6 mg/kg-day) (NTP, 2018, 2011)."

3M comments:

- Again, it is important to recognize that total T3 and total T4 measured in the blood represent mostly the biologically inactive fractions of thyroid hormones (Oppenheimer et al 1995 Mol Endo Bas Conc Clin Corr 249-268) and they alone do not represent the functional aspect of the thyroid.
- As explained in detail above with increased liver hypertrophy in conjunction with thyroid hormone displacement, PFBS likely can compete with T4 for protein binding in serum (similar to its congener, PFOS, as reported in Chang et al. 2007 Toxicology 234 21-33; Weiss

et al. 2009 Toxicol Sci 109 206-216). Therefore, decreased total T4 and T3 likely reflected increased liver-mediated metabolism of the thyroid hormones that had been displaced.

- Furthermore, because of the binding competition, when measuring for free T4 (the biologically active fraction of T4) in the presence of high PFBS concentration, equilibrium dialysis-based measurement for free T4 is required. If conventional analog assays were used instead of equilibrium dialysis, most likely the case with NTP data (2018; 2011), it would result in an artificially lowered value (negative bias) for free T4 due to binding interference. It behooves EPA to clarify with NTP whether an analog or an equilibrium dialysis method was used to measure free T4.
- Most importantly, when examining the thyroid-related parameters, the gold standard is thyroid histology (which is obviously more challenging to do so in humans) and serum TSH (Jahnke et al. 2004, Environ Health Perspect 112 363-368). It should be emphasized that NTP reported normal thyroid histology and TSH levels.

Page 26, section 4.1.2 of the EPA Draft PFBS Document: "Thyroid gland weight, thyroid histopathology, and TSH levels were not changed after 28 days of PFBS exposure in male or female rats at up to 1,000 mg/kg-day (NTP, 2018, 2011)."

3M comments:

This is a very important observation, indicating that the overall thyroid hormone balance was being maintained with the NTP study, as reflected by normal TSH (primary diagnostic indicator for thyroid hormone status) and normal thyroid histopathology.

Pages 27 – 28, section 4.2.2.1 of the EPA Draft PFBS Document: "Adult (PND 60) F1 females gestationally exposed to PFBS at doses greater than 200 mg/kg-day, however, exhibited fewer primordial follicles, primary follicles, secondary follicles, early antral follicles, antral follicles, and preovulatory follicles, as well as fewer corpora lutea compared to control (Feng et al., 2017). Importantly, no effects on the health (e.g., weight gain) of the exposed dams were observed at any dose (Feng et al., 2017). Lieder et al. (2009b) evaluated ovarian follicles in F1 females after they were mated and their pups had been weaned (i.e., lactation day [LD] 22), and observed no effects compared to controls at 1,000 mg/kg-day; however, the data were not reported."

3M comments:

The observations reported by Feng et al. (2017) were very different than those reported by Lieder et al. (2009b). Technical observations included:

- Effects reported by Feng et al. lacked dose-responses; the effects from 200 mg/kg-d were usually similar in magnitude to 500 mg/kg-d.
- The study design and PFBS dosing regimen by Lieder et al. (2-generation in rats) was more rigorous than Feng et al. (gestational only in mice) in terms of treatment duration, doses, as

well as direct treatments to developing fetuses and pups during sensitive life stages, see Table 4 below for comparison.

Table 4

			Lieder et al. 2009b	Feng et al. 2017
Species			Sprague Dawley rats	ICR mice
Test guideline			OECD 416 / OPPTS 870.3800 (2-gen)	None
GLP			Yes	No
Daily K⁺PFBS treatments (direct gavage)	Daily doses		30, 100, 300, 1000	50, 200, 500
	P-generation	Pre-mating, males	Yes, 70 days	No
		Pre-mating, females	Yes, 70 days	No
		Gestation, dams	Yes	Yes
		Lactation, dams	Yes	No
	F1-generation pups (before mating)	Weaning and on	Yes, ≥ 70 days	No

- It was not clear why Feng et al. did not include male offspring in their evaluation.
- The female mouse offspring in the Feng et al. study were not directly dosed with K^+PFBS, however, the reported myriad of adverse developmental outcomes occurred in these female mouse pups (e.g., reduced body weight and changes in reproductive organ morphology). In contrast, female rat offspring (from Lieder et al. 2009b) were not only exposed to PFBS during gestation and lactation, they were also directly dosed with PFBS (at higher dose levels than the Feng et al. study) after weaning and into their adulthood. There were no developmental effects noted in the female rat pups in Lieder et al. study.
- Regarding the alterations in ovary and uterus-related data, as reported by Feng et al:
    - Evaluation was reported for female pups at PND 60 only, not on PND 30; and not for dams (who were directly dosed with PFBS).
    - "Impaired" development reported by Feng et al. was based on decreased surface area (on microscopic slides) and limited morphological measurements. Surface area can be also attributed from different sectioning location (of the tissue). Feng et al. did not address how this was controlled among different animals. In addition, Feng et al. only provided relative organ-to-body weight data - there were no absolute organ weight data for the readers to interpret. Organ-to-brain weight data were not presented either.
    - Feng et al. did not take body weight into consideration when interpreting estrous cycle data which is unfortunate because they are related (Bermejo-Alvarez et al. 2012, Hum Reprod 27 3513-3522).

Overall, applying the criteria for evidence of integration and hazard characterization, as specified in the EPA Draft PFBS Document Section 2.3.6, there was a lack of concordance among the datasets reported by Lieder et al. (2009b) and Feng et al. (2017).

Page 28, section 4.2.2.3 of the EPA Draft PFBS Document: "The hormonal effects observed in the NTP (2018) and Feng et al. (2017) studies might be associated with adverse reproductive effects reported in these studies."

3M comments:

- NTP study (2018) did not evaluate reproductive effects directly. It was a 28-day repeated dose study where a statistically significant increased trend in testosterone was observed in females ($p \leq 0.05$), but not in males. In pairwise analyses, the increase in testosterone was not statistically significant for any individual dose group when compared to control (cf. page 28 of EPA Draft PFBS Document).
- In Feng et al. (2017), albeit there were changes in female reproductive organ morphology, functional aspects of reproduction appeared not to be affected according to study authors (i.e., maternal body weight, maternal body weight-gain, and various pregnancy outcomes).

Page 30, section 4.4 Renal Effects: The EPA states that Qin et al. (2016) was a "medium-confidence study."

3M comments:

The EPA's statement is incorrect. The overall confidence of this study was rated as deficient/low confidence in the EPA's evaluation of epidemiology studies (Figure 5, page 23).

Page 34, section 4.5.2 of the EPA Draft PFBS Document: "In general, serum biomarkers associated with altered liver function or injury, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were not significantly changed in male and female S-D rats across multiple oral gavage studies of varying exposure durations up to 90 days, at K+PFBS doses up to 1,000 mg/kg-day (Lieder et al., 2009a; 3M, 2001, 2000d). NTP (2018) and NTP (2011), however, reported increased serum ALT and AST in male (500 mg/kg-day only) and female (≥ 250 mg/kg-day for ALT; ≥ 500 mg/kg-day for AST) rats exposed to K+PFBS for 28 days."

3M comments:

There were apparent changes in serum liver enzymes in the NTP study that were not seen in the 90-day study by Lieder et al. (2009). Even more striking is that there was a large percentage of deaths that occurred in the NTP 28-day study. Mortality was not observed in the 28-day study by 3M (3M, 2001) with comparable doses (see Table 5 below).

Table 5					
Parameters		**3M Study**		**NTP Study**	
Doses evaluated (mg/kg/d)		100, 300, 900		62.5, 125, 250, 500, and 1000	
Dosing method		Oral gavage		Oral gavage	
Survival at the highest dose	Time	Study Day 22	Study Day 28	Study Day 22	Study Day 28
	Males	100% (15/15)	100% (15/15)	30% (3/10)	0% (0/10)
	Females	100% (15/15)	100% (15/15)	50% (5/10)	20% (2/10)

The 3M study (2001) had no mortality at the end of 28 days at the top dose of 900 mg/kg/d (3M 2001). In the 90-day study by 3M (Lieder et al. 2009), there was no mortality at 600 mg/kg/d. In the 2-generation study by 3M (Lieder et al. 2009b), where male rats were treated for at least 10 weeks (70 days) for two generations, there was no mortality at 1000 mg/kg/d. Hence it is perplexing what contributed to the mortality (at much shorter duration) in the NTP study, which adds difficulties and uncertainties in assessing the corresponding data, such as AST and ALT with the NTP study.

Page 34, section 4.6.2 of the EPA Draft PFBS Document: "PFBS studies have not particularly focused on perturbations in lipids or lipoproteins as a potential health outcome, as studies have typically focused only on measures of serum cholesterol and triglyceride as part of a broader panel of clinical chemistry measures in high- or medium-confidence rat studies of 10, 28, and 90 days (see Figure E-11) (3M (2000d)]; 3M (2001)]; and Lieder et al. (2009a)], respectively)."

3M comments:

This is not correct. PFBS has been carefully evaluated, mechanistically, for its effect in lipid metabolism by Bijland et al. (2011) using a humanized ApoE*3.Leiden.CETP transgenic mouse model which expresses human-like lipoprotein profile. Unlike longer-chain perfluoroalkyl sulfonates (PFHxS and PFOS) that markedly reduced plasma triglycerides, non-HDL-cholesterol, and HDL-cholesterol, PFBS modestly reduced plasma triglycerides only. Unlike PFHxS and PFOS, PFBS did not affect lipid metabolism-related gene expressions in the liver.

Page 35, section 4.4 Other Effects of the EPA Draft PFBS Document: The EPA states that one medium-confidence study was reported in five publications (Qin et al., 2017; Zhou et al., 2017b; Zhou et al., 2017a; Zhu et al., 2016; Dong et al., 2013b).

3M comments:

The EPA states that one medium-confidence study was reported in five publications (Qin et al., 2017; Zhou et al., 2017b; Zhou et al., 2017a; Zhu et al., 2016; Dong et al., 2013b), but does not reference the study of medium-confidence (Dong et al., 2013a). Further, the EPA did not include these 5 publications in their evaluation of epidemiology studies nor did they provide an explanation why the studies were excluded.

Page 43, Section 5.3 of the EPA Draft PFBS Document:

- See previous comments (vide supra)

Page 45, Section 5.5 of the EPA Draft PFBS Document:

- See previous comments (vide supra)

Page 53, Table 7 on asthma of the EPA Draft PFBS Document: The EPA refers to a "Medium-confidence case-control study (Zhou et al., 2016; Zhu et al., 2016; Dong et al., 2013b).

3M comments:

Given that the EPA did not include these individual studies in their evaluation of epidemiology studies, only the study by Dong et al (2013) should be referenced.

Page 55 (Section 6.1.1.) and pages F-4 to F-16 of the EPA Draft PFBS Document:

- See expert opinion by Mr. Bruce Allen (Appendix A)

Page 55, section 6.1.1. of the EPA Draft PFBS Document: "The EPA considered the 2014 *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* in determining interspecies and intraspecies UFs (UFAs and UFHs, respectively) (U.S. EPA, 2014c). Using the decision process described in Figure 2 of that guidance (U.S. EPA, 2014c), the EPA concluded that data are inadequate to support derivation of data-derived extrapolation factors. Specifically, given the lack of available models and data to address external dose and clearance in humans with any certainty or the magnitude of difference in half-life across species as a function of dose or time, the default approach of the use of $BW^{3/4}$ scaling to obtain a HED is considered appropriate in this case."

3M Comments:

3M agrees.

Pages 65 – 67, Section 6.1.2 of the EPA Draft PFBS Document: In the derivation of candidate chronic RfDs, specifically, on the UF allocations for UF_D and UF_S (Tables 14 and 15 of the EPA Draft PFBS Document), Table 6 below is reproduced, in part, to illustrate the allocation of UF_D and UF_S assigned to each study.

3M Comments:

Table 6		
	Feng et al. 2017 (Table 14)	**Lieder et al. 2009b (Table 15)**
	Thyroid effects	Kidney effects
UF_D	A **UF_D of 10** is applied to account for database deficiencies. The oral exposure database contains multiple short-term and subchronic-duration toxicity studies of laboratory animals (NTP, 2018; Bijland et al., 2011; NTP, 2011; Lieder et al., 2009a; 3M, 2001, 2000d), a two-generation reproductive toxicity study in rats (Lieder et al., 2009b), and multiple developmental toxicity studies in mice and rats (Feng et al., 2017; York, 2002). However, as thyroid hormone is known to be critical during developmental life stages, particularly for neurodevelopment, the database is limited by the lack of developmental neurotoxicity studies. **Further, due to the lack of chronic duration studies, there is additional uncertainty regarding how longer-term exposures might impact hazard identification and dose-response assessment for PFBS via the oral route (e.g., potentially more sensitive effects).** Lastly, as immunotoxicity is an effect of increasing concern across several members of the larger PFAS family, the lack of studies evaluating this outcome following PFBS exposure is a limitation in the database.	A **UF_D of 3** is applied due to database deficiencies. The oral exposure database contains multiple short-term and subchronic-duration toxicity studies of laboratory animals (NTP, 2018; Bijland et al., 2011; NTP, 2011; 3M, 2010; Lieder et al., 2009a; 3M, 2001, 2000d), a two-generation reproductive toxicity study in rats (Lieder et al., 2009b), and multiple developmental toxicity studies in mice and rats (Feng et al., 2017; York, 2002).However, the observation of decreased thyroid hormone is known to be a crucial element during developmental life stages, particularly for neurodevelopment, and the database is limited by the lack of developmental neurotoxicity studies. In addition, as immunotoxicity is an effect of increasing concern across several members of the larger PFAS family, the lack of studies evaluating this outcome following PFBS exposure is a limitation in the database.
UF_S	A **UF_S of 1** is applied because the POD comes from a developmental study of mice. The developmental period is recognized as a susceptible life stage in which exposure during certain time windows (e.g., gestational) is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991b). The additional concern over potential hazards following longer-term (chronic) exposures is accounted for under the UF_D above.	A **UF_S of 10** is applied to account for less than chronic-duration exposure because the POD comes from a subchronic duration study.

Based on the table shown above:

- For each study, the combined (UF_D x UF_S) is 10 for the Feng et al. study and 30 for the Lieder et al. study.
- When comparing the UF_D allocations, both studies were subjected to similar dataset deficiencies (i.e., developmental neurotoxicity and immunotoxicity data). However, the EPA Draft PFBS Document inferred a lack of chronic exposure duration with the Feng et al. study

hence an overall higher UF_D value of 10 was assigned (see bold underlined text shown in the table above).

- When comparing the UF_S allocations, according to the EPA Draft PFBS Document, Feng et al. has an UF_S allocation of 1 because it was a developmental study and additional uncertainty for it not being a chronic study had been adjusted with higher UF_D. The EPA Draft PFBS Document inferred a lack of chronic exposure duration with Lieder et al. study and an overall UF_S value of 10 was assigned to Lieder et al. study.
- The EPA Draft PFBS Document is <u>incorrect</u> in its assessment of UF_S allocations without valid scientific justifications. Feng et al. (2017) study was deemed to be a developmental study by the EPA Draft PFBS Document given that it was a gestation exposure study (direct K^+PFBS dosing was administered during gestation only to time-pregnant dams without additional dosing afterward). Unlike the study by Feng et al., Lieder et al. (2009b) was a 2-generation study with direct K^+PFBS dosing regiments that spanned from pre-mating, mating, gestation, lactation, and post-weaning. It not only had the gestation exposure period, the rigorous dosing schedules from Lieder et al. study (before and after gestation) unequivocally covered more life stages for pups than those reported by Feng et al. It is perplexing why Lieder et al. (2009b) was not considered as a developmental study. In addition, Feng et al. only carried one generation, Lieder et al. produced two generations with the same rigorous dosing schedules. Again, from all aspects of study design and robustness, a full-scale 2-generation study such as the one reported by Lieder et al. (2009b) is far more comprehensive in terms of evaluation during susceptible life stage when compared to the gestation-only study such as the one reported by Feng et al. (2017). A previously shown table (Table 4) is provided here again for illustration.

Table 4

			Lieder et al. 2009b	Feng et al. 2017
Species			Sprague Dawley rats	ICR mice
Test guideline			OECD 416 / OPPTS 870.3800 (2-gen)	None
GLP			Yes	No
Daily K^+PFBS treatments (direct gavage)	Daily doses		30, 100, 300, 1000	50, 200, 500
	P-generation	Pre-mating, males	Yes, 70 days	No
		Pre-mating, females	Yes, 70 days	No
		Gestation, dams	Yes	Yes
		Lactation, dams	Yes	No
	F1-generation pups (before mating)	Weaning and on	Yes, ≥ 70 days	No

Clearly EPA has inappropriately allocated an additional factor of 3 for the study by Lieder et al. (2009b) without sufficient justification. For all these scientific facts articulated herein, the current combined UF_D x UF_S value of 30 for Lieder et al. study should be re-assigned. At the very maximum, the combined UF_D x UF_S value should be the same as the combined UF_D x UF_S value of 10 or lower for the Lieder et al. study.

<u>Page 67, mathematical calculation of the EPA Draft PFBS Document</u>: EPA provided the following calculation for the Candidate Chronic RfD for kidney effects.

Candidate Chronic RfD for K^+PFBS (Kidney) = BMDL10 (HED) ÷ UFC

= 11.5 mg/kg-day ÷ 1,000
= 0.12 mg/kg-day
= 1×10^{-2} mg/kg-day

<u>3M Comments:</u>

There is a typo on the third line. It should be 0.0115 mg/kg-day, not 0.12 mg/kg-day

<u>Page F-17, Appendix F of the EPA Draft PFBS Document:</u> EPA selected the Dichotomous-Hill model for the model that best fit the papillary tubular/ductal epithelium hyperplasia in F0 female rats.

<u>3M Comments:</u>

We agree with EPA's selection of the Dichotomous-Hill model for the model that best fit the papillary tubular/ductal epithelium hyperplasia in F0 female rats, as shown in Table F-3 of the EPA Draft PFBS Document. This resulted in a $BMDL_{10}$ (HED) (mg/kg-day) of 11.4888.

Appendix A

Comments Related to BMD Analysis of PFBS

January 18, 2019

Introduction

I am an independent consultant and have been a practitioner in the field of risk assessment for 35 years. My emphasis has been on dose-response modeling, including benchmark-dose and statistical analysis. During that time I have contributed to the advancement of the science of risk assessment and have performed or responded to assessments of many chemicals suspected of posing problems for human health. Moreover, I have consulted with EPA regarding its BMDS program development, the software used by EPA for the analysis of perfluorobutane sulfonic acid (PFBS).

I was asked by 3M to independently review EPA's Draft Human Health Toxicity Values for Perfluorobutane Sulfonic Acid and Related Compound Potassium Perfluorobutane Sulfonate (EPA-823-R-18-307); hereafter referred to as the "EPA assessment." Specifically, I was asked to provide insight concerning EPA's benchmark dose modeling in its identification of points of departure (PODs) for PFBS, one based on kidney hyperplasia observed in Sprague Dawley rats and the other based on a decrease in total T4 levels in female ICR (CD-1) mice offspring at birth (postnatal day 1). I have been compensated by 3M for this review.

One specific item, posed by EPA to its selected peer-reviewers, was related to the modeling approaches used, with specific reference to the selection of benchmark response levels used to identify each POD. For decreased total T4 in female mice offspring, specifically, when considering species- and/or lifestage-specific differences in thyroid economy (e.g., differential reserve capacities for thyroid hormone in infants compared to adults and mice compared to humans), the reviewers were asked to comment on how EPA addressed these factors in the choice of a biologically based benchmark response level (i.e., level of change that characterizes the lower limit of biological significance compared with normal background responses, which EPA identified as a $BMDL_{20}$).

This document provides additional comments related to those concerns. Specifically, it addresses the choice of model and of a $BMDL_{20}$ for the T4 endpoint referenced above. In the following sections, we address the following issues:

- Concerns about choice of BMD model
- Lack of history for use of a $BMDL_{20}$ for POD derivation
- Decreased consistency associated with the use of a $BMDL_{20}$
- Lack of rationale for selection of $BMDL_{20}$ as a biologically based benchmark response level

Choice of BMD Model

For purposes of the discussion provided herein, values from Table F-2 (p. F-5) in EPA's draft toxicity assessment, reproduced in part, are summarized in the Table below:

Table: Modeling results for total T4 in PND 1 female offspring (litter n) exposed GDs 1–20

Model	Global p-value	AIC	$BMDL_{20}$ (HED) (mg/kg-d)
Linear	0.558	-4.72314	20.2211
Exp M2	0.7627	-5.34819	12.5215
Exp M4	0.8421	-3.85031	4.22705

Note: Other models not shown because they had no p-value for global fit (Hill and Exp M5) or because they devolved into one of the simpler forms shown here (i.e., polynomial and power models were identical to the simpler linear form; Exp M3 was identical to the simpler Exp M2).

For the sake of argument, we consider here the EPA's selected BMR (20% reduction in mean T4). We will argue later that this is a poor choice in itself, but the observations that follow in this section apply whatever the choice of BMR, and so our example calculations will focus on that BMR. EPA rationalizes the choice of the Exponential M4 model on the grounds that the BMDL estimates derived across the models differ by more than a factor of 3. Had it not been for that magnitude of difference, then the best fitting model (as judged by having the smallest AIC) would be the standard EPA basis for the choice of model and therefore of the BMDL.

It is not hard to demonstrate the logical inconsistency associated with the EPA model selection procedure. Suppose a "lazy modeler" had run just the Exponential model suite (as some in Europe are advocating). In that case, the Exp M2 model would still be the best fitting (based on AIC), but the difference in BMDL estimates is less than a factor of 3 (it equals 2.96), and so application of the EPA selection criteria would have resulted in the choice of Exp M2 and a BMDL of 12.5.

Now suppose that a "good modeler" adds to that analysis by being more thorough in considering model shapes; she adds to the set of models the Linear model (and the power and polynomial models, which devolve to the simpler Linear form). That addition results in another model that fits the data adequately and would be considered for selection (see table above). Moreover that model predicts a BMDL *greater* than either of the previous BMDL estimates. However, EPA's procedure would dictate that the selected BMDL would now be 4.23, even though the additional modeling results suggest that 12.5 might itself be too low.

That makes no sense. It leads to decisions that can never be changed in the direction of increasing a POD as more information is obtained and more modeling is completed. That is so because, if modeling results are added that predict higher BMDLs (which *should* tend to move the weight of evidence toward higher BMDL values), the paradoxical effect is that the lowest BMDL is more likely to be selected under this procedure.

The gist of the problem is that model *predictions* of a certain quantity (e.g., of a BMD or BMDL) have no bearing on how well the models fit the data. Clearly, we expect different models to predict different BMDs (otherwise we would not bother to run more than one model), but the ordering of those models with respect to BMD values is not inherently correlated with model fit and the associated model selection (or model averaging) process. The predictions are what gets selected, not the basis for that

selection process.

So, clearly, in this case a value of 12.5 would be selected as coming from the best fitting model. The Linear model, with a BMDL of 20.2, would be judged superior to Exp M4. Yet, the worst model (from an AIC perspective, which is a typical metric for model selection) is the one that EPA used to define the POD. A crude modeling averaging technique would suggest an even higher value could be used, 13.8 mg/kg-d.[1]

Choice of BMR

Irrespective of the model choice considerations discussed above, we are also concerned about the other choice EPA made when defining the POD, i.e., the use of a BMR of 20% relative deviation (20% reduction in mean T4) to derive what are labeled the $BMDL_{20}$ values. The comments in the following subsections indicate reasons why the $BMDL_{20}$ is not appropriate.

No History of Use

To our knowledge, no other EPA assessment has used a 20% relative deviation as the BMR.[2] It is not a BMR that is mentioned in EPA guidance. Its use here appears to be idiosyncratic except insofar as that choice can be supported as a biologically or toxicologically based decision. Comments related to that criterion are given in the "Biological Basis" subsection below.

Lacks Consistency

One of the main goals when the BMD approach was developed was to reduce the inconsistencies associated with the method prevailing at that time (called the LOAEL/NOAEL approach) (Crump, 1984).

An associated goal is to be consistent across compounds and endpoints. Only in that manner can we hope to derive RfDs (for example) that adequately reflect the relative risks across those compounds and endpoints. Such consistency allows us to believe that the costs associated with risk reduction can be rationally allocated and that higher risks are addressed more urgently than lower risks.

As mentioned above, the use of $BMDL_{20}$ is inconsistent with what has been done in other cases, for other compounds. Moreover, even internally to this PFBS assessment, the use of $BMDL_{20}$ makes it less consistent with the analysis of the other PFBS-induced effect modeled by EPA: papillary tubular/ductal epithelium hyperplasia in P0 female rats. The latter is a dichotomous effect, for which BMRs are typically defined in terms of extra risk. A $BMDL_{10}$ for a dichotomous effect, for example, is the BMDL associated with an increase in risk of 10%. This is different from the T4-associated $BMDL_{20}$, which is based on the magnitude of mean T4 change, not on a change in risk. Thus there is an inconsistency in terms of the metric for defining the POD.

But there is an approach for BMD analysis of continuous endpoints that is consistent with the risk metric used with dichotomous endpoints. It is the approach that expresses BMRs in terms of standard deviation (SD) "units" (Crump, 1995). Some results for this approach were presented in the EPA assessment, but they were not used to define the POD.

[1] A weighted average of the BMDLs from each model, with weights for that average equal to exp(-AIC). More sophisticated model averaging techniques are available from EPA-sponsored software; they have been evaluated favorably internally by EPA and by external peer-reviewers.

[2] We have not done a systematic search of the IRIS database with respect to selected BMR metrics.

Specifically, what Crump (1995) showed is the following, using the PFBS assessment of T4 and hyperplasia as the example.

- Suppose that it is possible to specify
    - the cut-point between "normal" and "low" T4 levels ***or***
    - the proportion of the (unexposed, control) population that would be considered low with respect to T4.
- Suppose you want to estimate the dose (BMD) that increases the extra risk of T4 abnormality by 10%. That is, you want to use the same metric you used for the kidney hyperplasia endpoint in this assessment.
- Then, you can calculate the change in the mean T4 that will give the target 10% extra risk of low T4, ***if*** you express that change in terms of the standard deviation, x*SD.

As an example, Crump (1995) showed that if you assume that 1% of the unexposed test population has low T4, then a reduction of the mean T4 by (1.1*SD), increases the risk of low T4 by 10%.[3] EPA has partially captured this relationship in their default choice in BMDS of the BMD_{1SD} for continuous endpoint analysis, incorporating a conservative rounding down from 1.1SD to 1SD.

It is our conclusion that, in the absence of additional information, the BMD_{1SD} results should have been used to determine PODs based on the T4 endpoint. This conclusion is on top of the conclusion above that model choice was not handled appropriately. Together they suggest that a T4-associated POD should have been based on a value of 23.4 (HED) mg/kg-d, the $BMDL_{1SD}$ from the Exp M2 model (see Table F-2, p. F-5 of the EPA assessment) if a model-selection (as opposed to a model-averaging) process is enacted.

Weak Support for $BMDL_{20}$ as a Biologically Based Benchmark Response Level

The EPA assessment ultimately relies on biologically based arguments in support of the $BMDL_{20}$ estimates. We consider the following lines of support offered for the choice of a 20% relative decrease as being biologically relevant (see pp. 55-56 of the EPA assessment):

a. "With regard to what level of decrease in thyroid hormone is sufficient for anatomical and/or functional alterations, particularly in neurodevelopment in developing fetuses or newborns, several studies have identified a fairly stable range across humans and experimental rodents. Neurodevelopmental and cognitive deficits have been observed in children who experienced a 25% decrease in maternal T4 during the second trimester in utero (Haddow et al., 1999)."
b. "In other studies, mild-to-moderate thyroid insufficiency in pregnant women was defined as having serum T4 levels below the 10th percentile for the study population, which was associated with a 15%–30% decrease relative to the corresponding median (Finken et al., 2013; Julvez et al., 2013; Román et al., 2013; Henrichs et al., 2010)."
c. "Similarly, decreases in mean maternal T4 levels of ~10%–17% during pregnancy and lactation have been found to elicit neurodevelopmental toxicity in rat offspring (Gilbert et al., 2016; Gilbert, 2011). As the lower end of the range of T4 changes associated with untoward developmental health outcomes (e.g., 10%) commonly falls within normal experiment-to-experiment variation in control values, a BMR of 20% RD from control mean was determined to

[3] All of these calculations make the same assumptions about endpoint distribution that are made by EPA in its BMDS runs (Appendix F), i.e., that T4 is normally distributed and that the variance is constant across dose groups.

be a minimally biologically significant degree of change when performing BMD modeling on thyroid hormone alterations in pregnant females and associated offspring."

a. 25% decrease in maternal T4 during second trimester

It should be immediately recognized that the 25% decrease cited is from a very specific scenario, confined to the second trimester. More importantly, the 25% decrease is compared to levels that pertained in each individual in the first trimester. They do not reference a change in mean levels. In fact, there is no way to determine what a mean change would be; all we can gather from this statement is that among those with the requisite 25% decrease, there were some cognitive deficits. We do not know how many deficits (what was the rate of response) even among those individuals. Nor do we know the proportion of individuals who had such decreases and therefore we have no basis for imputing a change in the mean T4.

Conclusion: this evidence provides no support for selecting a mean change of 20% as the BMR.

b. Thyroid insufficiency in women below the 10th percentile

This observation is tied to the determination that the 10th percentile is 15-30% below the population mean. Let us examine what those two observations entail.

Under the assumption of normally distributed T4 in the population (the same assumption used for the BMD modeling), the 10th percentile point would be at

$$\mu - 1.28*\sigma$$

where μ and σ are the mean and standard deviation of the T4 distribution, respectively. For the sake of this illustration, let us suppose that that was 20% less than the mean (equal to the relative deviation EPA has chosen to use for their T4 BMR, and within the range of 15-30% cited in their support). Therefore

$$\mu - 1.28*\sigma = 0.8*\mu$$

yielding the relationship

$$\sigma = (0.2/1.28)*\mu = 0.16*\mu.$$

Note that there is no more "simplification" that can be done here – we cannot solve for σ without knowing μ. Moreover, consider our contention that the BMR ought to be expressed in terms of SD units. This expression illustrates why that is the case: linking a population percentile for thyroid insufficiency (essentially a statement that 10% of women had T4 that was too low) to a change in mean T4 requires estimates of the SD for it to be translatable to extra risk.

But there is something more troublesome about this line of support for a 20% relative-deviation BMR. EPA's suggestion that that change be set as the BMR level is equivalent to specifying that the dose that decreases the T4 mean down to the 10th percentile of controls (the imputed cut-point for low T4) be the BMD. But if that is the mean T4 at the BMD, then by definition of the mean of a normal distribution, the probability of low T4 at the BMD is 50%. That is 44% extra risk. We contend that is the wrong level for any BMD, and (returning to an earlier point) is certainly inconsistent with other BMD analyses.

c. Decreases in mean T4 of 10-17% have elicited neurodevelopmental toxicity in rats

Once again we must note that, in the absence of information about the SD, there is no tie-in between the cited range of decrease and the change in proportion of rats who had adversely low T4.

But let us examine this statement using actual values from Feng et al. (2017). The control group mean T4 was 1.44 and the standard deviation in that group was 0.33. So how unlikely is it to see T4 values 10% to 17% below the mean? The following table shows that it is not at all unlikely to see such observations, nor indeed to see T4 values as much as 30% below the mean:

Percent below mean	T4 Value	Likelihood of observation below that value
10	1.296	0.331
17	1.1952	0.229
20	1.152	0.191
30	1.008	0.095

There is almost a 10% chance that an observation will be more than 30% lower than the mean in any random sample of control animals. There is nothing special about 20% decrease in that respect. With 19% of the observations in controls being expected to be less than 20% of the mean value, it is not as if picking a 20% relative deviation BMR defines a "critical range" that is numerically improbable even in the absence of exposure.

If anything, these calculations suggest that, if one desired to use relative deviation as the basis for BMR definition, then at least a 30% relative deviation is required to define a range of abnormal T4, i.e., the level below the mean predicted to have a low background rate (about 10% in this case). Importantly, however, note that this is not to say that the BMR should be set to 30% relative deviation; that would fall prey to the same issue addressed in point b above, i.e., that the probability of low T4 would go from about 10% to 50%. Here, as in the other cases discussed above, it is not possible to make consistent probabilistic statements without taking SD into consideration; merely examining relative deviations from the mean is not sufficient. The choice by EPA to use 20% relative deviation is shown here to have no support in that regard.

Suggested Alternative Analysis

Given the discussion above, we have a suggested alternative approach to the BMD analysis of the T4 endpoint. It incorporates the two major suggestions inherent in the above sections:

- Selection of a different dose-response model
- Definition of the BMR using a SD approach

If the default value of 1SD as the BMR was retained (as in EPA's reported-but-not-used analysis), then the POD for the T4 endpoint would be 23.4 (HED) mg/kg-d, as mentioned previously, just on the basis of model selection.

However, there is one piece of information mentioned by EPA that might be relevant to the choice of the BMR level, and that would suggest a non-default choice for the BMR. That is the observation provided in the EPA assessment that "thyroid insufficiency in pregnant women was defined as having serum T4 levels below the 10th percentile for the study population." We follow through with that additional input in the analysis below, using it to defend a choice of a 10% background rate of thyroid insufficiency. We recognize that there are some (perhaps major) assumptions associated with that

choice, including that that observation in humans is relevant to determining a background rate in the experimental animals. It is also an open question whether it is appropriate to use a response that has such a high background rate of abnormality. By that we mean that as the background rate of a purported "abnormality" increases, there is less chance that the "abnormality" under consideration (T4) bears any relation to adverse health outcomes.[4]

Nevertheless, by assuming a background rate of 10% and specifying the BMD to be the dose that gives 10% extra risk over and above that background (to be consistent with the analysis of the dichotomous endpoint), the methodology described in Crump (1995) dictates that the SD multiplier for the BMR should be approximately 0.4. I.e., if the mean T4 changes by 0.4*SD, then the extra risk associated with that change will be 10%.

We have run that version of the analysis using BMDS (results shown in the appendix for the Exponential models). It is still the case that the Exp M2 model fits the data best and is the single best model to select for POD estimation; that is not impacted by the choice of BMR. In that case, the $BMDL_{.4SD}$ is 8.3 (HED) mg/kg-d. Even with the more stringent conditions imposed by the choice of a higher background than the default background (10% as opposed to 1%), the resulting POD (8.3 (HED) mg/kg-d) is about twice the value of 4.2 (HED) mg/kg-d that EPA used to derive an RfD.

If we were going to conduct a full re-analysis, we would also recommend running the other continuous models from BMDS and averaging the results across models, with weights based on AIC values. A higher value of the POD would result from that approach, as it would factor in the Linear model, which happens to have both a greater BMD value and greater weight than the Exp M4 model. Until such model averaging is incorporated, we support a POD of 8.3 (HED) mg/kg-d for the T4 endpoint.

References

Crump, K. S. (1984). A new method for determining allowable daily intakes. *Toxicological Sciences*, *4*(5), 854-871.

Crump, K. S. (1995). Calculation of benchmark doses from continuous data. *Risk Analysis*, *15*(1), 79-89.

Bruce C. Allen
Independent Consultant
Chapel Hill, NC

[4] Consider the limiting case where the rate of purported "abnormalities" approaches 100% in controls: clearly, in that case the presence of the "abnormality" cannot be associated with the presence of adverse health endpoints (disease, lack of development, or death) since all or nearly all of the subjects had the "abnormality."

Appendix: Output from Alternative BMDS Run

```
================================================================
        Exponential Model. (Version: 1.11;  Date: 03/14/2017)
        Input Data File:
C:/Users/Bruce/Documents/BMDS/BMDS2704/Data/exp_Dax_Setting.(d)
        Gnuplot Plotting File:
                                          Thu Dec 20 10:32:13 2018
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   rho is set to 0.
   A constant variance model is fit.

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                              Initial Parameter Values

     Variable          Model 2             Model 3             Model 4          Model 5
     --------          -------             -------             -------          -------
     lnalpha          -1.29725            -1.29725            -1.29725         -1.29725
         rho                 0 *                 0 *                 0 *              0 *
           a          0.794588            0.945214               1.512            1.512
           b        0.00971785          9.5412e-005           0.0428586        0.0428586
           c                 0 *                 0 *          0.434618         0.434618
           d                 1 *                 2                 1 *                1

     * Indicates that this parameter has been specified


                              Parameter Estimates by Model

     Variable          Model 2             Model 3             Model 4          Model 5
     --------          -------             -------             -------          -------
```

lnalpha	-1.2837	-1.2837	-1.29626	-1.29725
rho	0 *	0 *	0 *	0 *
a	1.40224	1.40224	1.4541	1.44
b	0.0107117	0.0107118	0.0316353	0.0365363
c	--	--	0.416958	0.463035
d	--	1	--	1.24424

-- Indicates that this parameter does not appear in model
* Indicates that this parameter has been specified

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.0619412	0.0619412	0.0611684	0.0611078
rho	NA	NA	NA	NA
a	0.125025	0.127487	0.148456	0.165312
b	0.00345921	0.00367245	0.0322218	0.0287449
c	NA	NA	0.222523	0.208043
d	NA	NA	NA	1.32125

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	1.44	0.329
7.5	10	1.3	0.657
29.9	10	0.92	0.493
75	10	0.69	0.657

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	1.402	0.5263	0.2269
	7.5	1.294	0.5263	0.03612
	29.9	1.018	0.5263	-0.5885
	75	0.6279	0.5263	0.3729
3	0	1.402	0.5263	0.2269
	7.5	1.294	0.5263	0.03611
	29.9	1.018	0.5263	-0.5885
	75	0.6279	0.5263	0.3729
4	0	1.454	0.523	-0.08525
	7.5	1.275	0.523	0.151
	29.9	0.9355	0.523	-0.09388
	75	0.6853	0.523	0.02816
5	0	1.44	0.5228	-4.122e-007
	7.5	1.3	0.5228	4.546e-007
	29.9	0.92	0.5228	3.575e-007
	75	0.69	0.5228	2.451e-007

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	5.944999	5	-1.889998
A2	8.698072	8	-1.396144
A3	5.944999	5	-1.889998
R	0.3138778	2	3.372244
2	5.674097	3	-5.348194
3	5.674097	3	-5.348194
4	5.925156	4	-3.850311
5	5.944999	5	-1.889998

Additive constant for all log-likelihoods = -36.76. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	16.77	6	0.01017
Test 2	5.506	3	0.1383
Test 3	5.506	3	0.1383
Test 4	0.5418	2	0.7627
Test 5a	0.5418	2	0.7627
Test 5b	-1.733e-010	0	N/A
Test 6a	0.03969	1	0.8421
Test 6b	0.5021	1	0.4786
Test 7a	5.649e-013	0	N/A

Test 7b	0.5418	2	0.7627
Test 7c	0.03969	1	0.8421

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than .1. Model 2 seems to adequately describe the data.

The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is greater than .05. Model 4 does not seem to fit the data better than Model 2.

Degrees of freedom for Test 7a are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 7b is greater than .05. Model 5 does not seem to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

Specified Effect = 0.400000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	15.187	8.27351	37.4221
3	15.187	8.27351	44.9118
4	8.95774	2.58601	33.3712
5	10.8243	2.61069	39.7607

Attachment B

3M'S COMMENTS ON ATSDR TOXICOLOGICAL PROFILE FOR PERFLUOROALKYLS

John Banovetz, Ph.D.
Senior Vice President and
Chief Technology Officer

3M Research & Development

3M Center, Building 220-14-W-06
St. Paul, MN 55144-1000
651 736 9112 Office
jbanovetz@mmm.com

August 20, 2018

Division of Toxicology and Human Health Sciences,
Agency for Toxic Substances and Disease Registry,
1600 Clifton Rd. NE, MS F–57
Atlanta, GA 30329
Attn: Docket No. ATSDR– 2015–0004

Subject: 3M Company's Comments of ATSDR Draft Toxicological Profile for Perfluoroalkyls

The 3M Company (3M) appreciates the opportunity to review and provide comment on ATSDR's "Draft Toxicological Profile for Perfluoroalkyls" (Draft Profile). As we highlight here and address in our detailed comments, we believe there are major shortcomings with the current draft, especially with the proposed minimal risk levels (MRLs). Considering the strong interest by the general public and others, it is important that this profile reflect the best science and full weight of evidence known about these chemicals. At present, it does not.

3M's Voluntary Phase out and Declining PFOA, PFOS, and PFHxS

As a science-based company, 3M has substantial experience and expertise with the breadth of topics addressed by the Draft Profile. In fact, numerous 3M scientists are authors or contributors to many of the studies referenced in the report, especially in the areas of toxicology, pharmacokinetics, biomonitoring, and epidemiology. 3M also was first to sponsor the development of several physiologically-based pharmacokinetic models (PBPK) regarding perfluoroalkyls.

As you know, 3M announced in 2000 that it would voluntarily phase out the manufacture and use of PFOS and PFOA (and their related materials). This was completed worldwide by about 2008. 3M phased out these chemicals due to their persistence. We did not believe there was evidence of actual adverse health effects in humans at that time, and the body of literature available to date, when properly assessed, continues to confirm this position.

After 3M announced that it would voluntarily phase out of these chemistries, other manufacturers began to phase out of production and use of PFOA under EPA's Stewardship plan. As a result of the phase-out, the levels of PFOS and PFOA in the blood of the general population in the US have declined and are expected to continue to decline. Data from the American Red Cross show that, as of 2015, levels of PFOS and PFOA among these study subjects had declined 70-80% since 2000. Similar percentage have declined in the general U.S. population through 2013 – 2014 as published by NHANES. This is important

information for the public, which is absent in the current Draft Toxicological Profile for Perfluoroalkyls. Because people may erroneously equate presence with harm, levels found in the environment must be understood in the context of the weight of the evidence showing the lack of harm from perfluoroalkyl exposure at such levels.

The body of scientific evidence does not show adverse health effects in humans from perfluoroalkyls

The vast body of scientific evidence does not show that PFOS or PFOA cause any adverse health effects in humans at current exposure levels, or even at the historically higher levels found in blood. ATSDR acknowledges that there is no cause and effect, when it states: "The available human studies have identified some potential targets of toxicity; however, cause and effect relationships have not been established for any of the effects, and the effects have not been consistently found in all studies." However, ATSDR does not present this critical point until page 636 of the draft profile.

A recently released review of studies involving perfluoroalkyls exposed populations commissioned by the Australian government also supports the lack of evidence of harm. That May 2018 report by the Australian Expert Health Panel stated, "The Panel concluded there is mostly limited or no evidence for any link with human disease from these observed differences. Importantly, there is no current evidence that supports a large impact on a person's health as a result of high levels of perfluoroalkyl exposure." The report further stated: "After considering all the evidence, the Panel's advice to the Minister on this public health issue is that the evidence does not support any specific health or disease screening or other health interventions for highly exposed groups in Australia, except for research purposes."

ATSDR's Public Health Role Mandates that it Revise the Draft Toxicological Profile for Perfluoroalkyls

ATSDR's states that the "primary purpose" of the draft Toxicological Profile for Perfluoroalkyls is to provide "public health officials, physicians, toxicologists" and others "with an overall perspective on the toxicology of perfluoroalkyls" (p. 21). ATSDR does not meet this goal, especially with respect to the MRL development, because it relies on flawed and incomplete data and because the conclusions it draws are unjustified by the data on which it relies. These errors require a wholesale revision of the draft Toxicological Profile and a new round of comments on any revised profile.

For many stakeholders, MRLs may be the most important component of the draft Toxicological Profile for Perfluoroalkyls. Media accounts clearly show there is already great confusion among the public, Congress, the media and NGOs as to their meaning and how MRLs should or should not be used. Some erroneously believe that MRLs are a bright line between safe and unsafe. It is imperative, therefore, that ATSDR clearly educate readers on the use and meaning of MRLs in Chapter One, where the MRLs are first presented, and not where they currently appear, over 600 pages later deep in the technical appendices.

Stakeholders reading the draft profile need to clearly understand that ATSDR has said that:

- MRLs "are not intended to define clean up or action levels"
- MRLs are "intended only to serve as a screening tool"
- MRLs "are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects"
- "Exposure to a level above the MRL does not mean that adverse health effects will occur."
- An "MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals"
- "If someone is exposed to an amount above the MRLs, it does not mean that health problems will happen."

The Proposed MRLs Fail to Reflect the Best Available Science

Overall, the provisional MRLs proposed by ATSDR for PFOA, PFOS, and PFHxS were not derived using best available science. There were many deficiencies and unnecessarily conservative and scientifically flawed assumptions associated with these MRLs. They should be withdrawn or revised to reflect a more realistic and scientifically supported risk assessment. As more fully set forth in our comments, key concerns with these MRLs include, but not are limited to:

- Greater consideration should be given by ATSDR to the non-human primate studies that exist in the literature for PFOA and PFOS, as was done by ATSDR in 2015. In addition, ATSDR selection for PFOA and PFOS did not consider the more recently available human and non-human primate studies. 3M believes ATSDR should seriously consider these studies in their approach to MRL as they either do or more closely represent human physiology; and, have relevance to questions regarding thyroid, cholesterol, and liver evaluations. These include a Phase 1 clinical trial in humans for PFOA (Convertino et al. 2018) and a one-year evaluation of clinical chemistries in non-human primates for PFOS (Chang et al. 2017).

- ATSDR selected inappropriate studies to serve as basis for the proposed MRL for PFOA which ***lacked fundamental scientific rigor***, including such shortcomings as: (1) use of only single dose level, making it impossible to confirm a dose-response effect, or to determine the point of departure level; (2) involved too few animals to generate reliable results; (3) provided no details on the reproductive nor the developmental hallmarks; (4) litter bias; (5) used non-standard testing methods; and (5) provided no internal serum PFOA dosimetry data. The corresponding study results should not be used in any meaningful risk assessment for humans and are wholly inadequate to form the basis for a PFOA MRL.

- PFOA, PFOS, and PFHxS MRLs are biased (downward) because ATSDR used serum half-lives that do not accurately reflect the most reliable and current evidence on human serum half-lives applicable to the general population. Had it done so MRL

values would have ranged between 9 - 40% higher for PFOA, 12 – 38% higher for PFOS, and 14-38% higher for PFHxS;

- ATSDR applied scientifically flawed uncertainty factors that lowered the MRLs by as much as an order of magnitude or more, including: (1) use of an uncertainty factor of three for interspecies extrapolation (animal to human) for PFOA, PFOS and PFHxS, even though that rodents are known to be more sensitive than humans to the effects at issue; (2) use of an uncertainty factor of 10 in its PFOS and PFHxS MRL derivations to account for potential immunological effects that was arbitrary, not justified by toxicology and epidemiologic studies, and contrary to ATSDR's acknowledgement that the human evidence for immune effects is insufficient to support causation; and (3) use of an inappropriate uncertainty factor of 10 for PFOA for a LOAEL-to-NOAEL extrapolation because the study design was so deficient so as to preclude even establishing any LOAEL or NOAEL values.

Epidemiological Associations Claimed by ATSDR are Not Supported by the Science

In addition, the draft Toxicological Profile for Perfluoroalkyls identified eight potential epidemiological associations between perfluoroalkyl exposure and health outcomes. The relevant body of science for these chemicals does not support ATSDR's position. As our detailed comments show, the scientific evidence clearly refutes the claimed associations and shows that ATSDR must revisit its analysis. In addition, ATSDR actually acknowledges that none of these associations indicate causality (see above comment on page 2 of this letter). To minimize undue public misperceptions and undue fears, ATSDR must place this admission prominently at the beginning of the report, before any discussion of the alleged epidemiological associations between perfluroroalkyl exposure and health outcomes.

Many Other Concerns and Deficiencies Require Revisions to the Draft

Our detailed comments outline many other concerns with the draft Toxicological Profile for Perfluoroalkyls, including, but not limited to: (1) significant new studies were not considered by ATSDR; (2) a lack of transparency in ATSDR's synthesis of its weight-of-the-evidence review for the eight epidemiological associations or key toxicological endpoints; and (3) a failure to address declining levels of PFOS and PFOAs in the general population.

Finally, because of the 852-page length of the draft profile, along with its nearly 300-page supporting document, the 60-days provided to the public for review and comment was not adequate for detailed review and comment on every aspect of the draft Toxicological Profile for Perfluoroalkyls. Accordingly, the lack of comment on any particular detail or section within this ATSDR document does not necessarily imply agreement by 3M with that content.

ATSDR Must Further Review and Revise the DRAFT Toxicological Profile for Perfluoroalkyls

3M appreciates the opportunity to provide its comment on the draft Toxicological Profile for Perfluoroalkyls. The document represents a significant undertaking by ATSDR, but it needs

to be based on current, relevant and reliable scientific information to be accurate and useful to multiple stakeholders. As highlighted here and in our detailed comments, the shortcomings with the current draft, including the proposed MRLs require that ATSDR perform additional work to assure that the profile reflect the best science and full weight of evidence known about these perfluoroalkyls.

If there are questions or comments concerning this matter, please contact me.

Sincerely,

John Banovetz

John Banovetz, Ph.D.
Senior Vice President and Chief Technology Officer

Executive Summary of 3M's Comments

The 3M Company (3M) appreciates the opportunity to review and comment on the "Draft Toxicological Profile for Perfluoroalkyls". As authors or a sponsor of many of the human epidemiology and toxicology studies discussed in the draft documents, we offer these detailed comments for Health Effects in assisting with that effort. Given the magnitude of scientific literature that have become available since the last Draft was released in 2015, the following important scientific comments should be considered by ATSDR with the overall data integration.

A. The Public Comment Period was Too Short. The Draft Toxicological Profile is 852 pages long. Its support document is nearly 300 pages long. The 60-days provided to the public for review and comment was not adequate for detail review and comment on every aspect of the draft Toxicological Profile. Accordingly, the lack of comment on any particular detail or section within this ATSDR document does not necessarily imply agreement with that content.

B. MRL Meaning and Limitations Not Prominently Presented. ATSDR should be aware that for the public and regulators the Minimum Risk Levels (MRLs) will be an important component of the draft Toxicological Profile. Yet, ATSDR defers any explanation of what the MRLs mean and the limits on their use until deep in the technical appendices of this document (e.g., page 713 in Appendix A and page in Appendix C). Accordingly, it is very important that ATSDR features this information in Chapter 1, where ATSDR presents the MRL values. ATSDR should recognize that most readers will not go any further than this opening chapter. Media accounts show there is already great confusion among the general public, Congress, the media and NGOs as to what MRLs values mean and how they should or should not be used. There is a clear misperception that MRLs represent a line between safe and unsafe exposure to a chemical, which is incorrect.

ATSDR should include the following statements from the technical appendices in Chapter 1.

From Appendix A (page A-1, page 713 of the profile), ATSDR should include:

- *An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be* ***without appreciable risk of adverse noncancer health effects*** *over a specified route and duration of exposure. These substance-specific estimates, which are* ***intended to serve as screening levels****, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that* ***MRLs are not intended to define clean-up or action levels****.*
- *They are* ***below levels that might cause adverse health effects in the people most sensitive*** *to such chemical-induced effects.*
- *MRLs are generally* ***based on the most sensitive substance-induced endpoint*** *considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs.* ***Exposure to a level above the MRL does not mean that adverse health effects will occur****.*

- *MRLs are **intended only to serve as a screening tool** to help public health professionals decide where to look more closely.*
- *In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, **the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic** in laboratory animals*

From Appendix C (page C-1, page 835 of the profile), ATSDR should include:

- *These MRLs are **not meant to support regulatory action**, but to acquaint health professionals with exposure levels **at which adverse health effects are not expected to occur** in humans.*
- *MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.*

Finally, ATSDR's website includes a description of MRLs for the general public, which should also be included to help the lay public:

- *An MRL is an estimate of the amount of a chemical a person can eat, drink, or breathe each day **without a detectable risk to health**. MRLs are developed for health effects other than cancer. **If someone is exposed to an amount above the MRLs, it does not mean that health problems will happen**. When health assessors find exposures higher than the MRLs, it means that they may want to look more closely at a site.*

C. The PFOA, PFOS, and PFHxS MRLs are Critically Flawed, Lower than Appropriate or Necessary, Unsupported by the Science, and should be Withdrawn or Revised. Due to time limitations, 3M's review focused on the provisional Minimum Risk Levels (MRLs) for three perfluoroalkyls (PFOA, PFOS, and PFHxS). The selection of the critical toxicological endpoints and the derivation process in establishing these provisional MRLs lacked scientific rigor and that the best available science was not applied. The improper uses of studies and overly conservative assumptions used by ATSDR resulted in MRL values that are significantly lower than supported by the science. Key concerns with ATSDR's MRL development are presented below:

1) Toxicological endpoints and human relevance

Among the toxicological endpoints chosen by ATSDR for MRL calculations, they have not been observed in humans. ATSDR should explain the relevance of these effects, if any, to human health to avoid undue public misperception. Specifically, published mode of action data on xenosensor nuclear receptors have suggested that rodents may not be the most appropriate species for the hazard assessment of perfluoroalkyls on developmental toxicity in humans. In addition, rodent hepatocytes appeared to be more sensitive to xenosensor nuclear receptor activations than human hepatocytes. Therefore, ATSDR

should take this into consideration when performing human risk assessment using rodent data.

2) Best available science not applied

There are many technical uncertainties associated with the current MRL derivations for PFOA, PFOS, and PFHxS (all based on rodent studies), and ATSDR did not appear to apply the best available science. Specifically:

- For PFOA, the two studies selected by ATSDR lacked fundamental scientific rigor (*e.g.*, a single dose study without any dose-response, small sample size with only 6 pregnant dams; no details on the reproductive nor the developmental hallmarks, litter bias, non-standard testing methods, no internal serum PFOA dosimetry data…etc.). The corresponding study results should not be used in any meaningful risk assessment for humans. ATSDR is encouraged to consider evaluating a published phase 1 clinical trial data with PFOA in 49 human subjects for its assessment (Convertino et al. 2018).

- For PFOS, ATSDR should take maternal toxicity influence as well as human relevance under consideration. ATSDR is encouraged to consider evaluating a published clinical chemistry study with monkeys with PFOS for its risk assessment, given these non-human primates have much similar physiological resemblance to humans than those of rodents, and the effects of PFOS on 27 clinical chemistry parameters as well as the corresponding serum PFOS levels were followed for more than 400 days (Chang et al. 2017).

- For PFHxS, the thyroid histology finding in rats cannot be replicated in another rodent species (mice) under similar study conditions hence there is no conclusive evidence to suggest that PFHxS impacts thyroid homeostasis in rodents. ATSDR is encouraged to consider evaluating a published reproductive and developmental study in mice with PFHxS for its assessment (Chang et al. 2018). In addition, ATSDR should recognize that there are distinct differences in thyroid hormone regulations between rodents and humans; and similar to PPARα- or CAR/PXR-mediated hepatocellular hypertrophy noted in rats, thyroid findings in rodents are usually rodent-specific, usually not applicable to humans, and it requires careful (weight-of-evidence) interpretation when extrapolating to human risk assessment.

3) Excessive and unnecessary adjustment factors applied for point of departure (POD)

It is scientifically unjustified for ATSDR to apply a combined adjustment factor of 300 for PFOA, PFOS, and PFHxS MRLs in addition to the (large) dosimetric TK adjustments that had already been incorporated. The (very) large dosimetric adjustment factors (10,000, 14,400, and 15,500 for PFOA, PFOS, and PFHxS, respectively) more than adequately compensate for the difference between rodents and humans. The additional combined factor of 300 reflected an overall adjustment factor of 3,000,000 for PFOA, 4,320,000 for PFOS, and 4,650,000 for PFHxS from the point of departure (POD). The

extent of these adjustments, on the order of 10E6, is not made transparent by ATSDR and is excessive.

Specific uncertainty factors that are not scientifically justified include: (a) factor of 10 for immunotoxicity (PFOS, PFHxS); and (b) factor of 10 for use of LOAEL (PFOA)

4) Toxicokinetics and half-lives in humans

In their MRL calculations, ATSDR chose to use the arithmetic mean serum elimination half-life estimates for PFOA, PFOS, and PFHxS from Olsen et al. (2007) because the study of these retirees had a longer follow-up time. These retirees averaged 66 years of age at the end of the study. ATSDR was concerned that, based on a study by Seals et al. (2011), slower kinetics is likely to constitute a larger contribution to the terminal half-life. Olsen et al. had reservations of using arithmetic means to describe their data because of its right skewness; ATSDR chose to not acknowledge this limitation. In addition, ATSDR chose not to consider serum elimination half-lives that are dependent on other factors such as age of the study subjects, and not just follow-up time, because age is associated with the glomerular filtration rate (GFR). Renal clearance of perfluoroalkyls is largely a sum of three processes involving glomerular filtration, renal tubular secretion, and renal tubular reabsorption. Because PFOA and other perfluoroalkyls vary in their affinities to bind plasma proteins, glomerular filtration of perfluoroalkyls is a product of the unbound fraction of the perfluoroalkyls and GFR. Thus, the lower estimates of serum elimination half-lives based on the younger ages in the other study populations (Bartell et al. 2010; Li et al. 2018) may be due to the higher GFR of these younger study subjects. ATSDR also did not recognize that the proportion of the general population age $\geq$ 65 years old is approximately 15%. Therefore, other serum elimination half-lives should be considered in ATSDR's MRL calculations to reflect the overall general population and its greater GFR. At a minimum, ATSDR should present sensitivity analyses using these collective data (see below).

5) Underestimation of HEDs and MRLs by ATSDR using slower half-life

For PFOA, PFOS, and PFHxS, the corresponding HEDs (and subsequent MRLs) were likely to have been underestimated because ATSDR used the most conservative half-lives reported. These half-lives were based on a cohort of retired fluorochemical workers whose exposure source was occupational and the elimination profile was dependent upon a GFR reflective of older adults. ATSDR should use half-lives more closely matching the general population demographics and their GFR. This will correspond to increases in MRLs ranging between 9 - 40% higher for PFOA; 12 – 38% higher for PFOS, and 14-38% higher for PFHxS.

6) Chronic toxicology studies are available for PFOA and PFOS

Scientifically pertinent data such as 2-year chronic studies with PFOS (Butenhoff et al. 2012a) and PFOA (Butenhoff et al. 2012c) should be included by ATSDR for the weight-of-evidence consideration. In addition (to rodent data), in considering selection of

"chronic" studies, there are internationally-recognized guidance which states that "studies of 6 months duration in non-rodents are acceptable according to Council Directive 75/318/EEC, as amended" (EMEA 1999a). Therefore, non-human primate studies with PFOA (Butenhoff et al. 2002) and PFOS (Chang et al. 2017; Seacat et al. 2002) should also be considered by ATSDR. Most importantly, these studies not only encompassed extended study period (i.e., chronic exposure) but also illustrated similar toxicological endpoints.

D. Lack of comprehensive interpretation and synthesis of the epidemiological associations concluded by ATSDR

3M respectfully disagrees with the interpretation of the epidemiological associations concluded by ATSDR and offers scientific evidence to refute these opinions. Most importantly, 3M disagrees with the lack of highlighting by ATSDR that none of these associations indicate causality, as acknowledged by ATSDR (*cf.* pages 24 and 635-636). This (the absence of causation) should be highlighted on page 5 in front of the associations that ATSDR ultimately listed to minimize undue public misperception.

1) Epidemiological association: Pregnancy-induced hypertension and pre-eclampsia

ATSDR combined pregnancy-induced hypertension and pre-eclampsia into a single health outcome without providing scientific justification for combining these two distinct pregnancy outcomes. The evidence for an association between preeclampsia and PFOA/ PFOS exposure was limited to three epidemiologic studies with inconsistent findings; the strongest study methodologically reported no association. Similarly, only three studies examined the association between PFOA exposure and pregnancy-induced hypertension and also reported mixed results. The majority of studies, for both preeclampsia and pregnancy-induced hypertension, used unvalidated, self-reported pregnancy outcomes and could not establish temporality due to the cross-sectional study design. Overall, given these limitations and the inconsistencies in findings across studies, there is insufficient evidence for an association between preeclampsia and pregnancy-induced hypertension and PFOA/PFOS.

2) Epidemiological association: Hepatic enzymes

In citing an increase in liver enzymes is associated with PFOA, ATSDR neglected to simultaneously state there was no increased risk for liver disease, including enlarged liver, fatty liver, or cirrhosis. Thus, there is no liver disease-related causation with exposure to PFOA or PFOS. Furthermore, ATSDR grossly over interpreted the magnitude of influence of ALT by using the words "liver damage" associated with ALT at the concentrations reported in the literature. ALT is a leakage enzyme and may be increased due to necrosis, injury or repair. The human half-life of ALT is approximately 47 hours. Based on the recommendations of numerous regulatory authorities, increases in ALT activity of two-to threefold should be considered indicative of "hepatocellular damage**.**" Those epidemiological studies that have suggested an elevation of ALT

associated with PFOA or PFOS remain well-within the expected physiologic range of ALT, not 2 - 3 fold higher. Therefore, ATSDR's use of the term 'liver damage" is highly misleading. Furthermore, it is well-recognized in clinical pathology it is possible to have statistically significant modest increases in ALT that are not toxicologically relevant. Finally, ATSDR did not adequately mention the many confounding factors that should be considered in evaluating liver enzymes including age, sex, race, a reliable measure of obesity (not measured as just BMI), alcohol, diet, other diseases including diabetes, and genetics.

3) Epidemiological association: Increased serum total cholesterol and LDL

The ATSDR did not provide a rationale behind its suggestion of a possible biphasic response of serum cholesterol and PFOA (or likely PFOS). Although ATSDR recognized the preliminary abstract results of a phase 1 clinical trial of PFOA (ammonium salt) published in 2010 that stated observed reductions in LDL-cholesterol were consistent with a pharmacodynamic effect, ATSDR was unaware of the actual results from the clinical chemistry assessment from this phase 1 trial that have been publicly available via its Advance Access in Toxicological Sciences in February 2018 with final publication in the May 2018 issue (Convertino et al. 2018). ATSDR is strongly encouraged to carefully consider the Convertino et al. (2018) publication and its ramification(s) in ATSDR's weight of evidence review for PFOA related to cholesterols (as well as liver enzymes and thyroid hormones). The findings from this human phase 1 clinical trial showing that cholesterol is lowered at high doses of PFOA are consistent with some animal models and the hypolipidemic activity of the xenosensor nuclear receptor PPARα agonist PFOA. ATSDR should assess plausible noncausal roles of biology and physiology at the very low PFOA concentration (4+ orders of magnitude lower than Convertino et al.) that have been reported in the conflicting observational studies.

4) Epidemiological association: Increased risk of thyroid disease

There are no consistent associations reported across the studies found in the epidemiologic literature regarding thyroid hormones or specific thyroid disease (hypothyroidism, hyperthyroidism) as related to exposure to PFOA or PFOS. ATSDR's review of the thyroid literature is disjointed and provides minimal rationale to how ATSDR reached a decision that an association exists between PFOA/PFOS and increased risk of thyroid disease. This confusion is caused, in part, by the highly inconsistent evidence presented in the epidemiologic literature. Therefore, in the draft 2018 Toxicological Profile, ATSDR should acknowledge the lack of consistent evidence of an association.

5) Epidemiological association: Decreased antibody response to vaccines

Among the epidemiologic studies cited by ATSDR, antibody responses to 8 distinct vaccines were measured. Given that observed changes in antibody response to a particular vaccine type should not be interpreted as consistent with changes in the

antibody response to another vaccine type, the ATSDR should consider immune responses to individual vaccines as distinct health outcomes. Mostly null findings were reported across all studies for PFOA, PFOS, PFHxS, and PFDeA. Furthermore, most studies have found no association between PFAS levels and increased incidence of infectious disease (or lower ability to resist or respond to infectious disease). As such, the absence of clinical immunosuppression along with inconsistent findings both within and across studies, do not support the ATSDR conclusion "s*uggestive of a link between serum PFOA, PFOS, PFHxS, and PFDeA levels and decreased antibody responses to vaccines".*

6) Epidemiological association: Increased risk of asthma diagnosis

Prospective cohort studies have consistently reported no association between PFOA and asthma. Conversely, cross-sectional and case-cohort studies have reported inconsistent findings and were limited by temporal ambiguity, and unvalidated, self-reported asthma diagnosis. NTP (2016) recognized these limitations and concluded that "*there is low confidence that exposure to PFOA during childhood is associated with increased hypersensitivity responses based on the available studies*". The rationale for this conclusion was "*primarily due to the cross-sectional nature of the studies and uncertainty as to whether exposure levels reflect exposure prior to the development of hypersensitivity*." Therefore, collectively, the existing epidemiologic evidence does not support an association between PFOA exposure and asthma risk.

7) Epidemiological association: Increased risk of decreased fertility

ATSDR incorrectly concluded an association exists between increased perfluoroalkyls (PFOA, PFOS) and decreased fertility based on epidemiologic studies. In its 2018 draft Toxicological Profile, ATSDR failed to discuss methodological issues that have been repeatedly discussed in the published epidemiology literature, in particular, those surrounding the metric of time-to-pregnancy and the amount of interpregnancy time for reaccumulation of PFOA or PFOS. Women with longer interpregnancy intervals would have longer time for reaccumulation; thus the potential for reverse causation to be observed in parous women with time to pregnancy. As reviewed in their systematic review of the reproductive epidemiology literature regarding perfluoroalkyls, Bach et al. (2016) reported of the 8 epidemiologic studies reviewed related to time to pregnancy, only one study found an association when restricted to nulliparous women; 4 studies reported an association with parous women that Bach et al. (2016) concluded was not causal but likely the result of reverse causation and unmeasured confounding related to prior pregnancies and childbirths that could influence the measurement of PFAS.

8) Epidemiological association: Small decreases in birthweight

ATSDR incorrectly concluded that an association exists between lower birthweight (< 20 gm) and PFOA. ATSDR very briefly discussed two meta-analyses published by Johnson et al. (2014) and Verner et al. (2015). Unfortunately, several important issues were not discussed via the historical context of these two meta-analyses, including understanding

the relationship between maternal glomerular filtration and fetal growth. In addition, ATSDR was not aware of two more recent meta-analyses (Negri et al. 2017; Steenland et al. 2018). Negri et al. questioned the lack of a quantitative toxicological evidence to support the biological plausibility of a causal association in humans. The study abstract from Steenland et al. was recently published on-line in the journal *Epidemiology*. Based on their meta-analysis of 25 studies (that included one previously excluded large study), Steenland et al. reported an association of -1.0 grams (95% CI -2.4, 0.4) per ng/mL PFOA. Restricting the studies to where blood samples for PFOA measurement were collected in early pregnancy (or even shortly before conception), the time period identified by Verner et al. in their PBPK simulations where confounding by maternal glomerular filtration rate would be of least concern, Steenland et al. reported a meta-analysis nonsignificant estimate of -3.3 gm (95% CI -9.6, 3.0) per ng/mL PFOA; thus further indicating a lack of an association between lower birthweight and PFOA.

Detailed Comments on PFOA MRL

ATSDR position (page A-16)

MRL Summary: A provisional intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day was derived for PFOA based on altered activity at 5–8 weeks of age and skeletal alterations at 13 and 17 months of age in the offspring of mice fed a diet containing PFOA on GD 1 through GD 21 (Koskela et al. 2016; Onishchenko et al. 2011). The MRL is based on a HED LOAEL of 0.000821 mg/kg/day and a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability).

Selection of the Critical Effect: Intermediate-duration oral studies of PFOA in animals indicate that the liver, immune system, reproductive system, and the developing organism are the primary targets of toxicity because adverse outcomes were observed at lower doses than other effects and have been consistently observed across studies.

3M Conclusion

A. Studies by Onishchenko et al. (2011) and Koskela et al. (2016) should not be used to derive the PFOA MRL
B. The critical effects cited by ATSDR for the PFOA MRL derivation (altered activity and skeletal alterations in offspring in mice) were not supported by the available animal data, and they contradicted ATSDR's own evaluation of epidemiological data
C. PFOA does not affect the reproductive system in laboratory animals
D. The developmental effects reported in laboratory animals for PFOA were primarily mediated by maternal effects
E. Liver findings in rodents are not relevant for human risk assessment
F. Immune findings in rodents are not consistent; they lack concordance with epidemiological observation data
G. A study with one single dose group is not adequate in estimating point-of-departure
H. Serum PFOA concentrations in pups should be considered for POD instead of dams because critical effects chosen by ATSDR were based on (developing) pups
I. HED cannot be reliably estimated in the absence of serum concentration data
J. HED for PFOA will be higher when considering faster half-life
K. Wambaugh benchmark dose model used by ATSDR was not optimized
L. Uncertainty factors by ATSDR were conservative and not supported by scientific data
    1. Incorrect use of "10" for a LOAEL.
    2. Use of "3" for animal-to-human, in addition to large dosimetric TK adjustment, is conservative because humans are less sensitive than rodents with exposure to PFOA

ATSDR's overall interpretation on both toxicology and epidemiology data are inconsistent with the most current knowledge. Its application of uncertainty factors is not scientifically justified and the proposed PFOA MRL is not supported by the scientific data. The PFOA MRL derived for the human-health risk assessment is therefore inappropriate and not justified by an adequate scientific foundation.

3M Comments (Details):

A. Studies by Onishchenko et al. (2011) and Koskela et al. (2016) should not be used to derive PFOA MRL. The toxicology database for PFOA is quite comprehensive. Many of these studies included detailed information on the reproductive and developmental toxicity with these compounds across different PFOA dose levels as well as valuable insights on the role of maternal effects and its attribution to the developmental outcomes in laboratory animals. Comprehensive review on the potential developmental toxicity of PFOA in laboratory animals was reported in 2004 (Kennedy et al. 2004; Lau et al. 2004) and updated subsequently (Abbott 2015; Andersen et al. 2008; Lau 2012; Lau et al. 2007). Despite the wealth of data available, ATSDR chose mouse developmental studies reported by Onishchenko et al. (2011) and Koskela et al. (2016) as reference studies for its derivation of PFOA MRL (based on altered activity and skeletal alterations seen in offspring in mice).

   ATSDR's assessments on these studies (and the corresponding reported critical effects) failed to make clear to the public that the proposed MRL did not reflect the absence of an association between PFOA exposure and musculoskeletal outcomes or neurological outcomes in humans (cf. pages 141 – 145; pages 293-296). Furthermore, there are major technical concerns associated with these studies that preclude the results (from these studies) to be meaningful in any human risk assessment. They include:

   1. They are the same study. Albeit published five years apart, these two publications actually originated from one single study. From the same pregnant dams treated with dietary PFOA during gestation, the pups evaluated by Onishchenko et al. (2011) were litter-mates of the pups evaluated by Koskela et al. (2016). As such, it was really one study (in essence) and the corresponding outcomes (from both studies) should be consolidated when discussed.

   2. A single dose experiment cannot address (any) dose-response relationship. There was only one PFOA dose group used in these two studies and as such, it is impossible to interpret the experimental data reported by these authors in terms of any dose-response. Considering the inherent variations in biological responses in any animal study, the nature of a single-dose study simply does not allow any specific evaluation of any dose-and-effect responses or biological plausibility inference.

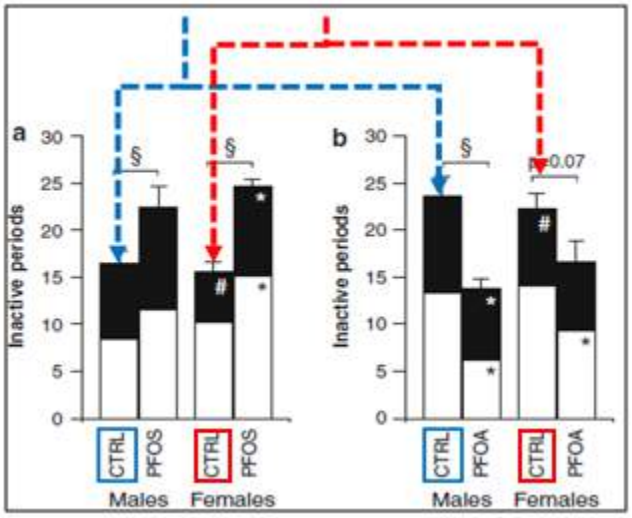
      Using a study that evaluated a single PFOA dose group was in absolute contradiction of what ATSDR stated in its MRL approach. On page A-6 of the draft profile, ATSDR explicitly stated that one of the MRL approach was to "*Identify laboratory animal studies that have evaluated dose-response relationship for toxicity targets identified in epidemiology studies*".

      Hence for PFOA, not only did ATSDR not identify musculoskeletal or neurological outcomes as sensitive endpoints in humans; it did not select a laboratory animal study that appropriately addressed or evaluated dose-response relationship.

3. <u>The study design was flawed and insufficient to support a NOAEL or LOAEL.</u> Again, given that there was only PFOA dose group used, the study design did not follow the fundamental practice of toxicology testing such as evaluation of a dose response relationship. Hence, given the lack of any dose-response, it is scientifically impossible to establish a realistic NOAEL and/or LOAEL for the data reported.

4. <u>Limited sample size.</u> There were only 6 dams that received PFOA diet to produce the pup cohort, and there was a total of 10 dams that received control diet; however, the control animals spanned from two (separate) blocks of individual experiments. The sample size for the study was quite small and given that only a single PFOA dose group was used, it is impossible to properly address biological plausibility (if any) and background variability.

   For example, regardless of sex, Onishchenko et al. (2011) reported a statistically significant difference between control and PFOA pups for the number of inactive periods (Figure 3b). However, on the accompanying graph (Figure 3a), they also reported a statistically significant difference between control and female pups from PFOS dose group for the number of inactive periods. Without looking at the treatment groups and just comparing the sex-matched control responses alone between Figure 3a and Figure 3b (see illustration below), it became very apparent the large variations exist even in the sex-matched control animals. This large variation (on the background control alone) most likely attributed to the statistical significance when compared to the treatment groups (either PFOS or PFOA).



   Another similar example is on the body weight. The absence of statistical power to address inherent biological variations due to the limited study design did not allow for a valid comparison of biological responses between control and treatment. While Koskela et al. (2016) reported an increase in the body weight in the female pups from PFOA-

treated group with statistical significance at 13 months and 17 months; however, the difference was already present at birth (as stated by the authors) hence the reported difference may well have reflected normal variation which cannot be adequately demonstrated as there were insufficient animals and litters.

5. Lack of reproduction (pregnancy) outcome information. Given the study design included the gestation and lactation periods, it was perplexing that very little information on the pregnancy or lactation outcomes were discussed by the authors (*e.g.*, gestation length, number of implantation, litter size, sex ratio, or lactation performance). All these are critical in evaluating the quality of the study.

6. Lack of litter outcome information. Given the study design included the developmental phase of pups, it was also perplexing as to why the authors did not disclose any detailed litter outcomes from dams received PFOA treatment (*e.g.*, survival, birth weight, anogenital distance, nipple retention, onset of number of implantation, gestation length, litter size, sex ratio, onset of sexual maturation…etc.) All these are critical in evaluating the quality of the study.

7. Questionable pup selection bias / litter bias. It was unclear as to how the pups were selected for the evaluations. To rule out litter-related effects, it is a standard practice for pups from the same litter to be evaluated as one single unit (rather than individual pups) in the assessment of reproductive and developmental outcomes in laboratory animals (OECD 2007, 2016). Given that there were only 6 dams that received PFOA treatment, therefore, the maximum number of pups from PFOA dose group should be 6 (*i.e.*, one pup per litter). Depending on the endpoints, the authors reported the data based on 6 – 10 pups, which would indicate that the pup selection was confounded by litter effect; and subsequently, the study findings were also confounded by litter effects.

8. Questionable dietary preparation. In the studies by Onishchenko et al. and Koskela et al., pregnant dams were administered with dietary PFOA throughout gestation for a total of 21 daily doses (as described by Koskela et al. 2016). According to the study authors, PFOA was dissolved in 95% ethanol first and then applied on food pellet. The pellets were kept on the bench for 2 hours (presumably at room temperature) to allow for ethanol evaporation prior to feeding them to the animals.

   This was a very crude method of preparing a dietary formulation – there were no information on the final PFOA concentration achieved in the diet and there was no information on the homogeneity distribution of PFOA in the diet. All these parameters were essential in contributing to a good dietary study and none of the information was available or explained by the study authors.

9. Possible residual ethanol present in the dietary PFOA chow. In addition to the crude dietary preparation method, the study authors assumed that the 95% ethanol used to dissolve PFOA would have been completely evaporated within 2 hours after sitting on the bench (presumably at room temperature), however, there were no supporting data to prove this. It is well-known that pure ethanol does evaporate faster than water on the

basis of higher vapor pressure, lower boiling point, and less hydrogen bonds (Innocenzi et al. 2008). When ethanol is mixed with water, more hydrogen bonds are created; and when ethanol-in-water mixture is further mixed with PFOA as well as applied onto the surface of food chow (such as this study), the additional intramolecular forces (between ethanol and water, ethanol-in-water and PFOA, and, ethanol-in-water and PFOA and food chow ingredients) would have reduced the overall volatility of ethanol. The authors should have obtained a quantitative measurement of the PFOA/chow mixture to demonstrate the absence of ethanol after 2-hour evaporation.

This verification step was critical for this study because the authors evaluated and reported neurobehavior endpoints as findings. Albeit the control animals also received food chow diet that had been applied with 95% ethanol followed by evaporation, however, the intramolecular force between ethanol, water and food chow (i.e., control food chow) would be different than the intramolecular force between ethanol, water, PFOA, and food chow (i.e., PFOA food chow). Given that ethanol is well-known for its effects on the central nervous system (Boschen and Klintsova 2017; Harrison et al. 2017) and 95% ethanol was used in the study, any ethanol that had not evaporated and remained on the food chow could have confounded the study results, especially on the neurobehavior parameters.

10. <u>There were no serum PFOA data reported in these studies.</u> ATSDR has determined that, rather than relying on external dose, serum PFOA concentration (internal dosimetry) is the appropriate exposure matrix when determining a point-of-departure (POD) for the MRL derivation with PFOA (*cf.* page A-16 and Table A-7 on page A-24 of the draft profile). Neither Onishchenko et al. (2011) or Koskela et al. (2016) reported any information on the serum PFOA concentrations; and this was a major deficiency of the study. Even though ATSDR "estimated" the time-weighted-average serum PFOA concentration based on its PBPK model, the absence of serum PFOA data preluded the verification of the ATSDR PBPK model, in addition to the other unknowns associated with the study (*i.e.*, no dose-response and no dose verification).

    It is also worth noting that the study authors had the technical capability to perform PFOA analysis because Onishchenko et al. (2011) reported PFOA concentrations in a subset of pup brain and liver samples.

11. <u>Timing of behavior assessments in pups were not appropriate.</u> In the study data reported by Onishchenko et al. (2011), numerous neurobehavior endpoints were evaluated by the study authors. Given that the study was done under non-GLP protocols and by a university research lab(s), most of the timings and behavior assessment procedures (as described by the study authors) did not appear to follow the conventional recommendations and methodology. As a result, it is difficult to determine the quality of the data that had been reported. For instance, compared to the OECD 426 test guideline (TG) for developmental neurotoxicity study (OECD 2007), these authors did not follow standardized timeline recommended to FOB evaluations for the developing pups. The table below is a side-by-side comparison between the OECD 426 TG recommendation timeline vs. what Onishchenko et al. did. It was apparent that Onishchenko et al. had

missed critical windows for the assessments on many key parameters (i.e., no behavior assessments were done prior to weaning) and there were no specific references or rationales to explain or justify their study design.

	OECD 426 TG Recommendation for developmental neurotoxicity study	Study by Onishchenko et al. 2011
Dosage	Control + 3 dose levels	Control + 1 dose level
Animal number	20 litters / group	6 litters / group
Detailed clinical observation	20 pups /sex (1 / sex/ litter)	6 – 10 pups / sex
Brain weight PND 11-22	10 pups / sex (1 / litter)	No data reported
Brain weight PND 70	10 pups / sex (1 / litter)	No data reported
Neuropathology PND 11-22	10 pups / sex (1 / litter)	No data reported
Neuropathology PND 70	10 pups / sex (1 / litter)	No data reported
Sexual maturation	20 pups /sex (1 / sex/ litter)	No data reported
Behavioral ontogeny (e.g., righting and reflex)	2X prior to weaning at PND 21	No data reported
Motor activity	1-3X prior to weaning at PND 21; 1X during PND 60-70	None prior to weaning; 1X during PND 35 – 56;
Motor and sensory function	1X during PND 23-27; 1X during PND 60-70	None prior to weaning; 1X during PND 90 - 120
Learning and memory (~ PND 23-27 and 60-70)	1X during PND 23-27; 1X during PND 60-70	None prior to weaning; 1X during PND 35 – 56;

12. Non-standard behavior assessment procedures used in pups. Among the behavior endpoints evaluated by Onishchenko et al., given that the study was done under non-GLP by university research lab(s) and it did appear that the tests were done on a single day without further repeat(s) later, it raised the question as to the overall reliability and reproducibility of the instruments and the corresponding data generated.

    For instance, to measure and record circadian activity in the home cage, the TrafficCage™ used by Onishchenko et al. is shown in the picture below (obtained from manufacturer's website). Compared to the conventional 3-D photo beam boxes where movements were recorded in vertical, horizontal, and lateral directions, the TrafficCage™ system lacks the ability to measure any vertical movements. In addition, the TrafficCage™ system has several "dead spots" without any sensors. The validity of the instrument and the corresponding results generated (circadian activity) are questionable.

Illustration of TrafficCage™

(Source: https://www.tse-systems.com/product-details/phenoworld/trafficage?open=3806#trafficage-3806)

13. No information on background data for bone morphology and bone density. Koskela et al. (2016) reported that female offspring from PFOA-treated dams had increased femoral periosteal area and decreased mineral density of tibias, hence ATSDR concluded that "skeletal alterations in offspring" was a critical effect with PFOA exposure in mice.

    Bone morphology is a collective description on the shapes (geometry) of the bones, such as long bones (*e.g.*, femur and tibia), short bones (*e.g.*, bones of the feet and hands), or flat bones (*e.g.*, calvaria or breast bones). There are many factors contributing to the morphological sizes of the bones. The morphology of bone is not a "fixed" static structure, rather, it is a composite structure that will continue to evolve like other organs in the body. While the components of the bones are maintained in a balanced manner, there are also inherent biological variability within each component that needs to be taken into account when determining the overall homeostatic status of the bones (Boskey and Coleman 2010; Jepsen 2009).

    It is well-known that age and body weight are two factors in establishing the size, mass, and strength of the bones (Iwaniec and Turner 2016). In the data reported by Koskela et al., there was a pre-existing difference in body weight in female pups at birth where higher body weight was consistently observed in these female pups from PFOA-treated groups; and that difference reached statistical significance at 13 months and 17 months (*vide supra*). Therefore, it should not be a surprise that increased bone sizes in offspring with higher body weight (*e.g.*, offspring from PFOA-treated dams) had increased periosteal and medullary areas in both femurs and tibias. On the other hand, given the small sample size of the animals used in this study, the inherent background variation cannot be ruled out. For example, compared to control, the study authors also reported a decrease in mineral density in tibias in offspring born from PFOA-treated dams. The extent of decrease was very minor (only 2.5%) and it was only observed in tibias, not in femurs. Because the study authors did not have any additional information on the

background data with regards to these parameters, this minor difference may be well within the normal biological variations (again, especially with such small sample size).

14. Mechanical determinants of bone functions were not affected in pups from PFOA-treated dams. Based on study data reported by Koskela et al. (2016), ATSDR concluded that there were skeletal alterations in offspring from PFOA-treated dams and deemed it to be a critical health effect. However, in the same cohort of pups, Onishchenko et al. (2011) reported motor and sensory function assessments (muscle grip strength and rotarod test) and found no differences in the outcomes between control and PFOA-treated groups. Given that muscle force is a strong determinant of bone integrity, the slight morphological difference noted by ATSDR possibly reflected the normal background variations in this strain of mice and not likely due to PFOA.

15. Lack of supporting evidence on the effect of PFOA and bone development. If PFOA exposure does have a direct (causal) effect on the bone development, then one would expect such effect to be even more pronounced under longer (repeated) dose conditions. This was not the case, as long-term toxicology studies in rodents and non-human primates have not identified bone as a target tissue with exposure to PFOA (Biegel et al. 2001; Butenhoff et al. 2002; Butenhoff et al. 2012b).

16. Other technical comments about the study data by Koskela et al. (2016).

    - In addition to the likely litter-bias that has been discussed earlier, it is unclear why Koskela et al. only included female offspring in their evaluation but not male offspring.

    - PFOA has a high affinity to binding with serum albumins and given that bone marrow is the hemopoietic origin of blood, one should not be surprised to find trace level of PFOA in the bone. Albeit Koskela et al. claimed that bone marrow had been flushed out and only the hard bones were powdered and analyzed for PFOA content, it is important to recognize that the bone consists of "live" mesenchymal cells with lots of protein components (chondrocytes, osteoblasts, and osteocytes), not just marrow (Boskey and Coleman 2010; Iwaniec and Turner 2016; Jepsen 2009).

    - The study authors only evaluated long bone morphology but not others. If bone is indeed a target tissue with exposures to PFOA, other bones (in addition to femur and tibia) also need to be included in the evaluation.

    - It is well-known that there are large inter-species differences in bone composition, density, quality, as well as genetic variability within the same species (Aerssens et al. 1998). Again, if bone is indeed a target tissue with exposures to PFOA, such cause-and-effect needs to be demonstrated in a dose-response fashion within the same animal model as well as other species.

- Other factors that can affect bone morphology and density should also be comprehensively evaluated before drawing a conclusion. For example, endocrine effects such as estrogen and IGF-1, essential nutrient status such as calcium and vitamin D3.

- The use of imaging devices in the assessment of bone morphology is not a new concept, and CT images have been used in both clinical settings as well as research settings. However, similar to the comments provided above on the behavior assessments provided above, Koskela et al. should have demonstrated that the validity of the micro-CT scanning technique used in their facility as well as their competency in using the instrument. Given the fact that a very small magnitude of surface area was being reported as a "statistically significant" change (in the range of 0.2 – 0.3 mm^2), it is important to validate the sources of these measurements. For example, was the instrument calibrated? Were the operator(s) trained in using the equipment? Were the acquired images analyzed by qualified radiologists who are trained in doing image interpretation?

- For any imaging-based scanning, it is absolutely critical that the object (or subject) remained steady for the duration of the scanning acquisition. Any movement during the scanning process will deviate the result. The study authors described that the bone was "*wrapped in a PBS-moistened tissue paper and inserted into a plastic tube, with the proximal end pointing upwards. The container was then placed into the chamber of the microCT device*". The description did not address attempts to prevent any movement of the bone (inside the plastic tube) during the scanning process. Given the asymmetrical shape of femurs and tibias, it is important to immobilize the bone inside the tube and any slight shift will artificially affect the image data during scanning.

Overall, the studies by Onishchenko et al. (2011) and Koskela et al. (2016) lacked scientific rigors to properly address the selected developmental endpoints and they should not be used for any human risk assessment.

B. <u>The critical effects cited by ATSDR for PFOA MRL derivation (altered activity and skeletal alterations in offspring in mice) were not supported by available animal data and contradicted ATSDR's own evaluation of epidemiological data.</u> There is insufficient evidence for an association between PFOA exposure and musculoskeletal outcomes or neurological outcomes in humans (cf. pages 141 – 145; pages 293-296). ATSDR should offer a plausible explanation as to why it believes these effects are relevant to human risk assessment.

C. <u>PFOA does not affect the reproductive system in laboratory animals.</u> It is incorrect for ATSDR to conclude that the reproductive system is one of the primary targets of toxicity with exposure to PFOA (cf. page A-16).

On the contrary, PFOA <u>did not</u> affect the functional aspects of male or female reproduction in laboratory animals. These included estrous cycles, sperm parameters, mating index, fertility index, and reproductive organ morphology. A number of studies on the reproductive

and developmental effects of PFOA in laboratory animals have been published (Abbott et al. 2007; Albrecht et al. 2013; Butenhoff et al. 2004; Gortner 1981, 1982; Lau et al. 2006; Staples et al. 1984; Yahia et al. 2010). Many of these studies included detailed information on the reproductive and developmental toxicity with these compounds across different PFOA dose levels as well as valuable insights on the role of maternal effects and its attribution to the developmental outcomes in laboratory animals.

The potential of PFOA to influence reproductive performance has been evaluated in mice, rats, and rabbits. Gestational exposure to ammonium PFOA did not affect the number of uterine implantation sites in various strains of mice such as CD-1, Sv129, PPARα knockout, and humanized PPARα (Abbott et al. 2007; Albrecht et al. 2013; Lau et al. 2006; White et al. 2007). At inhalation dose up to 25 mg/m^3/day of ammonium PFOA or oral doses up to 100 mg/kg/day given during gestation to rats did not affect mating, pregnancy, and implantation (Staples et al. 1984). Oral administration of ammonium PFOA up to 150 mg/kg/day in rats or 50 mg/kg/day in rabbits during GD 6 – 15 (period of organogenesis) also caused reduced body-weight gain, however, they did not affect the ovaries or the reproductive contents of the dams (Gortner 1981, 1982). In a two-generation reproduction/developmental study in rats (Butenhoff et al. 2004), the reproductive outcome was not affected with daily oral ammonium PFOA administrations up to 30 mg/kg/day (the highest dose used in the study). There were no effects on the mating or fertility indices in either male or female rats. Male rats had normal sperm parameters (count, motility, morphology) and female rats had regular estrous cycling with normal gestation lengths, and microscopic examination did not reveal any abnormalities in sex organs. Furthermore, effects of PFOA on reproductive organ morphologies in male non-human primates were evaluated from a six-month oral study and results indicated no abnormalities (Butenhoff et al. 2002).

D. <u>The developmental effects reported in laboratory animals for PFOA were primarily mediated by maternal effects</u>. While ATSDR concluded that developing organisms are primary targets of toxicity with exposure to PFOA (cf. page A-16), there are strong experimental evidences demonstrating that developmental effects associated with PFOA exposures in offspring are observed <u>only</u> where there were significant effects in the maternal animals. Because neither Onishchenko et al. (2011) nor Koskela et al. (2016) reported detailed maternal-related endpoints with regards to reproduction, no maternal influence discussion is possible. However, observations involving maternal effects in the outcome of the developmental toxicity, as seen in the disruption of maternal homeostasis, include the following examples:

Using the mouse developmental study data reported by Lau et al. (2006), which was the critical study chosen by U.S. EPA Office of Water for the derivation of the Lifetime Water Health Advisory for PFOA issued in 2016, there were statistically significant ($p \leq 0.05$), dose-related increases in maternal liver weight observed at doses 1 mg/kg/day ammonium PFOA or higher (the corresponding serum PFOA concentration was 21,900 ng/mL at the end of gestation). Various developmental effects were reported (*e.g.,* decreased postnatal survival, decreased body weight at birth and body-weight gain thereafter, and delays in eye openings) and they were only for litters from dams receiving 3 mg/kg/day or higher. Maternal responses clearly were present at doses that affected the fetus/neonate. In addition,

because the influence of body weight on sexual maturation is well-described in the literature, it is not surprising that Lau et al. noted altered pubertal maturations in the offspring.

The developmental toxicity of ammonium PFOA has also been studied in rats (Butenhoff et al. 2004; Gortner 1981; Staples et al. 1984) and rabbits (Gortner 1982). In these studies, no increase in malformations relative to controls was observed at oral doses up 150 mg/kg/day in rats and 50 mg/kg/day in rabbits, as well as inhalation concentrations up to 25 mg/m^3/day (6 hours/day). In the studies by Gortner and by Staples et al., any effects on fetal or pup body weight were present at dose levels equivalent to or higher than those causing effects such as body weight in the maternal animals. In a two-generation reproduction and developmental study in rats (Butenhoff et al. 2004), F1-generation pups from the highest dose group (30 mg/kg) had decreased birth weight and reduced viability that were in apparent relationship to the corresponding reduced body weight at birth and weaning. These latter effects are similar to those observed in mice by others (Abbott et al. 2007; Lau et al. 2006; Yahia et al. 2010). Even though similar to the observation by Lau et al. (2006) in that sexual maturation were slightly delayed (at the highest dose group only), there was no significant difference in F1 pups when days to sexual maturation was adjusted by (reduced) body weight.

Based on data from the large scale 2-generation reproductive and developmental studies (which are considered as the most comprehensive test by various agencies for evaluating endocrine functions), PFOA clearly did not alter the reproductive functions as the reproductive performances in both males and females were normal (*vide supra*). In addition, there is sufficient evidence in experimental animals (mammals) to suggest that rodents may not be the best model in evaluating the reproductive-related outcomes for human risk assessment. PFOA is a known activator for xenosensor nuclear receptors such as PPARα, constitutive androstane receptor (CAR), and pregnane X receptor (PXR) (Corton et al. 2014; Elcombe et al. 2010; Elcombe et al. 2014; Klaunig et al. 2003; Klaunig et al. 2012). It is well documented that PFOA causes hepatomegaly in rodents as a result of PPARα activation with some contribution from CAR and PXR. It is well-known that human liver is less responsive to the pleiotrophic effects of activation of PPARα or CAR (Gonzalez and Shah 2008; Klaunig et al. 2003; Klaunig et al. 2012; Lake 2009; Ross et al. 2010). Thus, with respect to PPARα and CAR-mediated effects in the liver and related metabolism, the human response is either attenuated or absent as compared to that of the rodents.

Mechanistic studies have demonstrated that many of the observed effects upon PFOA exposure, including those observed in developing mice, can be explained, in part, by the activation of PPARα. Many of the developmental effects were either absent or attenuated when PFOA was administrated to PPARα knockout mouse. The influence of PPARα on the fetal developmental effects of PFOA in the Sv/129 mouse strain (wild-type vs. PPARα knockout) was investigated by Abbott et al. (2007) and Albrecht et al. (2013). While it is not possible to rule out completely the contribution of other modes of action(s), many of the developmental effects with PFOA described above were attenuated and/or improved with PPARα knockout mice such as post-natal survival and body weight effects. Given that rodents are more responsive and susceptible than humans to PPARα-mediated biological effects (*vide supra*) and PPARα may not play a critical role in normal development

(Braissant et al. 1996; Lee et al. 1995), it calls into question the relevance of nuclear receptor-mediated effects in rodents and their biological significance to humans. Therefore, the developmental effects reported in the laboratory animals for PFOA were primarily mediated by maternal effects and based on the recent mode of action data, rodents may not be the most appropriate species for the hazard assessment of PFOA on developmental toxicity in humans.

E. Liver findings in rodents are not relevant for human risk assessment. While it is commonly acknowledged that liver is a primary target organ with exposure to PFOA, it is important to recognize that the liver effects observed in laboratory animals were adaptive in nature and there was no conclusive evidence to support that liver findings observed in laboratory animals with exposure to PFOA are relevant for human risk assessment. Given the known knowledge on the nuclear receptor activation and species relevance discussed earlier (*vide supra*), liver findings cited by ATSDR should not be deemed relevant for human risk assessment. For instance, in the study by Butenhoff et al. (2004), increased liver weights were reported in male rats of both the P and F1 generations at all dose levels.

The corresponding increases in liver weight in laboratory animals with exposure to perfluoroalkyls reflected the adaptive nature of liver, which is a natural phenomenon due to cytochrome P450 enzyme inductions in the liver. Given that PFOA is a known activator for several xenosensor nuclear receptors (as discussed above), microscopic changes in the liver of some PFOA-treated male rats such as hepatocellular hypertrophy and focal to multifocal necrosis were consistent with activation of these receptors and as discussed earlier, it is well-known that human liver is less responsive than rodents to the pleiotrophic effects of activation of these receptors (Gonzalez and Shah 2008; Klaunig et al. 2003; Klaunig et al. 2012; Lake 2009; Ross et al. 2010). Thus, with respect to PPARα and CAR-mediated effects in the liver and related metabolism, the human response is either attenuated or absent as compared to that of the rodents. Another federal agency, USEPA (in its assessments of PFOA in 2009 and again in 2016), as well as other international regulatory authorities such as European Chemical Agency Risk Assessment Committee (2015), European Food and Safety Authority (2018), and Australian Expert Health Panel (2018) also considered the liver weight findings in laboratory animal studies with PFOA (or other perfluoroalkyls) to be irrelevant for human risk assessments.

It should be noted that, acetylsalicylic acid (commonly known as aspirin) and alcohol can also elicit increased liver weight in laboratory animals similar to the observations reported with perfluoroalkyls in rodents (EMEA 1999b).

F. Mammary gland development findings in mice are inconsistent: Despite that the availability of several studies that have investigated the potential effects of PFOA on the developing mammary glands in mice as a consequence of exposure during either the *in utero* or postnatal/peripubertal (Albrecht et al. 2013, Tucker et al. 2014, White et al. 2007, White et al. 2009, White et al. 2011, Yang et al. 2009, Zhao et al. 2010), ATSDR is correct that this endpoint *cannot be consistently* described and quantified in mouse models. Given that 1) to

date, there is no standardized method or guideline of evaluating rodent mammary gland; and 2) there is a lack of concordance among all the available data on mammary gland development in mice as well as an absence of such findings in human epidemiological studies calls for question on the biological significance of this phenotype and its relevance to human health. This conclusion is consistent with the assessments from another federal agency, USEPA (in its assessments of PFOA in 2009 and again in 2016), as well as other international regulatory authorities such as European Chemical Agency Risk Assessment Committee (ECHA 2015), European Food and Safety Authority (EFSA 2018), and Australian PFAS Expert Health Panel (2018).

It should be noted that there are three epidemiologic studies that have examined the potential association between maternal PFAS exposure and shorter duration of breastfeeding or greater risk of stopping breastfeeding (Fei et al. 2010b; Romano et al. 2016; Timmermann et al. 2016). Fei et al (2010) measured PFOA and PFOS concentrations of 1400 women during early pregnancy. Self-reported data on the duration of breastfeeding (any and exclusive) were collected around 6 and 18 months after birth. While the study reported significant associations between PFOA concentrations and shorter duration of breastfeeding (before 3 and 6 months) among multiparous women, no significant associations were observed among primiparous women. The authors note that multiparous women who breastfed during prior pregnancies or breastfed longer may have had lower serum PFOA levels through excretion via breast milk. Consequently, reverse causation could not be excluded. The second study (Romana et al. 2016), observed a significant association between PFOA exposure and ending "any" breastfeeding by 3 and 6 months; however, no association was observed between PFOA exposure and ending "exclusive" breastfeeding by 3 and 6 months. More importantly, when stratified by parity, associations between PFOA and ending "any" breastfeeding at 3 and 6 months were largely attenuated for nulliparous women. Like Fei et al (2010), the significant associations observed among multiparous women were likely attributed to reverse causation. The third study (Timmerman et al. 2016), examined the potential association between PFOA exposure and duration of breastfeeding (both total and exclusive) among 1092 Faroese women with general population PFOA levels (median = 2.40 ng/mL). The authors reported that a doubling of maternal serum PFOA was significantly associated with a reduction in exclusive breastfeeding of 0.5 months. This association was observed among both primiparous and multiparous women (excluding the role of reverse causation). One important limitation of this study, worth noting, is that self-reported breastfeeding duration was collected 5 years after birth and was likely prone to misclassification error.

Finally, it is important to recognize that reduced breastfeeding duration in humans is not equivalent to "delayed mammary gland development" in rodents. In humans, numerous factors can influence breastfeeding duration other than diminished milk production (e.g., lack of prenatal education, inadequate lactation support from healthcare providers after delivery, medications incompatible with breastfeeding, lack of spousal/family support, short maternity leave, sore nipples/breasts, infant intolerance to breast milk, and individual choice). These factors were not considered in the epidemiology studies, and may have influenced the observed associations.

G. Immune findings in rodents are not consistent; and they lack concordance with epidemiological observation data. With exposure to PFOA, ATSDR also concluded that immunotoxicity is a primary target of toxicity based on decreased antigen-specific antibody responses in mice reported by DeWitt et al. (DeWitt et al. 2008; DeWitt et al. 2016) where PFOA suppressed T cell-dependent IgM antibody response (TDAR) but not the secondary IgG response. While ATSDR concluded that such findings were consistent with human epidemiology studies with regards to vaccine responses (see epidemiology discussion below), it is important to recognize that the humoral immune response to vaccinations, as measured in the human epidemiology studies, is mainly a secondary IgG memory response.

While suppression of the IgM response by PFOA was demonstrated in several studies where administered doses also induced signs of overt toxicity (i.e., reductions in body and lymphoid organ weight), the levels of IgG were not suppressed (either unchanged or enhanced). It is difficult to interpret why the primary IgM response was suppressed in mice by PFOA and yet the secondary IgG response was either not affected or enhanced. Collectively, human and animal bodies of evidence for antibody response are divergent. Mouse studies showed suppression of the IgM response with no impairment of the secondary antigen specific IgG response, which is in contrast to the epidemiological associations which suggested suppression by PFOA of IgG-mediated antibody titers to vaccinations in some studies for certain vaccines. Therefore, the weight of evidence and the lack of concordance between animal and human epidemiological data do not support the claim that PFOA induces immunotoxicity or caused decreased antibody response to certain vaccines. Finally, as noted above, the fact that the epidemiological data does not reveal a consistent association between exposure and response across all vaccines is further evidence that the animal and human data are not consistent.

Contrary to what ATSDR stated "the potential immunotoxicity of PFOA has not been investigated in chronic-duration studies" (*cf.* page A-30), it should be noted that the primary immune organs were evaluated microscopically in rats after 2 years of dietary treatment containing ammonium PFOA (Butenhoff et al. 2012c). In this study, representative primary immune organs were collected (mesenteric lymph node, spinal cord, bone marrow, and spleen) and evaluated microscopically by a board-certified veterinary pathologist at the end of a 2-year period. There were no neoplastic or non-neoplastic lesions observed in these immune organs. This is important because it demonstrated the absence of a direct effect on primary immune organs with chronic PFOA exposures in the rats. In addition, PFOA-treated rats had similar or higher percent survival compared to controls, which is contrary to chronic immunosuppression-mediated toxicity such as cyclosporin (a known immunosuppressant) that ultimately resulted in increased mortality in rats (Ryffel and Mihatsch 1986).

H. A study with one dose group is not adequate in estimating point-of-departure. ATSDR selected two mouse studies with developmental endpoints (Onishchenko et al 2011 and Koskela et al 2016) for the point-of-departure (POD) to derive the MRL value for PFOA (endpoints were altered activity and skeletal alterations in offspring of C57Bl/6 mice). These studies tested only a control group and one dose of 0.3 mg/kg, which was chosen as the LOAEL. As only one dose was tested, a dose-relationship cannot be evaluated.

Selection of studies with no information on dose-response for effects is not acceptable to establish a point-of-departure. ATSDR should follow its own guidance (as stated in pages A-6).

I. Serum PFOA concentrations in pups should be considered for POD instead of dams because critical effects chosen by ATSDR were based on (developing) pups. The studies chosen by ATSDR examined developmental endpoints that were measured in offspring, which are used as the basis for the MRL. In order to estimate steady-state plasma concentrations of PFOA, ATSDR used the Wambaugh model for PFOA that is parameterized for adult animals and cannot be used to predict concentrations in fetuses or pups. This model also does not account for life stage differences in physiology or pharmacokinetics, and can potentially over-predict as well as under-predict the area-under-the-curve (AUC). In addition, AUC and steady-state concentration are probably different in the offspring than in the dam. Overall internal exposure (as estimated by calculation of the AUC) may change with growth, and there could be a period of peak exposure. Use of the Wambaugh model (and thus use of the maternal plasma concentration as a surrogate for the offspring) introduces uncertainty in the MRL derivation as the offspring plasma concentration may be different that than of the maternal animals. Use of a physiologically-based model that incorporates fetal and pup compartments would provide an estimate of fetal and pup internal exposure (rather than use of the maternal concentration as a surrogate), which would reduce the uncertainty in the MRL value.

J. HED cannot be reliably estimated in the absence of serum concentration data. As discussed above, studies by Onishchenko et al. (2011) and Koskela et al. (2016) did not have any analytical verification on either the dietary PFOA level or the resulting serum PFOA concentrations in the mice. With the questionable reliability of the study design as well as the data gathered, there were a great number of inherent uncertainties associated with attempting to predict the mean serum concentrations using modeling approach.

Confirming that it is inappropriate to derive an MRL where there is an absence of serum concentration data, in its current draft profile for other perfluoroalkyls, ATSDR stated in several places that "…. Database was considered inadequate for derivation of an MRL … because … study did not measure serum [perfluoroalkyl] levels, which are needed to calculate / estimate HEDs" (cf. pages A-14, A-56, A-65, A-72, A-109).

K. HED for PFOA will be higher when considering faster half-life. In the MRL calculations, ATSDR chose to use the arithmetic mean serum elimination half-life estimate for PFOA from Olsen et al. (2007) over other studies because Olsen et al. had a longer follow up time and ATSDR was concerned that based on a study by Seals et al. (2011), slower kinetics is likely to constitute a larger contribution to the terminal half-life. For example, whereas Olsen et al. had an average follow-up of 5 years, Bartell et al. had a follow-up of a year and Li et al. had a follow-up of 2.3 years among those studies that followed individuals and were not cross-sectional analyses of populations. However, this line of reasoning by ATSDR for selection of the arithmetic mean from the Olsen et al. study fails to take into account several factors that likely biased upwards the ATSDR MRL estimates. These include the following points.

1. The ATSDR chose not to use the geometric mean estimate that was discussed in the Olsen et al. paper. Given the right skewness of their data, Olsen et al. were more favorable to use the geometric mean for a measure of central tendency. ATSDR provided no explanation as to why they chose the arithmetic mean vs. the geometric mean in this study. This decision is interesting (and curious) because ATSDR chose to report median initial and final concentrations in Table A2 rather than the arithmetic mean initial and final concentrations in Table A2. A median concentration would be better represented by a half-life estimate based on the geometric mean.

2. The Olsen et al. 2007 study comprised 26 retirees (end of study average age = 66 years) who likely would have had an average glomerular filtration rate lower than those calculated from younger ages as reported in Bartell et al. (average age 55) and Li et al. (age range 15 – 55). The average estimated glomerular filtration rate declines with age as shown in the table below.

Age range	Estimated GFR (ml/min/1.73 m^2)	Source:
1-6 months	77	Heilbron et al. 1991 Pediatr Nephrol. Jan; 5(1):5-11.
6-12 months	103	
12-19 months	127	
2-12 years	127	
20–29	116	https://www.kidney.org/sites/default/files/docs/11-10-1813_abe_patbro_gfr_b.pdf
30–39	107	
40–49	99	
50–59	93	
60–69	85	
70+	75	

Renal clearance of perfluorocarboxylates (and perfluorosulfonates) is largely a sum of three processes involving glomerular filtration, renal tubular secretion, and renal tubular reabsorption (Han et al. 2012). Because PFOA and other perfluorocarboxylates vary in their affinities to bind plasma proteins, glomerular filtration of perfluorocarboxylates (and perfluorosulfonates) is a product of the unbound fraction of the perfluorocarboxylate and the glomerular filtration rate (GFR). Thus, the higher estimates of GFR based on the younger ages in the other study populations, especially the younger Li et al. study which had approximately 50% of the follow-up time of Olsen et al., may be due to the age differences of the subjects, and not necessarily the shorter follow-up period considered in these studies. Thus, the serum elimination half-lives of other studies are likely equally valid for consideration in MRL calculations.

3. The Olsen et al. study had to consider, during the course of their follow-up, the possibility of retirees reentering the 3M Decatur and Cottage Grove manufacturing plants. Indeed, this resulted in Olsen et al. eliminating 1 study subject entirely, and truncating follow-up times for two retirees. This would have biased estimates upwards for the serum elimination half-lives due to the increased exposure. It is not likely that

ambient general population level concentrations would have biased these retiree's estimates substantially as discussed by Bartell et al. 2012. On the other hand, although Bartell et al. and Li et al. had shorter follow-up times, the primary exposure in these populations was through drinking water. Installation of GAC filters in these populations' affected municipal water supply would have immediately ceased their primary exposure to PFOA, PFOS, and PFHxS.

4. ATSDR suggests the Seals et al study indicates a lower clearance rate may occur as subjects are followed long-term post exposure; thus, the decision by ATSDR to use the study that had the longest follow-up time (Olsen et al. 2007). However, ATSDR did not mention the main limitations of the Seals et al. study: 1) the cross-sectional nature of the analysis. Individual subjects were not followed. Model-based estimates were instead calculated based on the initial concentrations; 2) there was the added assumption that there was uniform exposure based on the concentration of PFOA measured in each water district; and 3) subjects with initial PFOA concentrations < 15 ng/mL were excluded which maximized the probability of analyzing individuals with sufficiently high baseline PFOA concentrations that would not be at ambient levels. Seals et al. surmised their findings indicated the half-life for PFOA was between 2.3 and 3.8 years, not at the end of this range, as chosen by ATSDR via the arithmetic mean estimate from Olsen et al. Seals et al. did show their modeled estimates in clearance rates between low- and high-exposure water districts could suggest a possible concentration-dependent or time-dependent clearance process but could not rule out inadequate adjustment for background exposures.

5. Given the above additional considerations (beyond that of ATSDR's consideration about the length of follow-up), the MRLs, assuming same PODs from the same studies, are recalculated in the table below using the different serum elimination half-life values for PFOA, PFOS, and PFHxS that are reported in Olsen et al., Bartell et al., Li et al., and Seals et al. Accordingly, the percent of the MRL that might be overestimated by the ATSDR using in their most conservative serum elimination value (arithmetic means from Olsen et al. 2007) would then result in a range of overestimations of the MRL for PFOA between 9 and 40 percent. This type of sensitivity analysis is definitely needed in Appendix A for the MRL calculations to take into account the variation of serum elimination half-life estimates that have been reported in the literature that will be, in part, a function of the GFRs from the population studied. Given the fact that ATSDR has used developmental studies to calculate the PODs for their MRLs, it is therefore not justified to use the arithmetic mean half-life estimate based solely on retirees, in part, because the GFRs of older adults are markedly lower than adults of much younger age and people 65 years of age or older represent only approximately 15% of the general population Therefore the estimated half-lives should reflect the entire population, not just the upper tail, which can be a reflection of lower GFRs that occur with age. Thus, calculation of serum elimination half-lives may be age, sex, and concentration-dependent. MRLs, based in part on half-lives, should reflect this diversity of inputs in their calculations as shown in the table below.

Reference Study	Estimated Half-life		MRL (mg/kg/d)	% MRL over current ATSDR MRL
	Years	Days		
*ATSDR Estimate (arithmetic Mean from Olsen et al. 2007)	3.8	1400	2.74E-06	--
Olsen et al. 2007 (geometric mean)	3.5	1278	3.00E-06	9
Seals et al. 2011	2.9	1058	3.62E-06	24
Li et al. 2018	2.7	985.5	3.89E-06	30
Bartell et al. 2010	2.3	839	4.56E-06	40

As illustrated above, because HED and MRL are dependent of the clearance rate used, the resulting MRL for PFOA can differ substantially and could be 9 to 40% higher than the current provisional MRL proposed by ATSDR.

L. Uncertainties associated with Wambaugh benchmark dose model used by ATSDR. ATSDR relied on an animal PBPK model to predict subsequent POD of MRL derivation, but on the other hand, it has also explicitly stated that "Although physiologically based pharmacokinetic (PBPK) models have been developed for rodents and humans, these models are not sufficient to allow for comparisons between administered doses in laboratory animals and serum concentrations in humans" (*cf*. page 5 of draft profile). This statement indicated a great amount of uncertainty associated with the PBPK model used hence ATSDR needs to acknowledge this fact in its summary.

The supplementary information from Wambaugh et al. (2013) contains a table (Supplemental Table 3) that compares the agreement of the predicted final plasma concentration of PFOA with those measured from several animal studies. The plasma concentrations resulting from higher doses appear to be better predicted than those resulting from lower doses. For many of the studies that tested lower doses, a plasma concentration measurement was not available for comparison. However, one mouse study (Lau et al 2006) did have measured plasma concentrations available at lower doses; for these, the predicted values appear to overestimate the final plasma concentrations at the lower doses of 1 and 3 mg/kg/day. The predicted values are almost three times higher than those measured (a factor of 2 is generally accepted for model-predicted values). This introduces uncertainty around model predictions at these lower doses, which are closer to the dose used by ATSDR for derivation of the MRL than the higher values that appear to be better predicted by the model. Although ATSDR used the model to estimate serum concentrations at higher doses, the POD for derivation of the MRL was a dose of 0.3 mg/kg/day. As a result, the model predictions for serum concentration could be more uncertain in this low dose range. Although model predictions were not compared to measured steady-state concentrations by Wambaugh et al 2013, which was what was used to derive the POD plasma concentration, the overestimated predictions in the low dose range still introduces uncertainty into the assessment.

Although the Wambaugh model was used to estimate maternal serum concentrations from developmental datasets (Lau et al. 2006; White et al. 2009; Wolf et al. 2007), the model was not specifically parameterized for this, which is another factor contributing to the uncertainty in using this model to estimate an MRL for a developmental endpoint. The Wambaugh PFOA model was parameterized for male and female cynomolgus monkeys, male and female

SD rats, and female CD1 and C57Bl/6 mice. ATSDR states that they could not model some of the studies due to lack of parameters among different mouse models: Cheng et al. 2013 (Wistar rats), Loveless et al. 2008 (CD1 male mice), and Abbott et al. 2007 and Abrecht et al. 2013 (129S1/SvlmJ wild-type mice). While there are well-known differences in pharmacokinetics for male and female rats for PFOA and differences across species, ATSDR provides no evidence or support for sex or strain differences in pharmacokinetics for mice or differences in pharmacokinetics for different strains of rats. As ATSDR modeled only certain strains, this limits the studies it can use when relying on this model and introduces further uncertainty into the MRL value as several studies could not be considered.

In performing the benchmark dose modeling on the DeWitt et al. studies (2008; 2016), ATSDR used the Wambaugh model to estimate steady-state plasma concentrations of PFOA. These studies were conducted in C57Bl/6N mice, for which the Wambaugh model was not parameterized. ATSDR is not consistent in their modeling approaches with the Wambaugh model (i.e., they did not model some studies due to lack of strain-specific parameters but they modeled the DeWitt studies, which were conducted in a strain that the model was not parameterized for).

M. Uncertainty factors chosen by ATSDR were overly conservative and not supported by best available scientific data. They include:

1. Incorrect use of "10" for a LOAEL. ATSDR concluded that the studies by Onishchenko et al. (2011) and Koskela et al. (2016) did not have a NOAEL hence assigned an uncertainty factor of 10 for LOAEL to NOAEL extrapolation. However, given that there was only one PFOA dose group used in the study (in addition to the fact that there were very few animals studied), it was impossible to establish any meaningful dose-response relationship. ATSDR should recognize this limitation as a critical design flaw and it should also recognize that a NOAEL or LOAEL cannot be established under the study condition. This factor of "10" is not scientifically justified and should be removed by ATSDR should it insist on using the same dataset for its MRL derivation on PFOA.

2. Use of "3" for animal-to-human, in addition to large dosimetric TK adjustment, is not scientifically justified. While 3M agrees with ATSDR to adjust for toxicokinetic difference between human and rodent serum clearance of PFOA, 3M does not agree with the serum elimination half-life chose by ATSDR for the calculation (see toxicokinetic discussion above). In addition, while this TK clearance adjustment represented a factor of 10,000 based on ATSDR's derivation, 3M does not agree with ATSDR that an additional factor of "3" is needed to account for uncertainty in using laboratory animal data to derive human exposure levels. This, in fact, represents an adjustment of 30,000 when taking dosimetry into account. The use of an additional factor of 3 to account for rodent-to-human toxicodynamic difference is not necessary.

   More specifically, ATSDR has derived its proposed MRL based on the rodent developmental data. Because humans are considerably less sensitive to the pleiotrophic effects of xenosensor nuclear receptors such as PPARα, CAR/PXR activation compared to rodents (Corton et al. 2014; Elcombe et al. 2014; Gonzalez and Shah 2008; Klaunig

et al. 2003; Klaunig et al. 2012; Lake 2009; Ross et al. 2010), the qualitative differences brings into question the relevance of rodent developmental effects with exposure to PFOA and their biological significance to humans. For example, many of the developmental effects observed noted in wildtype mice when exposed to PFOA were attenuated when PPARα genes were knocked out (Abbott et al., 2007). This further supported the qualitative difference and human relevance between rodents and humans. Thus, the very large dosimetric adjustment of 10,000 more than adequately compensates for the additional factor of 3 for difference between rodents and humans. ATSDR should not apply another factor of 3 for animal to human extrapolation when this uncertainty is already embedded in the large adjustment for the dosimetric difference.

3. Additional factor of "10" for human variability is overly conservative. For PFOA MRL, ATSDR included a factor of 10 for human variability. If ATSDR could have developed a more appropriate PBPK model that accounted for life stage differences in humans (rather than relying on rodent model), this factor of 10 for human variability could potentially be reduced.

Detailed Comments on PFOS MRL

ATSDR Position (page A-36)

MRL Summary: A provisional intermediate-duration oral MRL of $2x10^{-6}$ mg/kg/day was derived for PFOS based on delayed eye opening and transient decrease in F2 body weight during lactation in the offspring of rats administered PFOS via gavage in a 2-generation study (Luebker et al. 2005a). The MRL is based on a HED NOAEL of 0.000515 mg/kg/day and a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability) and a modifying factor of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity).

Selection of the Critical Effect: The most sensitive targets of PFOS toxicity in laboratory animals are similar to those identified in longer term epidemiology studies. These effects include liver damage and increases in serum lipids, decreased antibody response to vaccines, and small decreases in birth weight; epidemiology studies have not consistently found neurological effects to be associated with serum PFOS levels.

3M Conclusion

A. The critical effect concluded by ATSDR with PFOS exposure (decreased pup body weight and delayed eye opening in rats) has been not shown in humans
B. ATSDR should recognize rodent-specific effects and their relevance to humans
C. PFOS does not affect the reproductive system in laboratory animals
D. The developmental effects reported in the laboratory animals for PFOS were primarily mediated by maternal effects
E. Liver findings in rodents are not relevant for human risk assessment
F. PFOS does not cause increase in serum lipid in laboratory animals
G. The nervous system is not a primary target organ with exposure to PFOS
H. Inconsistent immune findings in rodents were confounded by systemic toxicity
I. Inconclusive immune findings in human epidemiological data do not support ATSDR conclusions
J. Serum PFOS concentrations in pups should be considered for POD instead of dams because critical effects chosen by ATSDR were based on (developing) pups
K. HED for PFOS will be higher when considering faster half-life
L. Wambaugh benchmark dose model used by ATSDR was not optimized
M. Uncertainty factors by ATSDR were conservative and not supported by scientific data
    1. Use of "3" for animal-to-human, in addition to large dosimetric TK adjustment, is conservative because humans are less sensitive than rodents based on *in vitro* hepatocyte data (Bjork and Wallace 2009)
    2. Scientifically unjustified use of "10" for concerns on immunotoxicity

ATSDR's overall interpretation on both toxicology and epidemiology data are inconsistent with the most current knowledge. Its application of uncertainty factors is not scientifically justified and the proposed PFOS MRL is not supported by the scientific data. The PFOS MRL derived for the human-health risk assessment is therefore conservative and not scientifically justified.

3M Comments (Details):

A. The critical effect concluded by ATSDR with PFOS exposure (decreased pup body weight and delayed eye opening in rats) has been not shown in humans (see epidemiology discussion above). ATSDR should offer a plausible explanation as to why it believes these effects are relevant to human risk assessment.

B. ATSDR should recognize rodent-specific effects and their relevance to humans. For PFOS, the critical effect chosen by ATSDR are delayed eye opening and decreased pup body weight, based on the results from a 2-generation reproduction study in rats with PFOS (Luebker et al. 2005a). While the text of the proposed MRL derivation fails to make clear that none of the listed effects has been shown in humans (see epidemiology discussion above), the inclusion of some of the effects is incorrect even based on animal data alone. Many "effects" included by ATSDR are specific to rodents and often contrary to the current published literature. For instance, mechanistic research has shown that many metabolic effects to PFOS exposures in rodents can be explained by the activation of xenosensor nuclear receptors such as PPARα, constitutive androstane receptor (CAR), and pregnane X receptor (PXR) in the liver (Bjork et al. 2011; Bjork and Wallace 2009; Elcombe et al. 2012a; Elcombe et al. 2012b; Vanden Heuvel et al. 2006). Given that humans are considerably less sensitive to the pleiotrophic effects of PPARα or CAR/PXR activation compared to rodents (Corton et al. 2014; Elcombe et al. 2014; Gonzalez and Shah 2008; Klaunig et al. 2003; Klaunig et al. 2012; Lake 2009; Ross et al. 2010), the qualitative differences calls into question the relevance of rodent developmental effects and their biological significance to humans. For example, neonatal survival actually improved in mice when PPARα knockout mice were exposed to PFOS when compared to the wildtype (Abbott 2009; Abbott et al. 2009).

C. PFOS does not affect the reproductive system in laboratory animals. It is incorrect for ATSDR to conclude that reproductive system is one of the primary targets of toxicity with exposure to PFOS (cf. page A-36).

A number of experimental animal (mammalian) toxicological studies on the reproductive and developmental effects of PFOS have been published (Abbott et al. 2009; Butenhoff et al. 2009b; Case et al. 2001; Gortner et al. 1980; Grasty et al. 2005; Lau et al. 2003; Luebker et al. 2005a; Thibodeaux et al. 2003). These studies included detailed information on the developmental toxicity with these compounds as well as valuable insights on the role of maternal effects and its attribution to the developmental outcomes in laboratory animals. Comprehensive review on the potential developmental toxicity of the perfluoroalkyl acids was reported in 2004 (Lau et al. 2004) and updated subsequently (Abbott 2015; Andersen et al. 2008; Lau et al. 2004).

Overall, PFOS did not affect the functional aspects of male or female reproductive functions in the laboratory animals. These included estrous cycles, sperm parameters, mating index, fertility index, and reproductive organ morphology. The potential of PFOS to influence reproductive performance was evaluated in mice (Abbott et al. 2009; Thibodeaux et al. 2003), rats (Butenhoff et al. 2009; Luebker et al. 2005a), and rabbits (Case et al. 2001).

Gestational exposure to PFOS did not affect the number of embryonic implantation sites in several strains of mice (CD-1, Sv129, or PPARα knockout) (Abbott et al. 2009; Thibodeaux et al. 2003). Similarly, implantations were not affected in rabbits either when exposed up to 3.75 mg/kg-d during GD 7 – 20 (period of organogenesis) albeit decreased body-weight gain and food consumption were observed (Case et al. 2001). In rats, oral administration of PFOS up to 10 mg/kg-d during GD 6 – 15 (period of organogenesis) also caused reduced body-weight gain, however, they did not affect the ovaries or the reproductive contents of the dams (Gortner 1980).

In a two-generation reproduction/developmental study in rats (Luebker et al. 2005), potassium PFOS (given as potassium salt) doses as high as 3.2 mg/kg-d given to male and female rats for 6 weeks prior to mating, through mating and, for females, through gestation and lactation. PFOS did not adversely affect mating or fertility parameters in male or females, including fertility and pregnancy indices, estrous cycling, number of pregnancies per number of matings, number of days to inseminate, number of matings during the first week of cohabitation, epididymal sperm maturation, litter averages for corpora lutea, implantations, viable embryos, non-viable embryos, and reproductive organ histology. In particular, there were no statistically significant differences between control and potassium PFOS-treated females in the mean number of estrous cycles, rats with ≥6 consecutive days of diestrus or estrous during the 28-day evaluation period. In a developmental neurotoxicity study with PFOS, pregnant female rats received PFOS doses up to 1 mg/kg/day from gestation to lactation. No PFOS treatment-related effects were noted on maternal health or reproductive outcomes (Butenhoff et al. 2009). Furthermore, the morphologic effects of PFOS on reproductive organs in non-human primates were evaluated from a six-month oral study and results indicated no abnormalities (Seacat et al. 2002).

D. <u>The developmental effects reported in laboratory animals for PFOS were primarily mediated by maternal effects.</u> While ATSDR concluded that developing organisms are primary targets of toxicity with exposure to PFOS (cf. page A-36), there is strong experimental evidence demonstrating that developmental effects associated with PFOS exposures in offspring are observed <u>only</u> where there were significant effects in the maternal animals. Experimental evidence demonstrates that developmental effects associated with PFOS exposures in offspring are observed when maternal animals were affected such as body weights. Evidence involving maternal effects in the outcome of the developmental toxicity includes the following examples.

PFOS developmental toxicity has been evaluated in several laboratory species. In rabbits, oral PFOS administration ranging from 0.1 – 3.75 mg/kg/day was given from GD 6 – 20 and decreased maternal body-weight gain was observed at 1 mg/kg dose group or higher. No abnormal fetal effects were noted except decreased fetal body weight, which was observed with 2.5 and 3.75 mg/kg/day dose groups only. Study authors concluded that "The fetal effects occurred at maternally toxic dose levels and no fetal changes were present at nontoxic maternal doses" (Case et al. 2001). In mice, there was a statistically significant ($p \leq 0.05$), dose-related increase in maternal liver weight when pregnant dams were treated during gestation at a dose as low as 1 mg/kg potassium PFOS (Thibodeaux et al. 2003). Various develpmental effects were reported (e.g., decrased postnatal survival and growth deficits) but

primarily for litters from dams receiving 10 mg/kg/day potassium PFOS or higher (Lau et al. 2003). In addition to mice, the developmental toxicity of PFOS has also been evaluated in rats. Oral administration of PFOS during gestation to pregnant rats caused reduced maternal body-weight gain and fetal body-weight gain at 2 mg/kg-d maternal dose group or higher (Lau et al. 2003). In a two-generation reproduction/developmental study in rats by Luebker et al. (2005), described in detail above, the authors reported reduced body weight and body weight-gain at parental generation at 0.4 mg/kg or higher. Developmental hallmarks similar to those previously reported by others (*i.e.*, decreased fetal body weight, decreased postnatal survival, and developmental delays) were observed in pups from 1.6 mg/kg/day maternal dose groups or higher. Therefore, the developmental effects reported in the laboratory animals for PFOS were primarily mediated by maternal effects and based on the recent mode of action data, rodents may not be the most appropriate species for the hazard assessment of PFOS on developmental toxicity in humans.

E. Liver findings in rodents are not relevant for human risk assessment. The comments to follow are related to ATSDR's identification of "liver damage' in laboratory animal studies as sensitive target with exposure to PFOS. Similar to the comments provided earlier on PFOA, liver findings in rodents warrant careful consideration. Given that it is well recognized that there is distinct difference in mode-of-action between rodents and humans when it comes to liver changes mediated by xenosensor nuclear receptors, liver effects observed in rodents are scientifically unjustified and inappropriate for use as a critical effect for human risk assessment.

There is a well-established body of experimental evidence for activation of PPARα and CAR/PXR as a major factor in the rodent hepatic response to exposure to PFOS. As Elcombe et al. (Elcombe et al. 2012a; Elcombe et al. 2012b) point out, the hypertrophic and hyperplastic response of rat liver to PFOS exposure has clearly been demonstrated to be consistent with the criteria used to establish PPARα/CAR/PXR activation as a mode of action. The transcriptional signature (mRNA) for PPARα/CAR/PXR activation was also observed in livers from PND 21 male rat pups exposed via maternal gavage in the developmental neurotoxicology study reported by Butenhoff et al. (2009b) and Chang et al. (2009) as well as in adult male wild-type mice (Rosen et al. 2010). In the E3L.CETP mouse transgenic mouse model, dietary PFOS exposure of adult males resulted in transcriptional gene expression profiles and changes in lipid parameters consistent with activation of PPARα and PXR (Bijland et al. 2011). Rosen et al. (2009) observed the same transcriptional signature consistent with activation of PPARα/CAR/PXR in CD-1 mouse fetal liver after maternal exposure to PFOS during gestation.

There are fundamental differences between the responses of human and rodent liver from exposure to agents that increase activation of PPARα and CAR/PXR (Corton et al. 2014; Elcombe et al. 2014). The basis for the fundamental differences between the rodent and human liver response from exposure to agents that activate these receptors has become clearer with development of receptor knock-out and humanized receptor knock-in transgenic mouse models and the increased availability of human primary hepatocytes. When exposed to PPARα and CAR/PXR agonists, mice that have been genetically modified by removal of the natural mouse receptors and replacement with the natural human forms of the receptors

do not have the hyperplastic response observed in wild-type mice (Gonzalez and Shah 2008; Ross et al. 2010). Key differences between rodent and human hepatocytes, especially the lack of a hyperplastic response in human hepatocytes exposed to PPARα and CAR activators, have also been demonstrated (Elcombe et al. 1996; Goll et al. 1999; Hirose et al. 2009; Parzefall et al. 1991; Perrone et al. 1998).

As noted above, human hepatocytes respond to PPARα agonists differently than rodent hepatocytes, and activation of human PPARα does not appear to result in the characteristic hyperplastic response observed in rats and mice (Corton et al. 2014; Gonzalez and Shah 2008). Bjork and Wallace (2009), working with primary rat and human hepatocytes as well as the HepG2 human liver cell line in culture, demonstrated major differences between primary rat hepatocytes and human hepatocytes in response to exposure to PFOS in culture. In comparison to the large increase over control in mRNA for peroxisomal enzymes Cte/Acot1 and Acox, the human hepatocytes showed essentially no increase in transcripts. However, consistent with observations with other peroxisome proliferators, CYP4A11 mRNA was increased by PFOS exposure in human as well as Cyp4A1 in rat hepatocytes.

In addition to PPARα, Bjork et al. (2011) characterized the activation of several other hepatic nuclear receptors (PXR, CAR, the liver X receptor α (NR1H3 or LXRα), and the farnesoid X receptor (NR1H4 or FXR) by PFOS in primary rat and human hepatocytes. In rat hepatocytes, they demonstrated multiple nuclear receptors participate in the metabolic response to PFOS exposure, resulting in a substantial shift from carbohydrate metabolism to fatty acid oxidation and hepatic triglyceride accumulation. They concluded that, "while there is some similarity in the activation of metabolic pathways between rat and humans, particularly in PPARα regulated responses; the changes in primary human cells were subtle and possibly reflect an adaptive metabolic response rather than an overt metabolic regulation observed in rodents." Supporting this, the potential activation of human CAR3 isoform and human PXR has been studied. PFOS was not shown to activate directly either human nuclear receptor at concentrations up to 33 µM, with slight activation (much less than for positive control substances) of CAR3 and PXR occurring only at 100 µM (Ehresman et al. 2014).

Collectively, the established mode-of-action supports the liver hypertrophic effects in rodents from exposure to PFOS. The experimental evidence also shows the lack of a response, or a markedly reduced response, in human liver cells as compared to rodent liver. Furthermore, there were no adverse liver effects noted in humans (see epidemiology discussion above). The observational human data as well as a significant body of mechanistic experimental data that relates to the liver response to exposure to PFOS strongly suggests that use of rodent liver findings as an endpoint for the human-health risk assessment of PFOS is not scientifically justified. Other federal agency such as USEPA (in its assessments of PFOA in 2009 and again in 2016), as well as other international regulatory authorities such as European Chemical Agency Risk Assessment Committee (2015), European Food and Safety Authority (2018), and Australian Expert Health Panel (2018) also considered the liver weight findings in laboratory animal studies with PFOA (or other perfluoroalkyls) to be irrelevant for human risk assessments.

It should be noted that, acetylsalicylic acid (commonly known as aspirin), one of the most common over-the-counter drugs used in the world, can also elicit increased liver weight in laboratory animals similar to the observations reported with perfluoroalkyls in rodents (EMEA, 1999).

F. PFOS does not cause increase in serum lipid in laboratory animals. It is incorrect for ATSDR to conclude that "increases in serum lipid" is a sensitive target associated with exposure to PFOS. To the contrary, exposure to PFOS in laboratory animals has been consistently shown to decrease serum lipids (Butenhoff et al. 2012a; Chang et al. 2017; Elcombe et al. 2012a; Elcombe et al. 2012b; Seacat et al. 2003; Seacat et al. 2002). PFOS has been established as a hypolipidemic agent in mechanistic studies and reduction in serum cholesterol has been shown to be an early effect related to dosing with PFOS in toxicological studies with rodents and primates (Bijland et al., 2011; Elcombe et al., 2012a; Seacat et al., 2002, 2003). The hypolipidemic activity of PFOS occurs via the activation of xenosensor nuclear receptors peroxisome proliferator-activated receptor alpha (PPARα) and pregnane X receptor, which can influence fatty acid β-oxidation and lipid synthesis (Bijland et al. 2011; Bjork et al. 2011; Elcombe et al. 2012a; Elcombe et al. 2012b). Mechanistic study has elucidated how PFOS modulates the hypolipidemic responses. Using ApoE*3.Leiden.CETP mice, a humanized model having attenuated clearance of ApoB-containing lipoprotein and exhibiting human-like lipoprotein metabolism on a Western-type diet (ApoE*3 model paper), Bijland et al. (2011) demonstrated that high dietary doses of PFOS resulted in lower serum cholesterol by reducing VLDL production with enhanced triglyceride clearance (mediated by lipoprotein lipase) as well as decreased production of apolipoprotein B. PFOS also affected the rate of apolipoprotein A1 synthesis which ultimately resulted in the reduction of circulating HDL.

In a more recent study with non-human primates, Chang et al. (2017) confirmed the potential associations between serum PFOS and changes in serum lipid over a period of more than 1 year. With the highest serum PFOS achieved at approximately 165 ug/ml, only a slight reduction in serum cholesterol (primarily the high-density lipoprotein fraction), although not toxicologically significant, was observed and the corresponding lower-bound fifth percentile benchmark concentrations ($BMCL_{1sd}$) were 74 and 76 ug/ml for male and female monkeys, respectively.

Therefore, there is no evidence to suggest that PFOS causes an increase in serum lipid.

G. The nervous system is not a primary target organ in laboratory animals with exposure to PFOS. ATSDR also suggests that nervous system is a sensitive targets with exposure to PFOS per observations reported by Butenhoff et al. (2009b), this is incorrect.

In Butenhoff et al. (2009), the "increased motor activity and decreased habituation" was observed as a single, transient observation in male pups from 1.0 mg/kg-d maternal dose group on postnatal day (PND) 17. ATSDR failed to account for the lack of evidence for developmental neurological effects observed in the study as well as other corroborating studies. The use of this single, transient observation as a critical endpoint when more significant data are available as part of the same study (as well as other studies mentioned

below) that demonstrate normal neurological development is at odds with guidance for data interpretation for developmental neurotoxicity studies (Francis et al. 1990; USEPA 1998)These guidelines state that a weight of evidence approach and expert judgment should be used. It is evident that this has not been the case for PFOS.

Locomotor activity was one of many developmental neurotoxicological endpoints evaluated in the study by Butenhoff et al. (2009). While habituation (a primitive form of learning) and higher learning and memory were evaluated in three phases of the Biel maze swimming assessment on PNDs 22 through 28. The tri-phasic Biel maze swimming trial test paradigm to evaluate learning and memory did not reveal an effect of PFOS on the studied parameters in pups (20 / sex / dose groups). There were no other observations among the many recorded that were suggestive of a neurotoxicological effect of PFOS on development through the PND 66 observation period. A functional observation battery (FOB) was performed with the same sets of 20 rats per sex per group on PNDs 4, 11, 21, 35, 45, and 60; and it included various stages of development permitting: ease of cage removal; ease of handling in hand; lacrimation/chromodacryorrhea; salivation; piloerection; appearance of fur; palpebral closure; respiratory rate/character; red, crusty deposits; mucous membranes/skin color; eye prominence; eye color; mobility; muscle tone; convulsions/tremors; hindlimb extension; grooming; arousal; bizarre/stereotypic behavior; urination/defecation; pupillary response; backing; forelimb/hindlimb grip strength; tail pinch response; gait; and air righting. None of these FOB endpoints was affected by treatment with PFOS.

The lack of an effect on learning and memory is also supported by the results of Lau et al. (2003) and Luebker et al. (2005a). In the study by Lau et al., PND 22 rat pups from dams given 3.0 mg/kg/d throughout gestation did not differ from controls when tested using a T-maze with alternation. In the study by Luebker et al., F_1-generation pups were tested for learning, short-term retention, and memory in a passive avoidance paradigm beginning on PND 24, and, beginning on approximately PND 70, were evaluated in a water-filled M-maze for neuromuscular coordination, swimming ability, learning, and memory. No effects of treatment were observed.

H. Inconsistent immune findings in rodents which were confounded by systemic toxicity**.** With exposure to PFOS, ATSDR also concluded that immunotoxicity (as decreased antibody responses to vaccines) is one of the most sensitive targets. Similar to the discussion with PFOA, these are based on the decreased antigen-specific antibody responses in mice where PFOS suppressed T cell-dependent IgM antibody response (TDAR) but not the secondary IgG response (Dong et al. 2011; Dong et al. 2009; Guruge et al. 2009; Peden-Adams et al. 2008). A key principle in conducting a robust immunotoxicity study is to avoid / minimize systemic toxicity, including body weight loss.

Toxicological studies cited by ATSDR for reduced immune findings are confounded by overt toxicity and should not be included in the interpretation of immune findings. For example, in the studies by Dong et al. (2009; 2011), exposure to PFOS has also been associated with suppression of NK cell activity, a dose-dependent decrease in IgM PFC responses, but no evidence in IgG suppression were noted. It is important to note that the reported suppressions with exposures to PFOS appeared to be a high dose phenomenon where

systemic effects (i.e., body weight reduction) were present. This confounded the overall study interpretation in the immunotoxicity studies because reduced body weight as well as increased corticosterone serum levels were known immunosuppressive factors. The data presented by Dong et al. also lacked scientific validity to support the conclusion that PFOS suppresses immune responses. Concordance between several key immune parameters should be systematically illustrated in these immunotoxicity studies. Again, using the study by Dong et al. (2009) as an example, they did not properly address the following:

1. It is well known that body weight plays a critical role in studying immune response and any factors that can influence body weight will likely indirectly affect immune responses. Although Dong et al. claimed that body weight was not affected in the first two lower dose groups (0.5 and 5 mg/kg TAD), in looking at Table 1 in the Dong et al. paper, there appeared to be a difference in mean body weight change between the control group (3.10) and the 0.5 mg/kg group (2.58). By taking the summary data for each treatment group to replicate the ANOVA and Dunnett's t tests by computing 1-sided critical values for Dunnett's test, the final body weights in the 0.5 mg/kg treatment group were significantly lower than the control group at $\alpha=0.10$ ($0.05 < p < 0.10$).

2. It is also well known that the antibody titers to vaccinations are secondary IgG antibody isotype. The study data reported by Dong et al. (as well as others) was the primary IgM antibody response only, which did not reflect what the status of the secondary (memory) IgG antibody was.

3. It is important to emphasize that, not only was the secondary IgG response not measured by Dong et al, it was not appropriately induced to elicit a *bona fide* memory response as antigen was challenged only once in the study.

4. As an extension from above, Dong et al. did not evaluate the production of other immunoglobulin isotypes and they did not take the time-based progression of IgM → IgG antibody class switching into consideration. The normal progression of antibody development involves the IgM production by B cells first as primary immune response. The B cells will subsequently proliferate and become activated when further challenged by antigen, which, ultimately leads to antibody class switching to produce IgG, which is the clinical measurement for the assessment of antibody titer.

5. While Dong et al. claimed that the antibody response was reduced based on IgM PFCR data; the IgM PFCR activity was only evaluated in spleen cells only. The authors should have also looked at thymus and serum for IgM levels to illustrate that the responses are consistent.

6. By way of similar rationale listed in point #3, Dong et al. should have looked at IgG in addition to IgM, as well as evaluated IgG levels in thymus and serum.

7. While the immune cell populations were reported by Dong et al. in spleen and thymus, they did not look at these cell populations in another key immune organ: bone marrow. That was a major omission by the study authors.

8. While Dong et al. reported NK cell activity in their study for the spleen, they did not examine the thymus.

9. The LDH assay is not a standard assay used to assess NK cell activity and the LDH values reported by Dong et al. should not be interpreted as NK cell activity data. LDH measurement is associated with cell membrane integrity and it is a non-specific assay. The standard assay for NK cell activity is flow cytometry, which Dong et al. did not perform.

10. Dong et al. reported a negative effect of PFOS and the splenic lymphocyte proliferation as a way of demonstrating that the immune cells were not "proliferating" upon challenge. However, the specific problem with this piece of data is that MTT assay is not a measurement of cell proliferation. It is simply an indicator of cell's mitochondrial respiration state and it does not reflect any proliferative responses at all. The standard assay for cell proliferation would be something like BrDU assay, which was not evaluated by Dong et al.

11. The antigen challenge substance used by Dong et al. was sheep red blood cell (SRBC) and in the field of immunology, responses from SRBC challenge are very crude and non-specific to T cell activation. There are many T-cell dependent antigens available for use in the immunology research (i.e., ovalbumin) and Dong et al. failed to recognize this.

12. No information on blood lymphocyte counts was provided (part of the standard CBC panel parameters).

13. No histological evidence for thymus, spleen, or bone marrow was provided.

14. Dong et al. only evaluated male mice; they should have also looked at female mice to rule out any gender-specific difference in the immune response.

As discussed above, antibody response is IgG isotype, not IgM. If PFOS was truly an immunosuppressing agent, one would expect similar suppressive immune responses to be observed in major key organs such as decreased IgM and IgG in spleen, thymus, and serum concurrently. Dong et al. evaluated IgM in spleen only but did not provide any concurrent IgM status in other key organs such as thymus or serum. As an immunosuppressing agent, one would expect decreased immune cell populations in spleen, thymus, blood, and bone marrow and Dong et al. only looked at spleen and thymus. As an immunosuppressing agent, one would expect decreased proliferation in immune cells and Dong et al. did not use the correct methods to evaluate these responses. If one is to rely on Dong et al. data as the basis for their evaluation, they need to justify why, when compared to the concurrent control with an overall body weight gain of 3. 1 g over 60-day dosing period, a significant lower overall body weight-gain of 2.58 g in the lowest dose group mice (0.5 mg/kg/ TAD) did not confound the immunological responses reported.

Peden-Adams et al. (2008) reported increased lymphatic NK cell activity was seen in male B6C3F1 mice but not females; however, NK cell activity was not measured in other key immune organs such as spleen, thymus, or serum. They also reported suppression of IgM but did not evaluate IgG. The study by Guruge et al. (2009) reported that exposure to PFOS was associated with reduced ability of animals to respond to infectious disease, which was based on the resistance of female B6C3F1 mice to influenza virus A/PR/8/34 (H1N1) after exposure to PFOS. However, the study was confounded by mortality.

Collectively, these studies cannot be conclusively interpreted as demonstrating an effect of PFOS on immune functions and there is no robust scientific evidence to support the claim that PFOS is associated with immune suppression in mice.

On page A-44 of the draft Toxicological Profile (for PFOS MRL), contrary to what ATSDR stated that "Immune function was not examined following chronic-duration oral exposure in laboratory animal studies", it should be noted that the primary immune organs were evaluated microscopically in rats after 2 years of dietary treatment containing potassium PFOS (Butenhoff et al. 2012a). In this study, representative primary immune organs were collected (femur with bone marrow, lymph node (mesenteric), spinal cord (cervical, thoracic, and lumbar); spleen; sternum with bone marrow, and thymus) and evaluated microscopically by a board-certified veterinary pathologist at the end of a 2-year period. There were no statistically significant findings (neoplastic or non-neoplastic) for these immune organs in either male or female rats fed potassium PFOS in diet when compared with respective control group rats. This is important because it demonstrated the <u>absence</u> of a direct effect on primary immune organs with chronic PFOS exposures in the rats. In addition, PFOS-treated rats had similar or higher percent survival compared to controls, which is contrary to chronic immunosuppression-mediated toxicity such as cyclosporin (a known immunosuppressant) that ultimately resulted in increased mortality in rats (Ryffel and Mihatsch 1986).

I. <u>Inconclusive immune findings in human epidemiological data.</u> While ATSDR concluded that such findings in rodents were consistent with human epidemiology studies with regards to vaccine responses (see epidemiology discussion above), it is important to recognize that the humoral immune response to vaccinations, as measured in the human epidemiology studies, is mainly a secondary IgG memory response, not IgM. While suppression of the IgM response by PFOS was demonstrated in several animal studies where administered doses also induced signs of overt toxicity (i.e., reductions in body and lymphoid organ weight), it is difficult to interpret why the primary IgM response was suppressed in mice by PFOS and yet the secondary response was either not affected or enhanced. Collectively, the aforementioned studies suggest that PFOS impairs immune cell activity in laboratory animals at very high doses which may be mediated in part by overt toxicity as suggested by increased corticosterone serum levels, decreased body and lymphoid organ weights and decreased lymphoid tissue cellularity. The animal studies do not support that PFOS suppresses immune cell activity in the absence of overt toxicity.

J. <u>Serum PFOS concentrations in pups should be considered for POD because critical effects chosen by ATSDR were based on (developing) pups.</u> ATSDR selected a rat 2-generation study (Luebker et al. 2005a) for the point-of-departure to derive the MRL value for PFOS

(endpoints were decreased pup bodyweight and delayed eye opening in offspring of SD rats). Similar to PFOA, the study chosen by ATSDR for the PFOS POD examined developmental endpoints that were measured in offspring, which are used as the basis for the MRL. In order to estimate steady-state plasma concentrations of PFOS, ATSDR used the Wambaugh model for PFOS, which is parameterized for adult animals and cannot be used to predict concentrations in fetuses or pups. This model also does not account for life stage differences in physiology or pharmacokinetics. The area-under-the-curve (AUC) and steady-state concentration are probably different in the offspring than in the dam. Overall internal exposure (as estimated by calculation of the AUC) may change with growth, and there could be a period of peak exposure. Use of the Wambaugh model introduces uncertainty in the MRL derivation as the offspring plasma concentration may be different that than of the maternal animals. Use of a physiologically-based model that incorporates fetal and pup compartments would provide an estimate of fetal and pup internal exposure (rather than use of the maternal concentration as a surrogate), which would reduce the uncertainty in the MRL value.

K. <u>HED for PFOS will be higher when considering faster half-life.</u> In the MRL calculations, ATSDR chose to use the <u>arithmetic mean</u> serum elimination half-life estimate for PFOA from Olsen et al. (2007) over other studies because Olsen et al. had a longer follow up time and ATSDR was concerned that based on a study by Seals et al. (2011), slower kinetics is likely to constitute a larger contribution to the terminal half-life. For example, whereas Olsen et al. had an average follow-up of 5 years, Bartell et al. had a follow-up of a year and Li et al. had a follow-up of 2.3 years among those studies that followed individuals and were not cross-sectional analyses of populations. However, this line of reasoning by ATSDR for selection of the arithmetic mean from the Olsen et al. study fails to take into account several factors that likely biased upwards the ATSDR MRL estimates. These include the following points.

1. The ATSDR chose not to use the geometric mean estimate that was discussed in the Olsen et al. paper. Given the right skewness of their data, Olsen et al. were more favorable to use the geometric mean for a measure of central tendency. ATSDR provided no explanation as to why they chose the arithmetic mean vs. the geometric mean in this study. This decision is interesting (and curious) because ATSDR chose to report median initial and final concentrations in Table A2 rather than the arithmetic mean initial and final concentrations in Table A2. A median concentration would be better represented by a half-life estimate based on the geometric mean.

2. The Olsen et al. 2007 study comprised 26 retirees (end of study average age = 66 years) who likely would have had an average glomerular filtration rate lower than those calculated from younger ages as reported in Bartell et al. (average age 55) and Li et al. (age range 15 – 55). The average estimated glomerular filtration rate declines with age as shown in the table below.

Age range	Estimated GFR (ml/min/1.73 m^2)	Source:
1-6 months	77	Heilbron et al. 1991 Pediatr Nephrol. Jan;5(1):5-11.
6-12 months	103	
12-19 months	127	
2-12 years	127	
20–29	116	https://www.kidney.org/sites/default/files/docs/11-10-1813_abe_patbro_gfr_b.pdf
30–39	107	
40–49	99	
50–59	93	
60–69	85	
70+	75	

Renal clearance of perfluorocarboxylates (and perfluorosulfonates) is largely a sum of three processes involving glomerular filtration, renal tubular secretion, and renal tubular reabsorption (Han et al. 2012). Because PFOA and other perfluorocarboxylates vary in their affinities to bind plasma proteins, glomerular filtration of perfluorocarboxylates (and perfluorosulfonates) is a product of the unbound fraction of the perfluorocarboxylate and the glomerular filtration rate (GFR). Thus, the higher estimates of GFR based on the younger ages in the other study populations, especially the younger Li et al. study which had approximately 50% of the follow-up time of Olsen et al., may be due to the age differences of the subjects, and not the shorter follow-up period considered in these studies. Thus, the serum elimination half-lives are likely equally valid for consideration in MRL calculations.

3. The Olsen et al. study had to consider, during the course of their follow-up, the possibility of retirees reentering the 3M Decatur and Cottage Grove manufacturing plants. Indeed, this resulted in Olsen et al. eliminating 1 study subject entirely, and truncating follow-up times for two retirees. This would have biased estimates upwards for the serum elimination half-lives due to the increased exposure. It is not likely that ambient general population level concentrations would have biased these retiree's estimates substantially as discussed by Bartell et al. 2012. On the other hand, although Bartell et al. and Li et al. had shorter follow-up times, the primary exposure in these populations was through drinking water. Installation of GAC filters in these populations' affected municipal water supply would have immediately ceased their exposure to PFOA, PFOS, and PFHxS.

4. ATSDR suggests the Seals et al study indicates a lower clearance rate may occur as subjects are followed long-term post exposure; thus, the decision by ATSDR to use the study that had the longest follow-up time (Olsen et al. 2007). However, ATSDR did not mention the main limitations of the Seals et al. study: 1) the cross-sectional nature of the analysis. Individual subjects were not followed. Model-based estimates were instead calculated based on the initial concentrations; 2) there was the added assumption that there was uniform exposure based on the concentration of PFOA measured in each water district; and 3) subjects with initial PFOA concentrations < 15 ng/mL were excluded

which maximized the probability of analyzing individuals with sufficiently high baseline PFOA concentrations that would not be at ambient levels.

5. Given the above additional considerations (beyond that of ATSDR's consideration about the length of follow-up), the MRLs, assuming same PODs from the same studies, are recalculated in the table below using the different serum elimination half-life values for PFOA, PFOS, and PFHxS that are reported in Bartell et al., Li et al., and Seals et al. Accordingly, the percent of the MRL that might be overestimated by the ATSDR using in their most conservative serum elimination value (arithmetic means from Olsen et al. 2007) would then result in a range of overestimations of the MRL for PFOS between 12 and 38 percent. This type of sensitivity analysis is definitely needed in Appendix A for the MRL calculations to take into account the variation of serum elimination half-life estimates that have been reported in the literature that will be, in part, a function of the GFRs from the population studied. Given the fact that ATSDR has used developmental studies to calculate the PODs for their MRLs, it is therefore not justified to use the arithmetic mean half-life estimate based solely on retirees, in part, because the GFRs of older adults are markedly lower than adults of much younger age and people 65 years of age or older represent only approximately 15% of the general population Therefore the estimated half-lives should reflect the entire population, not just the upper tail, which can be a reflection of lower GFRs that occur with age. Thus, calculation of serum elimination half-lives may be ages, sex, and concentration-dependent. MRLs, based in part on half-lives, should reflect this diversity of inputs in their calculations.

Reference Study	Estimated Half-life		MRL (mg/kg/d)	% MRL over current ATSDR MRL
	Years	Days		
*ATSDR Estimate. (arithmetic Mean from Olsen et al. 2007)	5.4	2000	1.72E-06	--
Olsen et al. 2007 (geometric mean)	4.8	1752	1.96E-06	12
Li et al. 2018	3.4	1241	2.77E-06	38

As illustrated above, because HED and MRL are dependent of the clearance rate used, the resulting MRL for PFOS can differ substantially and could be 12 to 38% higher than the current provisional MRL proposed by ATSDR.

L. Wambaugh benchmark dose model used by ATSDR was not optimized. ATSDR relied on animal PBPK model to predict subsequent POD of MRL derivation, but on the other hand, it has also explicitly stated that "Although physiologically based pharmacokinetic (PBPK) models have been developed for rodents and humans, these models are not sufficient to allow for comparisons between administered doses in laboratory animals and serum concentrations in humans" (*cf*. page 5 of draft profile). This statement indicated a great amount of uncertainty associated with the PBPK model used hence ATSDR needs to acknowledge this fact in its summary.

Although the Wambaugh model was used to estimate final maternal plasma concentrations in rats from developmental datasets (Butenhoff et al. 2009b; Chen et al. 2012; Luebker et al. 2005a; Luebker et al. 2005b; Thibodeaux et al. 2003), the model was not specifically

parameterized for this, which is another factor contributing to the uncertainty in using this model to estimate an MRL for a developmental endpoint.

The Wambaugh PFOS model was parameterized for male and female cynomolgus monkeys, male and female SD rats, and male and female CD1 mice. ATSDR states that they could not model some data sets as the studies were conducted in strains that the model was not parameterized for. Specifically, they state that they could not model the following studies: Long et al. 2013 (C57BL/6 mice), Dong et al 2009 and 2011 (C57BL/6 mice), Guruge et al. 2009 (B6C3F1 mice), Peden-Adams et al. 2008 (B6C3F1 mice), Wang et al. 2015 (Wistar rats), Onishchenko et al. 2011 (C57BL/6 mice), and Yahia et al. 2008 (ICR mice). ATSDR provides no evidence of sex or strain differences in pharmacokinetics for mice or rats. As ATSDR modeled only certain strains, this limits the studies they can use when relying on this model and introduces further uncertainty in MRL values.

M. Uncertainty factors by ATSDR were overly conservative and not supported by scientific data. They include:

1. Use of "3" for animal-to-human, in addition to large dosimetric TK adjustment, is not scientifically justified. While 3M agrees with ATSDR that adjusting for toxicokinetic difference between human and rodent serum clearance of PFOS is appropriate; 3M does not agree with the serum elimination half-life chose by ATSDR for the calculation (see toxicokinetic discussion above). While this represented a factor of 14,400 based on ATSDR's MRL derivation, 3M does not agree with ATSDR that an additional factor of "3" is needed to account for uncertainty in using laboratory animal data to derive human exposure levels. This, in fact, represents an adjustment of 43,000 when taking dosimetry into account. The use of an additional factor of 3 to account for rodent-to-human toxicodynamic difference is not scientifically justified and unnecessary.

   More specifically, ATSDR has derived its proposed MRL based on the rodent developmental data. Because humans are considerably less sensitive to the pleiotrophic effects of xenosensor nuclear receptors such as PPARα, CAR/PXR activation compared to rodents (Corton et al., 2014; Elcombe et al., 2014; Gonzalez and Shah, 2008; Klaunig et al., 2003; Klaunig et al., 2012; Lake, 2009; Ross et al., 2010), the qualitative differences brings into question the relevance of rodent developmental effects with exposure to PFOS and biological significance to humans. Thus, the very large dosimetric adjustment of 14,400 more than adequately compensates for the additional factor of 3 for difference between rodents and humans. ATSDR should not apply another factor of 3 for animal to human when this uncertainty is already embedded in the large adjustment for the dosimetric difference.

2. Additional factor of "10" for human variability is overly conservative. For PFOS MRL, ATSDR included a factor of 10 for human variability. If ATSDR could have developed a more appropriate PBPK model that accounted for life stage differences in humans (rather than relying on rodent model), this factor of 10 for human variability could potentially be reduced.

3. Scientifically unjustified use of "10" for concerns on immunotoxicity. As discussed earlier, to the extent that exposure to PFOS influences immune cell activities at very high doses in laboratory animals and as such, these systemic effects indirectly affect immune responses. In addition, long-term subchronic studies in non-human primates (Chang et al. 2017; Seacat et al. 2002) as well as 2-year chronic study in rats (Butenhoff et al. 2012a) did not identify the immune system being the target organs. As a matter of fact, the survival rates in the 2-year chronic study in PFOS-treated rats were higher than the concurrent control. The animal studies do not support that PFOS suppresses immune cell activity in the absence of overt toxicity and an uncertainty factor of "10" is not scientifically justified and should be removed by ATSDR.

   [NOTE: It should be noted that the 2-generation reproductive and developmental study in rats with exposure to PFOS (Luebker et al. 2005) was the same critical study chosen by U.S. EPA Office of Water for the derivation of the Lifetime Water Health Advisory for PFOS issued in 2016. EPA's conclusion on the immunotoxicity is included below:]

   > *"Both human and animal studies have demonstrated the potential impact of PFOS on the immune system; however, uncertainties exist related to MOA and the level, duration, and/or timing of exposure that are not yet clearly delineated. The animal immunotoxicity studies support the association between PFOS and effects on the response to sheep red blood cells as foreign material and on the natural killer cell populations; however, the doses with effects are inconsistent across studies for comparable endpoints. When both males and females were evaluated, the males responded at a lower dose than the females. Because of these uncertainties, EPA did not quantitatively assess this endpoint."*

Detailed Comments on PFHxS MRL

ATSDR Position (page A-49)

MRL Summary: A provisional intermediate-duration oral MRL of 2x10-5 mg/kg/day was derived for PFHxS based on thyroid follicular cell damage in adult male rats administered via gavage PFHxS for a minimum of 42 days (Butenhoff et al. 2009a; Hoberman and York 2003). The MRL is based on a HED NOAEL of 0.0047 mg/kg/day and a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability) and a modifying factor of 10 for database limitations.

Selection of the Critical Effect: Two intermediate-duration studies in laboratory animals have been identified for PFHxS. In a developmental toxicity study, increased incidences of thyroid follicular cells hypertrophy, and hyperplasia were observed in F0 male rats administered ≥3 mg/kg/day (Butenhoff et al. 2009a; Hoberman and York 2003). Increased liver weight and centrilobular hepatocellular hypertrophy were also observed in the males at ≥3 mg/kg/day. No reproductive or developmental effects were reported. Liver effects (decreases in serum lipids, increases in hepatic triglyceride levels, and increases in liver weight) were also observed in mice exposed to 6 mg/kg/day PFHxS in the diet for 4–6 weeks (Bijland et al. 2011). Using the Hall et al. (2012) criteria (see Section 2.9 for a discussion of the criteria), the liver effects were not considered relevant for human risk assessment. Thus, the lowest LOAEL identified in intermediate-duration studies was 3 mg/kg/day for thyroid effects.

3M Conclusion

A. The critical effect concluded by ATSDR with PFHxS exposure (thyroid follicular cell damage) has been not shown in humans
B. No conclusive evidence to suggest that PFHxS impacts thyroid homeostasis in rodents
C. ATSDR should recognize rodent-specific thyroid effects and their relevance to humans
D. HED for PFHxS will be higher when considering faster half-life
E. Wambaugh benchmark dose model used by ATSDR was not optimized
F. Uncertainty factors by ATSDR were overly conservative and not supported by scientific data
    1. Use of "3" for animal-to-human, in addition to large dosimetric TK adjustment, is conservative because humans are less sensitive than rodents based on *in vitro* hepatocyte data (Bjork and Wallace 2009)
    2. Scientifically unjustified use of "10" for concerns on database limitations, especially on immunotoxicity and general toxicity

ATSDR's overall interpretation on both toxicology and epidemiology data are inconsistent with the most current knowledge. Its application of uncertainty factors is not scientifically justified and the proposed PFHxS MRL is not supported by the scientific data. The PFHxS MRL derived for the human-health risk assessment overly conservative and not supported by adequate scientific foundation.

3M Comments (Details):

A. The critical effect concluded by ATSDR with PFOA exposure (thyroid follicular cell damage) has been not shown in humans. ATSDR needs to offer a plausible explanation as to why it believes these effects are relevant to human risk assessment.

B. No conclusive evidence to suggest that PFHxS impacts thyroid homeostasis in rodents. Based on findings from a reproductive and developmental study with PFHxS in rats (Butenhoff et al. 2009a), ATSDR concluded that the thyroid follicular cell damage findings in rats was the critical effect and used that as the basis for its derivation of PFHxS MRL. This is not the correct interpretation.

It is incorrect for ATSDR to conclude that there was "thyroid follicular cell damage" based on the study findings reported by Butenhoff et al. (2009a). The descriptor "increased incidence of thyroid follicular epithelium hypertrophy/hyperplasia" does not mean "thyroid follicular cell damage". In that study where rats received daily doses of potassium PFHxS at either 0, 0.3, 1, 3, or 10 mg/kg/day, increased incidence of thyroid follicular epithelium hypertrophy/hyperplasia was noted in the 10 mg/kg/day dose group male rats after 42 days of treatment (see table below). Because histomorphometrically, there is a distinct difference between hypertrophy (increases in cell size) vs. hyperplasia (increases in cell number), it is impossible to determine whether there was actual thyroid hyperplasia associated with PFHxS exposure in the rats because, following standard practice at the time of the study, both hypertrophy and hyperplasia were reported as one category by the original study pathologist.

		Potassium PFHxS Doses (mg/kg/day)				
		0 (control)	**0.3**	**1.0**	**3.0**	**10**
Number of F_0 male rats evaluated		10	10	10	10	10
Microscopic Thyroid hypertrophy/hyperplasia (follicular epithelium)	Minimal	0	1	1	2	0
	Mild	2	2	1	2	3
	Moderate	0	0	0	0	4
	Total Incidence	**2**	**3**	**2**	**4**	**7**

Given that the systemic circulating thyroid hormones levels were not measured in that study, as stated by the study authors, the overall thyroid hormone status was difficult to interpret because the combined histological categorization added additional uncertainty. In addition, because thyroid gland dysfunction could potentially affect the reproductive functions in the animals, but yet there were no treatment-related effects on mating or fertility in any of the PFHxS-treated rats, there was no strong evidence to support thyroid-related effects based on this study.

In addition, ATSDR should recognize that in rodents, increased hepatocellular hypertrophy due to activation of hepatic nuclear receptors is often accompanied by increased thyroid follicular epithelial hypertrophy/hyperplasia (Capen 1997). This is a well-documented in rodents and it is primarily due to the increased hepatocyte mass (hypertrophy) overall will result in an increase in overall liver metabolism. The increased liver metabolism is capable of directing the circulating thyroid hormone for rapid turnover (with increased hepatic UDP-glucuronyl transferase). Consequently, to compensate for the higher turnover rate of thyroid hormones, there will be an increase in thyroid gland activity hence it is common to see hepatocellular hypertrophy and thyroid hypertrophy concurrently. Again, this observation is particularly well-known phenomenon in rodents but not in humans (see detailed discussion below) (Capen 1997; Curran and DeGroot 1991). Therefore, the observed increase in mild to moderate thyroid follicular epithelial hypertrophy and hyperplasia in the 10 mg/kg-d treatment group males was consistent with the increase in centrilobular hepatocellular hypertrophy associated with exposure to PFHxS. Again, it reflected the activation of xenosensor nuclear receptor activation in rats when exposed to PFHxS (Bijland et al. 2011; Bjork et al. 2011; Bjork and Wallace 2009; Chang et al. 2018).

Recognizing this uncertainty as well as the difference in serum toxicokinetics between female rats and female mice, a separate OECD 422 study was reported by Chang et al. (2018) and they demonstrated that thyroid hormone status in mice exposed to PFHxS (based on TSH levels and thyroid histopathology) was not altered. In that study, there was no effect of PFHxS on TSH in the adult F_0 mice or in the F_1 pups when serum TSH was measured at multiple times during their development; and, most importantly, there were no effect on thyroid histopathology. Therefore, there is no evidence to suggest that PFHxS impacts thyroid homeostasis.

C. ATSDR should recognize rodent-specific thyroid effects and their relevance to humans. In addition, there are significant differences exist in thyroid hormone physiology between rodents and humans. In human and non-human primates, circulating thyroid hormones are bound primarily to thyroid binding globulin (TBG) and this high-affinity binding protein is absent in rodents (Oppenheimer et al. 1995). Rodents mainly rely on serum albumin, which has lower affinity than TBG, as thyroid hormone carriers. The plasma thyroid hormone half-life is considerably shorter (12 – 24 hours) than in humans (5 – 9 days) (Capen 1997). It has been well demonstrated that, between rodents and humans, these difference in plasma half-lives of thyroid hormones and binding affinity to carrier proteins attribute to a greater sensitivity of rodents (but not humans) in developing hypertrophic and hyperplastic lesions (Capen 1997; Curran and DeGroot 1991).

In summary, ATSDR should recognize that there are distinct differences in thyroid hormone regulations between rodents and humans; and similar to hepatocellular hypertrophy noted in rats, thyroid findings in rodents require careful (weight-of-evidence) interpretation when extrapolating to human risk assessment.

D. HED for PFHxS will be higher when considering faster half-life. In the MRL calculations, ATSDR chose to use the arithmetic mean serum elimination half-life estimate for PFOA from Olsen et al. (2007) over other studies because Olsen et al. had a longer follow up time and ATSDR was concerned that based on a study by Seals et al. (2011), slower kinetics is likely to constitute a larger contribution to the terminal half-life. For example, whereas Olsen et al. had an average follow-up of 5 years, Bartell et al. had a follow-up of a year and Li et al. had a follow-up of 2.3 years among those studies that followed individuals and were not cross-sectional analyses of populations. However, this line of reasoning by ATSDR for selection of the arithmetic mean from the Olsen et al. study fails to take into account several factors that likely biased upwards the ATSDR MRL estimates. These include the following points.

1. The ATSDR chose not to use the geometric mean estimate that was discussed in the Olsen et al. paper. Given the right skewness of their data, Olsen et al. were more favorable to use the geometric mean for a measure of central tendency. ATSDR provided no explanation as to why they chose the arithmetic mean vs. the geometric mean in this study. This decision is interesting (and curious) because ATSDR chose to report median initial and final concentrations in Table A2 rather than the arithmetic mean initial and final concentrations in Table A2. A median concentration would be better represented by a half-life estimate based on the geometric mean.

2. The Olsen et al. 2007 study comprised 26 retirees (end of study average age = 66 years) who likely would have had an average glomerular filtration rate lower than those calculated from younger ages as reported in Bartell et al. (average age 55) and Li et al. (age range 15 – 55). The average estimated glomerular filtration rate declines with age as shown in the table below.

Age range	Estimated GFR (ml/min/1.73 m^2)	Source:
1-6 months	77	Heilbron et al. 1991 Pediatr Nephrol. Jan;5(1):5-11.
6-12 months	103	
12-19 months	127	
2-12 years	127	
20–29	116	https://www.kidney.org/sites/default/files/docs/11-10-1813_abe_patbro_gfr_b.pdf
30–39	107	
40–49	99	
50–59	93	
60–69	85	
70+	75	

Renal clearance of perfluorocarboxylates (and perfluorosulfonates) is largely a sum of three processes involving glomerular filtration, renal tubular secretion, and renal tubular reabsorption (Han et al. 2012). Because PFOA and other perfluorocarboxylates vary in their affinities to bind plasma proteins, glomerular filtration of perfluorocarboxylates (and perfluorosulfonates) is a product of the unbound fraction of the perfluorocarboxylate

and the glomerular filtration rate (GFR). Thus, the higher estimates of GFR based on the younger ages in the other study populations, especially the younger Li et al. study which had approximately 50% of the follow-up time of Olsen et al., may be due to the age differences of the subjects, and not the shorter follow-up period considered in these studies. Thus, the serum elimination half-lives are likely equally valid for consideration in MRL calculations.

3. The Olsen et al. study had to consider, during the course of their follow-up, the possibility of retirees reentering the 3M Decatur and Cottage Grove manufacturing plants. Indeed, this resulted in Olsen et al. eliminating 1 study subject entirely, and truncating follow-up times for two retirees. This would have biased estimates upwards for the serum elimination half-lives due to the increased exposure. It is not likely that ambient general population level concentrations would have biased these retiree's estimates substantially as discussed by Bartell et al. 2012. On the other hand, although Bartell et al. and Li et al. had shorter follow-up times, the primary exposure in these populations was through drinking water. Installation of GAC filters in these populations' affected municipal water supply would have immediately ceased their exposure to PFOA, PFOS, and PFHxS.

4. ATSDR suggests the Seals et al study indicates a lower clearance rate may occur as subjects are followed long-term post exposure; thus, the decision by ATSDR to use the study that had the longest follow-up time (Olsen et al. 2007). However, ATSDR did not mention the main limitations of the Seals et al. study: 1) the cross-sectional nature of the analysis. Individual subjects were not followed. Model-based estimates were instead calculated based on the initial concentrations; 2) there was the added assumption that there was uniform exposure based on the concentration of PFOA measured in each water district; and 3) subjects with initial PFOA concentrations < 15 ng/mL were excluded which maximized the probability of analyzing individuals with sufficiently high baseline PFOA concentrations that would not be at ambient levels.

5. Given the above additional considerations (beyond that of ATSDR's consideration about the length of follow-up), the MRLs, assuming same PODs from the same studies, are recalculated in the table below using the different serum elimination half-life values for PFOA, PFOS, and PFHxS that are reported in Bartell et al., Li et al., and Seals et al. Accordingly, the percent of the MRL that might be overestimated by the ATSDR using in their most conservative serum elimination value (arithmetic means from Olsen et al. 2007) would then result in a range of overestimations of the MRL for PFHxS between 14 and 38 percent. This type of sensitivity analysis is definitely needed in Appendix A for the MRL calculations to take into account the variation of serum elimination half-life estimates that have been reported in the literature that will be, in part, a function of the GFRs from the population studied. Given the fact that ATSDR has used developmental studies to calculate the PODs for their MRLs, it is therefore not justified to use the arithmetic mean half-life estimate based solely on retirees, in part, because the GFRs of older adults are markedly lower than adults of much younger age and people 65 years of age or older represent only approximately 15% of the general population Therefore the estimated half-lives should reflect the entire population, not just the upper tail, which can

be a reflection of lower GFRs that occur with age. Thus, calculation of serum elimination half-lives may be age, sex, and concentration-dependent. MRLs, based in part on half-lives, should reflect this diversity of inputs in their calculations.

Reference Study	Estimated Half-life		MRL (mg/kg/d)	% MRL over current ATSDR MRL
	Years	Days		
*ATSDR Estimate (arithmetic Mean from Olsen et al. 2007)	8.5	3100	1.57E-05	--
Olsen et al. 2007 (geometric mean)	7.3	2665	1.82E-05	14
Li e al. 2018	5.3	1935	2.51E-05	38

As illustrated above, because HED and MRL are dependent of the clearance rate used, the resulting MRL for PFHxS can differ substantially and could be 14 to 38% higher than the current provisional MRL proposed by ATSDR.

E. Wambaugh benchmark dose model used by ATSDR was not optimized. Similar to comments provided above for PFOS and PFOA, the MRL is largely based on uncertainty rather than on supportable science derived from Wambaugh model. Again, ATSDR relied on animal PBPK model to predict subsequent POD of MRL derivation, but on the other hand, it has also explicitly stated that "Although physiologically based pharmacokinetic (PBPK) models have been developed for rodents and humans, these models are not sufficient to allow for comparisons between administered doses in laboratory animals and serum concentrations in humans" (*cf*. page 5 of draft profile). This statement indicated a great amount of uncertainty associated with the PBPK model used hence ATSDR needs to acknowledge this fact in its summary.

F. Uncertainty factors used by ATSDR were overly conservative and not supported by scientific data. They include:

1. Use of "3" for animal-to-human, in addition to large dosimetric TK adjustment, is not scientifically justified. While 3M agrees with ATSDR in principle to adjust for toxicokinetic difference between human and rodent serum clearance of PFHxS, which represented a factor of 15,500 based on ATSDR's derivation, 3M does not agree an additional factor of "3" is needed to account for uncertainty in using laboratory animal data to derive human exposure levels. This, in fact, represents an adjustment of 46,000 when taking dosimetry into account. The use of an additional factor of 3 to account for rodent-to-human toxicodynamic difference is unnecessary and not scientifically justified.

   More specifically, ATSDR has derived its proposed MRL based on the rodent developmental data. Because humans are considerably less sensitive to the pleiotrophic effects of xenosensor nuclear receptors such as PPARα, CAR/PXR activation compared to rodents (Corton et al. 2014; Elcombe et al. 2014; Gonzalez and Shah 2008; Klaunig et al. 2003; Klaunig et al. 2012; Lake 2009; Ross et al. 2010), the qualitative differences brings into question the relevance of rodent developmental effects with exposure to PFHxS and biological significance to humans. Thus, the very large

dosimetric adjustment of 15,500 more than adequately compensates for the additional factor of 3 for difference between rodents and human extrapolation. ATSDR should not apply another factor of 3 for animal to human when this uncertainty is already embedded in the large adjustment for the dosimetric difference.

2. Additional factor of "10" for human variability is overly conservative. For the PFHxS MRL, ATSDR included a factor of 10 for human variability. If ATSDR could have developed a more appropriate PBPK model that accounted for life stage differences in humans (rather than relying on rodent model), this factor of 10 for human variability could potentially be reduced.

3. Scientifically unjustified use of "10" for concerns on database limitations, especially on immunotoxicity and general toxicity. ATSDR stated that there is limited toxicology database on PFHxS, especially with regards to immunotoxicity and general toxicity. This is not correct.

   Albeit the number of publications on PFHxS is fewer than PFOS or PFOA, the available studies (to date) on PFHxS have addressed many key toxicity endpoints such as liver and cholesterol under repeated dose conditions following comprehensive macroscopic and microscopic examinations (Bijland et al. 2011; Butenhoff et al. 2009a; Chang et al. 2018). ATSDR is incorrect in stating that there are limited "general toxicity" information on PFHxS.

   Furthermore, with regards to the immunotoxicity, ATSDR has not justified the relevance of existing studies to human risk assessment. Studies by Butenhoff et al. (2009a) and Chang et al. (2018), repeated oral treatments of PFHxS to either adult male rats or mice for 42 days, and, pregnant dams from the beginning of gestation to the end of lactation, had no effects on the weights (absolute or relative) or the histology of the primary immune organs, including thymus, spleen, lymph nodes, or bone marrow. These data clearly support an absence of effects on immune function, which was the conclusion by ATSDR (on Table 2-5 of the draft profile).

   Therefore, the default database uncertainty factor of "10" is not scientifically justified and should be removed by ATSDR.

Detailed Comments on Pregnancy-induced hypertension / pre-eclampsia (PFOA, PFOS)

ATSDR Position

ATSDR concluded there is "*suggestive epidemiological evidence for an association between serum PFOA and PFOS and pregnancy-induced hypertension/pre-eclampsia*." For PFOA, evidence was based on 6 studies: 4 cross-sectional (Nolan et al. 2010; Savitz et al. 2012a; Savitz et al. 2012b; Stein et al. 2009) 1 prospective cohort (Darrow et al. 2013) and 1 case-cohort (Starling et al. 2014). For PFOS, evidence was based on 3 studies (Stein et al. 2009; Darrow et al. 2013; Starling et al. 2014).

3M Comments on Preeclampsia

It is unclear why ATSDR combined pregnancy-induced hypertension and pre-eclampsia into a single health outcome. While both diseases are defined by new onset of hypertension that develops after the 20th week of pregnancy, preeclampsia is a far more serious complication of pregnancy often characterized by proteinuria and/or signs of clinical pathology to another organ system. Further, the American College of Obstetricians and Gynecologists recognizes pregnancy-induced hypertension and pre-eclampsia as two distinct types of hypertensive disorders with differing diagnostic criteria and disease management strategies (American College of Obstetricians and Gynecologists 2013). The ATSDR provided no scientific justification for combining these two distinct pregnancy outcomes.

Of the 6 studies referenced by ATSDR, only 3 specifically evaluated preeclampsia in relation to maternal exposure levels of PFOA and/or PFOS (Stein et al. 2009; Savitz et al. 2012a; Starling et al. 2014). These studies differed by several important factors (which were not addressed in the ATSDR draft profile) including study design, exposure assessment and preeclampsia assessment. These differences are discussed below.

Both Stein et al. (2009) and Savitz et al. (2012a) were cross-sectional studies of a highly exposed community population in the Mid-Ohio Valley region (C8 Health Study). In both studies, self-reported preeclampsia was obtained via questionnaire. This was a major deficiency of these studies given that self-reported preeclampsia has a low positive predictive value (~50-60%) when validated against medical records (Stuart et al. 2013). Further, study participants were aware of their exposure status (i.e. PFOA and PFOS levels), which likely introduced some level of recall bias. In addition, Stein et al. (2009) obtained self-reported preeclampsia outcomes between 2000-2006, which preceded PFOA, and PFOS serum measurements by approximately 5 years (*i.e.*, temporality would be difficult to establish). Savitz et al. (2012a), on the other hand, examined pregnancy outcomes from 1990 to 2004 in relation to <u>modeled</u> PFOA exposure. The model was based on serum PFOA measurements in 2005, residential histories, historical information on PFOA releases, environmental distribution and pharmacokinetic modeling. The authors reported an overall correlation of 0.67 between predicted (modeled) and observed serum PFOA levels measured in 2005-2006 and stated that "our estimates undoubtedly

introduced some misclassification" (Savitz et al. 2012a). This study observed a significant positive association for risk of preeclampsia when modeled PFOA was analyzed per 100 ng/mL increase (OR = 1.08, 95%CI: 1.01-1.15); however, no significant findings were observed when estimated serum PFOA concentrations were evaluated in quintiles (i.e., no dose-response) or per interquartile increase in the log transformed estimates. (Note: The ATSDR did not cite these null findings in the draft profile). Additionally, Stein et al. (2009) reported no significant association between self-reported preeclampsia and measured PFOA levels. Preeclampsia was, however, significantly associated with PFOS levels above the median (OR = 1.3, 95%CI: 1.1-1.7) and levels above the 90th percentile (OR = 1.6: 95%CI: 1.2-2.3), but not for levels below the 90th percentile or when PFOS was examined per increase from the 25th to the 75th percentile. (Note: Again, ATSDR failed to cite these findings in the draft profile).

The most recent study (Starling et al. 2014) to examine the potential association between preeclampsia and PFAS levels was a case-cohort study of 976 women enrolled in the Norwegian Mother and Child Cohort. Unlike studies by Stein et al. (2009) and Savitz et al. (2012a), Starling et al. (2014) was the only study to measure maternal plasma PFOA levels during mid pregnancy. Furthermore, it was the only study to use medically validated preeclampsia cases (466 cases and 510 non-cases) and include nulliparous women. Since parity is an important risk factor for preeclampsia, the exclusion of parous women was a notable strength of the study. Moreover, the inclusion of nulliparous women ensured that measured PFAS levels were not affected by recent declines in body burden due to prior pregnancies and lactation (Starling et al. 2014). This study reported no significant associations between risk of preeclampsia and measured PFOA and PFOS when analyzed in quartiles and as a continuous variable. It is important to note that while PFOA and PFOS levels in this study represented general population levels, the median PFOS concentration was approximately equal to the Mid-Ohio River Valley levels reported by Stein et al (2009).

3M Conclusion on preeclampsia

The evidence for an association between preeclampsia and PFOA and PFOS exposure is limited to 3 epidemiologic studies with inconsistent findings. When considering the important limitations of 2 studies (Stein et al. 2009; Savitz et al. 2012a), and the null findings of the methodologically strongest study (Starling et al. 2014), there is insufficient evidence of an association between preeclampsia and PFOA and PFOS exposure.

3M Comments on pregnancy-induced hypertension

Like the preeclampsia studies, only 3 studies specifically examined the association between pregnancy-induced hypertension (PIH) and PFOA and PFOS levels: 2 cross-sectional studies (Nolan et al. 2010; Savitz et al. 2012b) and one prospective cohort, with some cross-sectional analysis (Darrow et al. 2013). All three studies examined a highly exposed community population in the Mid-Ohio Valley region. Again, the ATSDR draft profile failed to acknowledge notable limitations (or strengths) of these studies and

provided no interpretation of the results. As such, study limitations and overall findings are briefly discussed below.

Nolan et al. (2009) examined the relationship between PIH and residential drinking water with elevated PFOA levels from the Little Hocking Water Association (LHWA). While this study was strengthened by use of medically validated cases of PIH, it was severely limited by lack of individual PFOA exposure measurements. Rather, water service category (LHWA only versus partial LHWA) served as a proxy for high versus low PFOA exposure. The study reported a nonsignificant unadjusted OR = 1.2, 95% CI: 0.7-2.0 and concluded that PFOA was not associated with an increased risk of maternal risk factors (Nolan et al. 2009).

Savitz et al. (2012b) examined the potential relationship between modeled serum PFOA estimates and PIH obtained from birth records in two separate analyses. Both analyses used modeled serum PFOA of the mother at 4 months of gestation. As stated previously, the study authors acknowledged that this modeling approach "undoubtedly introduced some misclassification" of PFOA exposure (Savitz et al. 2012a). In the first analysis (Study 1), models were based exclusively on the residential address listed on birth certificates. In the second analysis (Study 2), birth records were linked with lifetime residential history based on self-reported survey data. In Study 1, the authors reported "no consistent evidence of an association between estimated PFOA exposure and still birth, pregnancy-induced hypertension, preterm birth, or indices of fetal growth" and in Study 2, the authors reported that "PFOA was unrelated to pregnancy-induced hypertension" (Savitz et al. 2012b).

Darrow et al. (2009) was a prospective analysis of measured maternal PFOA and PFOS serum levels (2005-2006) and PIH cases (n=106) ascertained from birth records between 2005 and 2010). It is important to note, however, that 25% of the births preceded PFOA and PFOS serum measurements. Furthermore, PFAS levels measured in 2005-2006 may not have reflected PFAS levels at the time of follow-up (2008-2011), especially among women with reduced PFAS body burden due to multiple pregnancies and lactation. PFOA and PFOS were analyzed as continuous variables (per unit increase and per interquartile increase), and as quintiles among all births and separately for the first pregnancy conceived after serum measurement among nonpregnant women. For PFOA, among all births, significant associations were observed between PIH and PFOA analyzed as per in unit increase and as quintiles (with a significant dose-response). No associations were observed when PFOA was analyzed as per interquartile increase. More importantly, no significant associations were observed for any PFOA metric among first pregnancies conceived after serum measurement. (Note: this information was not cited in the ATSDR draft profile). For PFOS, among all births, significant associations were observed between PIH and PFOS analyzed as per in unit increase and as quintiles (with no significant dose-response), but not when PFOS was measured as per interquartile increase. Among first pregnancies conceived after serum measurement, significant associations were observed for both continuous variables and for quintile 3 only with no significant trend. Overall, inconsistent results were observed within the study and no evidence of a monotonic increase in risk was reported. The authors concluded that

"*results provide some evidence of positive associations between measured serum perfluorinated compounds and pregnancy-induced hypertension*" but also acknowledge that "*more refined outcome classification is warranted*".

3M Conclusion on Pregnancy-induced Hypertension

Only three studies have examined the association between PFOA exposure and PIH and have reported mixed results. Although Darrow et al. (2013) observed significant positive associations, the other two studies (Nolan et al. 2009; Savitz et al. 2012b) did not. Given the inconsistency in findings within the Darrow et al. (2013) study and across all 3 studies, and the fact that no independent confirmation of these findings outside the community population in the Mid-Ohio Valley region exists, the evidence of an association between PIH and PFOA exposure is limited. Further, given that Darrow et al. (2013) is the only study to have examined PIH in relation to PFOS exposure and reported mixed findings with no significant trend, therefore there is insufficient evidence of an association between PIH and PFOS exposure.

Detailed Comments on Hepatic Enzymes (alanine aminotransferase, ALT)

ATSDR position.

On page 5, ATSDR wrote, "Although a large number of epidemiology studies have examined the potential of perfluoroalkyl compounds to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causation. Based on a number of factors including the consistency of findings across studies, the available epidemiology studies suggest associations between perfluoroalkyl exposure and several health outcomes."

According to the ATSDR, this includes "liver damage, as evidenced by increases in serum enzymes and decreases in serum bilirubin levels (PFOA, PFOS, PFHxS)." Noted on page 147, ATSDR wrote, "Occupational exposure and community studies did not find increased risk of liver disease associated with PFOA or PFOS. As assessed by serum enzyme and bilirubin levels, the epidemiology studies provide suggestive evidence of liver damage. Increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) levels and decreases in serum bilirubin levels have been reported in occupational, community and/or general population studies. Although there is considerable variability across studies, the evidence is adequate for PFOA, PFOS, and PFHxS, particularly for ALT levels." Presented on pages 148-149 is Table 2-10, which displays a summary of liver disease in humans. On pages 150-156 is a summary of alterations in serum hepatic enzymes and bilirubin levels in humans. There were 13 cross-sectional studies (not counting duplicate references) and 3 longitudinal studies. [Note: Some of these studies are mislabeled as cohort studies in the draft Supporting Document for Epidemiological Studies when they are, in fact, cross-sectional studies. See Table 7 (Gilliland and Mandel 1996; Mundt et al. 2007; Olsen et al. 2000, 2003; Olsen et al. 1999) (both cross-sectional and cohort).] Liver disease and hepatic enzyme findings are discussed for PFOA on pages 170-172 with summary on page 186 where ATSDR wrote, "Exposure to PFOA does not appear to be associated with increased risks of liver disease in workers or highly exposed community members. The epidemiology studies have found associations between serum PFOA levels and increases in serum ALT, AST, and GGT enzyme levels and decreases in serum bilirubin levels. However, the results have not been consistently found, and serum enzyme levels were typically within normal range. Four studies examined the risk of serum enzyme levels outside of the normal range; the results were mixed for the risk of elevated ALT, with two studies finding and increased risk and two studies finding no association." For PFOS, the discussion of liver disease and hepatic serum enzymes and bilirubin is found on pages 187-188 with the ATSDR summary on page 196 where ATSDR wrote, "The available occupational exposure studies or general population studies do not consistently suggest an association between PFOS exposure and increases in the risk of liver disease or biliary tract disorders. A small number of occupational exposure studies have not found associations between serum PFOS levels and increases in ALT, AST, or GGT levels." The only mention of PFHxS is on page 197 were ATSDR cited the Lin et al. (Lin et al.

2010) study and that they did not find associations between ALT and GGT levels with PFHxS levels in the NHANES data set that they analyzed.

3M Comments

ATSDR mischaracterized the epidemiological data as it relates to ALT and PFOA and its use of the phrase "liver damage". ALT is a "leakage" enzyme and may be increased due to necrosis, injury or repair (Cattley and Cullen 2013). Increases of two- to four-fold in rodents, canines, non-human primates, and humans indicate hepatic injury. As defined by (Hall et al. 2012),"Based on the recommendations of regulatory authorities, (EMEA 2010; FDA 2009; HED 2002) increases in ALT activity of two-to threefold should be considered as indicated of 'hepatocellular damage.' As will be discussed below, those studies that have suggestion of an elevation of ALT remain well-within the expected physiologic range of measured ALT. Using the term 'damage' in this context is therefore highly misleading. It is also possible to have quite modest but statistically significant increases in ALT that are not toxicologically relevant (Cattley and Cullen, 2013). It should be noted that the human half-life of ALT is approximately 47 hours with significant variation of 10 – 30% on a day-to-day basis with significant circadian variation (Cordoba et al. 1999; Kim et al. 2008). ATSDR failed to mention this when cohort studies are conducted examining estimated serum PFOA concentrations over time when there is only a single ALT value reported. Finally, it should be noted that nonalcoholic fatty liver disease is the most common cause of mild elevations of liver enzymes (Giannini et al. 2005).

Several studies are worthy of careful evaluation in this ATSDR Toxicological Profile as it relates to ALT and PFOA either because: 1) the size of the population studied that was exposed to PFOA via the drinking water, 2) the study concerned occupational populations, or 3) the study was experimental and based on a phase 1 clinical trial in humans designed to ascertain the maximum tolerated dose of PFOA (ammonium salt). Three studies concerning exposure to PFOA via drinking water were from the C8 Science Panel (one cross-sectional (Gallo et al. 2012), and the other two were cross-sectional and longitudinal based on an estimated cumulative serum (ng/mL-year) model (Darrow et al. 2016). Four studies were occupational studies including two cross-sectional studies (Olsen and Zobel 2007; Sakr et al. 2007a) and two longitudinal studies (Olsen et al. 2012; Sakr et al. 2007b). One study was an experimental phase 1 clinical trial (Convertino et al. 2018). Collectively, these studies do not suggest "liver damage" (see above 2 to 4-fold increase) as measured by ALT associated with increasing serum concentrations of PFOA. Although some studies' regression coefficients for PFOA may be statistically significant, the percent variation explained of ALT by PFOA is minimal, at best, and the elevation of ALT very modest (generally an increase of 1 to 3 IU ALT). Nor is there any evidence of increased mortality from increased liver disease in epidemiologic analyses of community-based exposure to PFOA (Darrow et a. 2016) or in occupational cohort mortality studies (Steenland and Woskie 2012); (Raleigh et al. 2014).

Several types of studies are discussed below.

Community studies (n = 2)

Gallo et al. (2012). Gallo et al. reported on the C8 Health Project cross-sectional data collected in 2005-2006. They found a positive association between PFOA and serum ALT. Based on 3 different regression models, Gallo et al. reported statistically significant ln-PFOA (ng/mL) beta coefficients in models where lnALT was the independent variable. What is most important to note is that these three models had an increasing number of covariates (2, 7, and 11) besides PFOA in each model. The R^2 of these three models were 0.170, 0.174, and 0.265, respectively. However, the partial R^2 for PFOA (difference between R^2 including and excluding PFOA) remained 0.002, 0.001, and 0.002 for these three models, respectively. This clearly does not suggest that PFOA was a substantive contributor to the increase of ln ALT as it only explained between 0.1 and 0.2 percent of the variance of ln ALT, although the coefficient was statistically significant because of the study sample size (N = 47,092). The ATSDR failed to mention this very low partial R^2 in the regression modeling that was done by Gallo et al. Based on their fitting values of ALT by deciles of PFOA (given the mean values of the covariates), Gallo et al. showed a mean (untransformed) ALT of approximately 20.9 IU/L reported at 6 ng/mL PFOA that increased to approximately an ALT of 22.2 IU/L at 30 ng/mL PFOA (+1.3 IU/L increase in ALT) but plateaued thereafter. The highest decile was 23 IU/L ALT associated with approximately 320 ng/ml PFOA. It should be noted that the upper reference range (depending on laboratory) for ALT is approximately 45 IU/L.

Darrow et al. (2016). In their cross-sectional analysis, they suggested the results of the C8 Science Panel's community worker cohort study were consistent with the Gallo et al. (above) showing an increasing trend in the β coefficients across quintiles where estimated serum PFOA in 2005-2006 was Quintile 1 (2.6-<5.8 ng/mL PFOA; Quintile 2 5.8-<11.4 ng/mL; Quintile 3 11.4-<26.7 ng/mL PFOA; Q4 26.7-<81.5 ng/mL PFOA; and Q5 81.5-3558.8 ng/ml PFOA. There were up to 11 covariates in these models, which were the same as model 3 in Gallo et al. Darrow et al. did not provide R^2 or partial R^2 values in these cross-sectional analyses.

In their analysis of estimated cumulative exposure of PFOA in the C8 Science Panel's community and worker study on liver function and disease (Darrow et al. 2016), Table S1 (see supplement) of Darrow et al. provided the linear regression coefficients for ln-transformed ALT per ln PFOA. These coefficients for PFOA for the 3 models were Model 1 (β = 0.003); Model 2 (β =0.012); and Model 3 (β = 0.011) adjusted for the same number of covariates in addition to PFOA (2, 7, and 11). The R^2 for these 3 models were 0.15, 0.232, and 0.235 respectively, similar in magnitude to Gallo et al. (see above paragraph) of 0.170, 0.174, and 0.265 for the same models adjusted for the covariates in their cross-sectional analysis, although PFOA in Darrow was an estimated cumulative ng/mL-year metric versus

measured (ng/mL). However, unlike Gallo et al., Darrow did not show the partial R^2 for PFOA. Because the coefficients of determination for the Darrow et al. models 1, 2, and 3 are very similar to Gallo et al. (despite a different metric for PFOA), it is highly likely the partial R^2 for PFOA in the Darrow et al. study also remained in the extremely low range of 0.001 (0.1%) to 0.002 (0.2%), thus ln PFOA (ng/ml-years) probably explained very little of the variance of ln ALT in the Darrow et al. paper in Table S1.

Darrow et al. also estimated, via modeling, the estimated cumulative serum PFOA concentration (ln ng/mL-year) and reported (compared to the reference quintile) the following percent change in ALT per increased quintiles of estimated cumulative PFOA where: Quintile 1 (reference); Quintile 2 (191.2-<311.3 ng/mL-years PFOA) 2.3%; Quintile 3 (311.3-<794.1 ng/mL-years PFOA) 3.6%; Quintile 4 (791.4-<3997.6 ng/mL-years PFOA) 4.0%; and Quintile 5 (3997.6-205667.3 ng/mL-years PFOA 6%. In other words, at least a 10X (one order of magnitude or higher) increase in estimated cumulative PFOA in this C8 Science Panel's community workers cohort study resulted in a 6% increase (95% CI 4% to 7.9%) in the ALT. For example, if Quintile 1 reference had an ALT value of 25 IU/L, the ALT value for Quintile 5 would be 26.5 IU/L, adjusted for the 11 covariates. If the ALT value would have been 45 IU/L (upper end of normal) for ALT for Quintile 1 adjusted for the 11 covariates, the corresponding ALT value for Quintile 5 (at least an order of magnitude higher in cumulative PFOA concentration) would be 47.7 IU/L. Given the very slight change in these ALT values over a large range (at least 10X) of estimated cumulative serum PFOA concentrations, a change of just 6% in an ALT would be, for all purposes, considered clinically insignificant. This point should be emphasized by ATSDR because Darrow et al. did not report any increased risk for any liver disease or the subcategory of enlarged liver, fatty liver or cirrhosis as related to PFOA in this community worker cohort study. Based on a 10-year lagged exposure, the hazard ratios (95% CI) for these three liver diseases were Quintile 1 (reference); Quintile 2: 1.04 (0.82, 1.50); Quintile 3: 0.91 (0.64, 1.31); Quintile 4: 0.84 (0.59, 1.21); and quintile 5: 0.87 (0.61, 1.25). The hazard ratio for those prospectively followed since 2006 were Quintile 1 (reference); Quintile 2 (1.19 (0.75, 1.88); Quintile 3: 1.02 (065, 1.61), Quintile 4 (0.94 (0.60, 1.48), and Quintile 5: 0.92 (0.58, 1.47).

Thus, it would be highly inappropriate for ATSDR to continue to suggest that the enzyme findings from the Darrow et al. (or Gallo et al.) suggest "liver damage" is associated with PFOA. In fact, the C8 Science Panel (2012) stated the obvious as they interpreted their own research,

> "*From our studies of patterns of diagnosed liver disease there is no evidence of any increased risk of liver disease in relation to PFOA exposure. Based on our studies of liver enzymes and inconsistent findings in reported literature there is some evidence of small shifts in liver function, mainly within the normal physiologic range, being associated with increasing PFOA exposure. It is uncertain if PFOA is the cause of*

> *the association, but if so there is no evidence that this is reflected in any increase in overall incidence of diagnosed liver disease. Therefore, the Science Panel does not find a probable link between exposure to PFOA and liver disease."*

Furthermore, this line of reasoning by the C8 Science Panel is in agreement with the ATSDR Toxicological Profile (page 24), which stated,

> *"It should be noted that although the data may provide strong evidence of an association, it does not imply that the observed effect is biologically relevant because the magnitude of the chance may be within the normal limits or not indicative of an adverse health outcome."*

[NOTE: The C8 Science Panel findings were based on "probable link" assessments that were defined as part of a settlement agreement and do not indicate causation (Steenland et al. 2014)]

Occupational Studies (n = 4)

Sakr et al. (2007a) conducted a cross-sectional analysis of 1,025 active workers at the DuPont Washington Works plant. Median serum PFOA concentrations among 259 of the workers assigned in PFOA (ammonium salt) production areas was 494 ng/mL (range 17 – 9,550). Lesser exposed groups with more intermittent or past exposures had median PFOA concentrations ranging from 114 to 195 ng/mL. Based on a linear regression analysis with 6 other covariates (model R2 = 0.276), the regression coefficient for ALT was not statistically significant (β= 0.023, p = 0.124). Examining only those workers not taking cholesterol lowering medications (n = 840), the regression coefficient became β = 0.031, p = 0.071.

Sakr et al. (2007b) also conducted a longitudinal analysis of ALT and PFOA that involved 231 workers and their measured ALT. The regression coefficient for PFOA was not statistically significant (β= 0.54, 95% CI -0.46, 1.54).

Olsen and Zobel (2007) reported on a cross-sectional study of 506 male 3M workers, not taking cholesterol lowering medications, working at 3 different production sites. Analyzed by deciles, they reported the adjusted mean of the 1st decile was 29 IU/L (95% CI 25 – 33) compared to the mean of the 10th decile (95% CI 30 – 38). These means were not statistically significantly different. The median PFOA concentrations were 60 ng/mL (range 7 – 130) in the first decile compared to 4,940 (range 3,710 – 92,030) in the 10th decile. An adjusted (age, BMI, alcohol) regression analysis that examined ln ALT and ln PFOA resulted in a coefficient for ln PFOA of 0.0249 (p-value 0.06). A different analysis that substituted triglycerides for BMI resulted in an adjusted coefficient of 0.0115 (p-value 0.40). The latter was examined because ALT can also be elevated due to dyslipidemia (see below discussion).

Olsen et al. (2012) conducted a longitudinal analysis of workers who were engaged in the decommissioning, demolition and removal of production buildings that were involved with the production of perfluoroctanesulfonyl fluoride (POSF) and PFOA. This remediation work occurred over a 2-year time period although not all workers were engaged for that period of time. Baseline clinical chemistries and perfluoroalkyl measurements were taken before a worker became involved with the project, which was followed by similar end-of-project measurements. Of 120 workers with baseline concentrations < 15 ng/mL PFOA and < 50 ng/mL PFOS, their median increase at end-of-project was 5.3 ng/mL (mean 44.2 ng/mL) ($p < 0.0001$) and 0.7 ng/mL PFOS (median 4.2 ng/mL) ($p<0.0001$). Given these modest increases in serum PFOA or PFOS concentrations, there was no change in median ALT and the mean ALT change was -0.7 IL/L ($p = 0.53$).

Experimental study (n = 1)

Convertino et al (2018). A 6-week phase one clinical trial was conducted in Scotland to determine the maximum tolerated dose that could be provided with the weekly oral administration of PFOA (ammonium salt) for ultimately evaluating the chemotherapeutic potential of PFOA in solid tumors (Convertino et al. 2018). The study was a standard 3+3 dose escalation phase 1 study. Forty-nine subjects participated. Subjects received PFOA (ammonium salt) on a single weekly dose as high as 1200 mg week. Monitoring of clinical chemistries, including ALT, AST, GGT, alkaline phosphatase and total bilirubin were done. Based on analysis of the probability distribution functions, ALT was unchanged for any categorization with the highest PFOA category at 870 – 1530 μM (~360,000 – ~632,000 ng/mL) where a reduction of serum cholesterol consistent with a pharmacodynamic effect was evident. Given the study conditions, these authors concluded liver enzymes were not altered at PFOA concentrations that are 5 orders of magnitude greater than the general population measurements of PFOA.

General Population (NHANES) studies

It should be noted that several of the studies reported by ATDSR analyzed NHANES data. The challenges of using NHANES biomonitoring data to incorporate into any form of risk assessments has been well-described by Sobus et al. (2015). In this regard, both Lin et al. (2010) and Gleason et al. (2015) have analyzed multiple 2-year cycle NHANES cross-sectional data with liver enzymes and PFOA or PFOS. Due to its study design, ATSDR is well-aware that temporality cannot be determined in these NHANES cross-sectional studies. However, an equally important methodological limitation that has not been addressed by either Lin et al. or Gleason et al. with their analysis of NHANES data, or this ATSDR Toxicological Profile, relates to the analysis of liver enzyme data in relation with serum lipids. As shown by Deb et al. (2018), in their

analysis of NHANES data from 1999-2012 there is an association between measured liver enzymes and lipid levels. Deb et al. reported that LDL was associated with a 2-fold increase in odds of an elevated ALT and AST measurements. Thus, any association between perfluoroalkyls measurements and liver enzymes should consider at least adjusting for age, sex, race/ethnicity, and lipids. If lipids are associated with liver enzymes then lipids might be a confounder in studying the association between perfluoroalkyls and liver enzymes. However, some may suggest PFOA may be associated with lipids (at lower PFOA concentrations). Therefore, lipids, at low concentrations, might be on the causal path between the exposure (perfluoroalkyls) and increased liver enzymes. On the other hand, there is less evidence to suggest this path (higher lipids) exists at substantively higher perfluoroalkyl concentrations (see Convertino et al. 2018). Thus, the intermediate path of serum lipids might need to be considered in studying the association between perfluoroalkyls and liver enzymes. ATSDR offered no insights into this issue between perfluoroalkyls, lipids, and liver enzymes. What is certain, however, is there has not been reported to be an increased risk of self-reported liver disease in NHANES data (Melzer et al. 2010), in the Canadian Health Measures Survey (Fisher et al. 2013) as well as with medically validated liver disease with exposure to PFOA in the C8 Health Panel study (Darrow et al. 2016), including fatty liver disease. In this regard, with a lack of any increased risk for liver disease, it is inappropriate to infer very weak associations with ALT and measured perfluoroalkyls in populations whose serum PFAS concentrations can be orders of magnitude different. Thus, numerous confounding factors must be considered in analyses of ALT, including age, sex, body mass index (preferably waist-to-hip ratio as a measure of abdominal obesity), triglyceride level, total cholesterol, alcohol, glucose (women), physical activity, and smoking (the latter two are negatively correlated) (Kim et al. 2008).

3M Conclusion

There is no association between either PFOA or PFOS and liver disease including enlarged liver, fatty liver, or cirrhosis. Small percentage changes in ALT have been reported, albeit inconsistently in epidemiology studies across vastly different perfluoroalkyl concentrations, but are within normal physiological ranges. This small magnitude of change, if it is even present, does not indicate liver damage by any standard clinical practice of medicine. Confounding cannot be ruled out as a possible explanation for this observation due to the many factors that can influence ALT. Thus, there is insufficient evidence of an association with ALT.

Detailed Comments on Cholesterol

ATSDR position on PFOA and cholesterol

On page 5, the ATSDR wrote, "Although a large number of epidemiology studies have examined the potential of perfluoroalkyl compounds to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causation. Based on a number of factors including the consistency of findings across studies, the available epidemiology studies suggest associations between perfluoroalkyl exposure and several health outcomes." According to ATSDR, this included "increases in serum lipids, particularly total cholesterol and low-density lipoprotein (LDL) cholesterol (PFOA, PFOS, PFNA, PFDeA)." On pages 156-169 is Table 2-12, which provides a summary of serum lipid outcomes in humans. For various studies: Figure 2-9 is a graph of percent change in total cholesterol relative to PFOA levels; Figure 2-10 provides elevated cholesterol adjusted risk relative to PFOA; Figure 2-11 is a graph of percent change in LDL relative to PFOA levels; Figure 2-12 provides elevated LDL adjusted risk relative to PFOA. Based on these figures and studies presented in the ATSDR text (pages 172, 177-182), ATSDR concluded (page 186), "studies examining the change in cholesterol per change in serum PFOA levels have found greater increases in serum cholesterol levels associated with serum PFOA levels at the lower range of PFOA levels and the dose-response curve suggests a biphasic relationship. Positive associations have also been observed for LDL cholesterol, although associations have not been consistently found. In general, no consistent associations were found between serum PFOA and HDL cholesterol or triglyceride levels." On page 187, ATSDR recognized "In contrast to the results observed in epidemiology studies, an experimental study in humans exposed to PFOA (MacPherson et al. 2011) and human exposure to other PPARα agonists, such as fibrates (Roy and Pahan 2009), suggest that hypolipidemic effects, similar to those observed in rodents, may occur in humans exposed to PFOA, although humans may not be as sensitive as rodents."

3M Comments on PFOA and Cholesterol

The ATSDR recognized (pages 181, 187) the preliminary results of a phase 1 clinical trial of PFOA (ammonium salt) that was published in 2010 as an abstract by MacPherson et al. (2011) in the J Clinical Oncology. The abstract stated "Reductions in LDL-cholesterol consistent with a PD effect were observed." The phase 1 trial was a dose escalation study with the highest weekly dose administered at 1200 mg PFOA (range 50mg – 1200 mg). ATSDR was not certain whether this effect occurred at all dose levels as such clarification was not present in the abstract. ATSDR was not aware that the results from the clinical chemistry assessment from this phase 1 trial have been available via Advance Access and published on February 16, 2018 in *Toxicological Sciences* with hardcopy publication in the May 2018 issue, (Convertino et al. 2018). ATSDR is strongly encouraged to carefully consider the Convertino et al. (2018) publication and its ramification(s) in ATSDR's weight of evidence review for PFOA as related to lipids (as well as liver enzymes and thyroid hormones).

According to Convertino et al. (2018), this phase 1 dose-escalation study assessed the chemotherapeutic potential of perfluorooctanoate (ammonium salt). There were 49 primarily solid-tumor cancer patients who failed standard therapy that received weekly doses of PFOA (50 – 1200 mg) for 6 weeks. The primary purpose of this study was to determine the dose limiting toxicity of PFOA. However, no more than one subject demonstrated a dose limiting toxicity at any dose level so a maximum tolerated dose was not reached. The 1000 mg weekly dose was the recommended phase 2 dose based on tolerability. Standard clinical chemistry measurements were performed at baseline examination and weekly thereafter. Not all subjects took the weekly dose so measured serum PFOA concentration, internal dosimetry, not dose administered, was considered the metric of choice. Statistical analyses included generalized estimating equations a probabilistic analysis using probability distribution functions at various PFOA concentrations, and a 2-compartment pharmacokinetic/pharmacodynamic model. According to Convertino et al., total cholesterol (and free T4 – see under thyroid) showed a negative trend with increased serum PFOA concentrations with a clear transition in shape and range of the probability distribution functions for a decrease in total cholesterol at approximately 420 and 565 μM PFOA (approximately 175,000 – 230,000 ng/mL PFOA). The effect observed involved LDL, not HDL, and is consistent with the toxicological evidence in rodents observed at approximately an order of magnitude lower concentration. The PFOA concentrations, however, reported by Convertino et al. in the phase 1 clinal trial are several orders of magnitude higher than those reported to occur in workers, an exposed West Virginia community, and the general population.

Based on the study abstract that was available to ATSDR (Macpherson et al. 2010), ATSDR speculated about the possibility of a biphasic response in the human with decreased cholesterol reported at higher PFOA concentrations and elevated cholesterol at markedly lower levels. However, the ATSDR did not offer any possible modes of action explanation for a biphasic response whereas Convertino et al. did. The ATSDR should offer their explanations for a biphasic response. At the high concentrations of PFOA administered and measured where the decrease became clear with total cholesterol, Convertino et al. suggested this hypolipidemic response was consistent with a xenosensor nuclear receptor PPARα-mediated mode of action. They then suggested the inconsistency with the observational epidemiological studies showing positive associations between cholesterol and markedly lower PFOA concentrations are likely the consequence of one or more noncausal biological explanations. These would include the inherent variability in the glomerular filtration rate which confounds other associations that have been reported with PFOA including lower birthweight and chronic kidney disease; organic transporters in the gastrointestinal tract that may share binding affinity with lipids and PFOA; saturation of an underling physiologic mechanism given the nonlinear association observed n between PFOA and cholesterol reported by Steenland et al. (2009) and Frisbee et al. (2010) that was also mentioned by the ATSDR (page 181); and PFOA binding to lipoproteins (also mentioned by ATSDR on page 181). Convertino et al. cautioned that the latter may not have been thoroughly examined as Butenhoff et al. (2012d) had an extremely low sample size (n = 1) and should be replicated in much larger numbers. Convertino et al. also urged examination of plausible biologic modes of action that could support the hypercholesterolemia positive association reported at low ng/mL

PFOA. They wrote, "these observational studies have reported contrary associations, but currently understood biology does not support the existence of such conflicting effects." And, in fact, many of the authors of the papers cited in Figures 2-9 through 2-12 discounted the contrary animal data as not being relevant to humans. This can no longer be accepted practice in the literature given the publication of Convertino et al. (2018). Clearly, more cross-sectional studies are highly unlikely to be enlightening to any scientific understanding. ATSDR agrees with this recommendation when they wrote on page 635, "Interpretation of the human data is limited by the reliance of cross-sectional studies, which do not establish causality, and the lack of exposure data."

ATSDR also wrote on page 635, "Studies of serum lipids suggest that the dose-response curve is steeper at lower concentrations and flattens out at higher serum perfluoroalkyl concentrations (Steenland et al. 2010), additional studies that could be used to establish dose-response relationships would be valuable. Mechanistic studies examining the association between perfluoroalkyl exposure and serum lipid level would also provide insight." Therefore, ATSDR and the scientific community (both toxicologists and epidemiologists) are urged to reassess the dose response curve in humans based on the one and only experimental study done in humans (Convertino et al. 2018).

In this regard, ATSDR should consider whether the associations observed in many epidemiologic studies (primarily cross-sectional) at the much lower general population and community levels for PFOA may actually be a reflection of underlying, yet-to-be identified, physiological processes that result in a noncausal lipid/PFOA biological associations. This includes ATSDR's desire, so stated above, to describe the mode of action likely at these low doses that results in the association with higher cholesterol that is entirely inconsistent with the animal and human toxicological evidence that has demonstrated at sufficiently high concentrations of PFOA results in hypolipidemia. Convertino et al. offered several possible noncausal explanations (see above) but other possibilities are also worthy of investigation. For example, not stated by Convertino et al., is the fact that thyroid disease and chronic kidney disease can both affect GFR. Both of these conditions are also associated with dyslipidemia. All three may affect the glomerular filtration rate. Dyslipidemia, itself, has also been associated with altered GFR. Therefore, a lowered GFR may maintain a higher amount of PFOA – creating the association observed in some epidemiology studies.

In summary, given the recent publication of Convertino et al., the ATSDR should acknowledge the consistency of pharmacodynamic effects (decreased cholesterol and LDL) in both animals and humans with high exposure to PFOA. It is therefore inaccurate to have written what ATSDR provided on page 634 when stated, "The effects observed in rodents differ from those observed in humans. In humans, exposure to PFOA, PFOS, PFNA, and PFDeA appear to result in increases in serum lipid levels, particularly total cholesterol levels."

3M Conclusion on PFOA and cholesterol

There is no association between PFOA and coronary artery disease, cerebrovascular disease (stroke), and hypertension. Very high concentrations of PFOA will unequivocally result in lowered serum total cholesterol involving LDL, not HDL cholesterol in experimental studies in both animals and humans. The mode of action is likely via PFOA acting on xenosensor nuclear receptors, including PPARα, which is common to many species, including humans. Fibrate pharmaceuticals that lower serum cholesterol in humans also bind to this same nuclear receptor family. The contrary association of higher cholesterol associated with low PFOA concentrations, as reported in several but not all observational epidemiology studies, remains yet to be understood as to its biological (causal or noncausal) plausibility.

ATSDR position on PFOS and cholesterol

ATSDR presented information on PFOS and cholesterol on pages 188-196, with figures presented on total cholesterol change (%) relative to serum PFOS level in Figure 2-13, risk of abnormal cholesterol with PFOS levels in Figure 2-14, and LDL cholesterol change (%) relative to serum PFOS level in Figure 2-14. Unlike PFOA, there are fewer studies presented in these figures for PFOS. Neither the occupational studies nor the community study (which was not exposed to PFOS in the drinking water) are presented in these figures. The ATSDR wrote there were positive associations reported between PFOS and cholesterol with the occupational (page 188) and community (page 188-189) studies but the results were mixed in the general population studies (page 193-194).

3M Comments on PFOS and Cholesterol

ATSDR cited the Olsen et al. 2003a study as well as Steenland et al. 2009 study as evidence for positive associations reported between PFOS and cholesterol. Not discussed by the ATSDR was the concern expressed by both investigators that although PFOS may have been significant predictors of lipid levels, PFOS did contribute much to the variance of the prediction. For example, Steenland et al. wrote, "It should be noted that although PFOA and PFOS are highly significant predictors of lipid levels (our study had high power to detect statistically significant differences compared with prior smaller studies), the perfluorinated compounds themselves did not explain a large portion of the variance in lipids." For total cholesterol, the most important predictors were age, gender, and body mass index, not serum levels of PFOS. Olsen et al. stated for their model of cholesterol where the $R^2 = 0.06$, the partial R^2 for PFOS was < 0.01.

Similar to the PFOA phase 1 clinical trial discussed above, the ATSDR should recognize (which it has not) the findings from Chang et al. (2017) regarding a non-human primate study where a slight reduction in serum cholesterol (primarily HDL) was reported with administration of PFOS (potassium salt) in a 6-month study of non-human primates. The corresponding lower bound 5^{th} percentile benchmark concentration was 74,000 and 86,000 ng/mL for these male and female monkeys (cynomolgus), respectively. This

finding would suggest that at sufficiently high concentrations, PFOS is likely to result in lower (HDL, not LDL) serum cholesterol concentrations in humans.

3M Conclusion on PFOS and cholesterol

There is insufficient evidence to conclude an association exists between PFOS and lipids in the epidemiology literature.

Detailed Comments on Thyroid Disease

ATSDR position

On page 5 and 6, ATSDR wrote, "Although a large number of epidemiology studies have examined the potential of perfluoroalkyl compounds to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causation. Based on a number of factors including the consistency of findings across studies, the available epidemiology studies suggest associations between perfluoroalkyl exposure and several health outcomes." According to the ATSDR, this includes "increased risk for thyroid disorders. (PFOA, PFOS)". Similar statement was provided on page 25. ATSDR provides Table 2-15 (pages 223-237) as a summary of thyroid outcomes in humans. This table contains both studies that reported both thyroid hormones as well as thyroid disease (self-reported as well as medically validated) in occupational, community-based and general populations. Study designs are not listed in these tables and the reader is referred to the supporting information. For PFOA (correcting for the study design misidentification discussed earlier in the supporting information), it appears that of the 21 studies listed in Table 2-15, 20 are cross-sectional with one study a cohort. For PFOS, 18 studies in Table 2-15 were cross-sectional and 1 study had a cohort component. ATSDR did not comment on this preponderance of cross-sectional studies as they discussed thyroid. The text presents a mixture of findings but no rationale of understanding provided by ATSDR. Unlike other sections, there are no summary statements in the thyroid section for either PFOA or PFOS.

3M Comments

ATSDR's review of the thyroid is disjointed and did not explain how it decided that an "association" exists between PFOA/PFOS and an increased risk of thyroid disease. This confusion is caused, in part, by the inconsistent evidence presented in the scientific literature. The lack of a summary statement by ATSDR indicate the lack of scientific support for the conclusion that ATSDR makes.

Primary hypothyroidism is clinically characterized by a high serum thyrotropin (TSH) concentration and a low serum free thyroxine fT4 concentration. Subclinical hypothyroidism is generally defined as a normal Ft4 in the presence of an elevated TSH. Hyperthyroidism is defined as a decreased TSH level and elevated free T4 and free T3 levels. Measuring specific antibodies, such as anti-TSH-receptor antibodies in Graves' disease, or anti-thyroid peroxidase in Hashimoto's thyroiditis — a common cause of hypothyroidism — may also contribute to the diagnosis.

As ATSDR wrote (page 238), there were "no associations between serum PFOA and TSH or T4 levels found in the general population studies except for Lewis et al. (2015). On page 222, ATSDR also wrote, "the occupational exposures do not suggest an association between serum PFOA and alterations in thyroid hormone levels." Further, ATSDR conceded that although TSH, T3 or T4 have been reported, "*the results are not consistent across studies (page 222).*" Thus, on a population analysis basis, trends in

thyroid hormone levels, in particular TSH (the primary clinical diagnostic indicator to diagnose hypo-or hyperthyroidism), is lacking with exposure to PFOA or PFOS.

In the abovementioned phase 1 clinical trial of PFOA (ammonium salt) (Convertino et al. 2018), the physicians examined for TSH and free T4, the usual two thyroid tests done for clinical thyroid assessment. The phase 1 trial study is described above in the lipids section. Based on the probability distribution functions, there was no change in TSH even at the highest concentrations of PFOA measured (highest category range was 870 μM - 1530 μM (μM (~360,000 ng/mL – ~632,000 ng/mL) PFOA. There appeared to be an increase in free T4 (fT4) at a higher PFOA transition point than reported for cholesterol. This increase with no apparent effect on TSH suggested to Convertino et al. that the increase in fT4 was not clinically significant but may be due to displacement of the thyroid bound hormone by PFOA. Such an effect is reported for PFOS in rats where displaced thyroxine from binding proteins transiently increases free thyroxine without altering overall thyroid hormone homeostasis (Chang et al. 2007,20008; Weiss et al. 2009).

In their analysis of NHANES data, Melzer et al. (2010) reported associations for females categorized as having "*current thyroid disease with thyroid medication*". However, they did not delineate by type of thyroid disorder (hypothyroidism, hyperthyroidism). Given the high prevalence of hypothyroidism in females, it can be presumed the majority of these prevalent female cases were hypothyroid. This finding was <u>not</u> supported by Winquist and Steenland (2014) in their analysis of the mid-Ohio river valley population who were exposed to drinking water that contained PFOA. Winquist and Steenland (2014) wrote in their study Abstract:

> *"Associations were observed for hyperthyroidism and hypothyroidism among women*."

However, this was not supported by their Discussion section where they wrote:

> *"We found evidence of an association between PFOA exposure and functional thyroid disease, especially for hyperthyroidism among women (in retrospective analyses) and for hypothyroidism among men (in prospective analyses)."*

This quote, however, is not supported by the ATSDR review of Winquist and Steenland (2014) where the ATSDR wrote on page 238, "*No associations between cumulative serum PFOA and hyperthyroidism or hypothyroidism were found in retrospective analysis (Winquist and Steenland 2014b). However, in prospective analysis, an association between cumulative serum PFOA and hypothyroidism was found in men (Winquist and Steenland 2014b)."*

Indeed, analysis of the Winquist and Steenland 2014 supporting information tables (see the eTable 1 through eTable 6 in Winquist and Steenland 2014) reported <u>no</u> statistically significant trends ($P < 0.05$) for hypothyroidism in women in either their retrospective, retrospective qualifying year, or prospective analyses. (This would be in direct conflict

with the findings from Melzer et al.). Altogether, there were 12 trend test analyses conducted (log linear model trend test p-values) in these supporting tables. For hypothyroidism, there were 0 trend tests among women with p-values < 0.05; 1 trend test with a p-value >= 0.05 and < 0.1; 3 trend tests with a p-value between >= 0.1 and < 0.2; and 8 trend tests with a p-value >= 0.2. These observations do not support an association between PFOA and hypothyroidism among women.

On the other hand, for hyperthyroidism among women, there were 4 trend tests with a p-value < 0.05; 2 trend tests with a p-value between >= 0.05 and < 0.1; 4 trend tests with a p-value between 0.1 and < 0.2; and 2 trend tests with a p-value >= 0.2. Among males, there were 4 trend tests with a p-value < 0.05 for hypothyroidism but none for hyperthyroidism.

ATSDR also reported (see page 222) that in a study published in 2015, Steenland et al. "did not find an association between serum PFOA and the risk of thyroid disease in male or female workers at the Washington Works facility," In fact, what Steenland et al. wrote, was "there was a positive non-significant trend for male hypothyroidism" where the 10 year lag trends in relative risk were 1.00 reference, 1.64, 1.13, 2.16 (p value trend via categories p = 0.06), however, their table presented this information as "thyroid disease" not differentiated to the type. Not discussed by Steenland et al. or by ATSDR, is the fact that there was an equally negative trend (not significant) in women for thyroid disease where the 10-year lag trends in relative were 1.0 reference, 0.79, 0.87, and 0.23; p value trend via categories p = 0.13).

3M Conclusion on thyroid disease

Given the inconsistencies in the literature regarding associations of thyroid hormones and thyroid disease, there is insufficient evidence to conclude an association exists as related to exposure to PFOA or PFOS.

Detailed Comments on Decreased Antibody Response to Vaccines (PFOA, PFOS, PFHxS, PFDeA)

ATSDR Position

The ATSDR draft document concluded that "*evidence is suggestive of a link between serum PFOA, PFOS, PFHxS, and PFDeA levels and decreased antibody responses to vaccines*". Evidence for this conclusion comes from 8 epidemiologic studies (4 cross-sectional and 4 prospective cohort) in which antibody titers to vaccinations were quantified in combination with measurements of serum PFOA, PFOS and other PFAS levels, coupled with supportive animal studies. Among the epidemiologic studies, antibody responses to 8 distinct vaccines (i.e., diphtheria, tetanus, mumps, measles, rubella, influenza A/H1N1, influenza A/H3N2 and influenza B) were measured. The most commonly studied vaccine response was to the tetanus vaccine with 5 studies (Grandjean et al. 2012; Grandjean et al. 2017; Granum et al. 2013; Kielsen et al. 2016; Mogensen et al. 2015) followed by 4 diphtheria studies (Grandjean et al. 2012; Mogensen et al. 2015; Kielsen et al. 2016; Grandjean et al. 2017), two rubella and measles studies (Granum et al. 2013; Stein et al. 2016b) and two influenza A/H3N2 studies (Looker et al. 2014; Stein et al. 2016a)). Antibody responses to mumps (Stein et al., 2016b), *H. influenza* (Granum et al., 2013), influenza B and influenza A/H1N1 (Looker et al., 2014) were each examined in only 1 study.

3M Comments

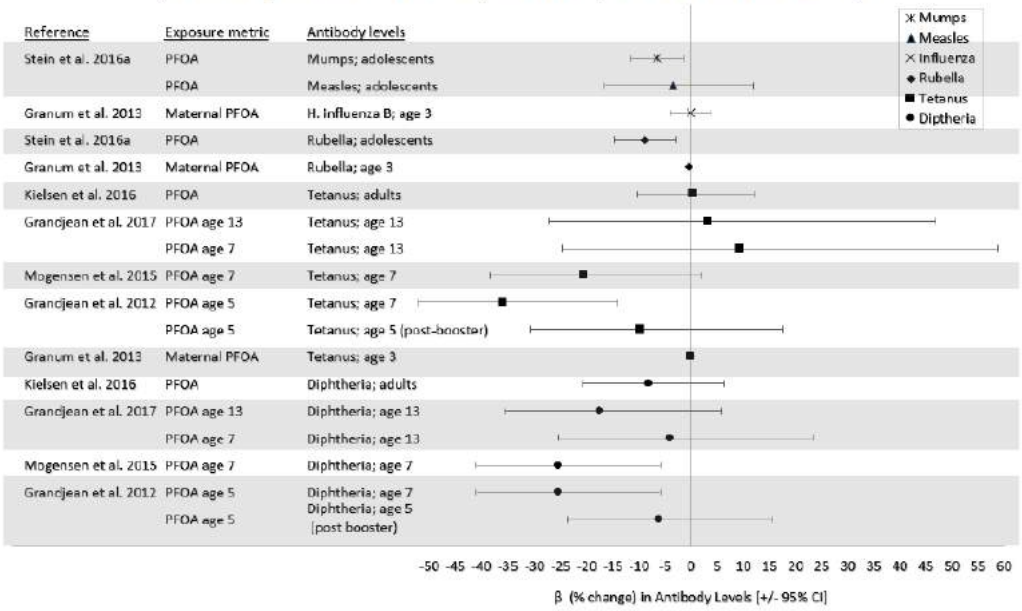
It is inappropriate for ATSDR to interpret antibody responses to these 8 distinct vaccines as a single health outcome (i.e., "decreased antibody responses to vaccines"). Commercially available vaccines differ depending on the nature of the vaccine antigen. Tetanus and diphtheria, for example, are toxoid vaccines whereas measles, mumps and rubella are live attenuated vaccines. Influenza vaccines are inactivated (killed), conjugate or live attenuated depending on the strain and method of administration (e.g., intranasal, injectable). Consequently, each vaccine type elicits an immune response through various molecular and cellular mechanisms of the immune system. Additionally, all vaccines contain various excipients including adjuvants to improve the antibody response, preservatives, stabilizers, and vehicles for delivering the vaccine which may differ substantially depending on the vaccine (Baxter 2007).

The National Toxicology Program acknowledged the differences in immune response across vaccines, and stated that "*The strength of an antibody response in terms of antibody level and length of time that an elevated/effective antibody response is maintained is known to differ across vaccines*" (NTP 2016). Granum et al (2013), a study cited in the ATSDR draft profile, also concluded that "*different vaccines may stimulate different components of the immune system, which can explain the vaccine-dependent differences in the effect of PFAS exposure*". Therefore, observed changes in antibody response to a particular vaccine should not be interpreted as consistent with

changes in the antibody response to another vaccine. **The ATSDR draft document should consider immune responses to individual vaccines as distinct health outcomes.**

The ATSDR draft profile graphically presents epidemiologic study findings (i.e., the changes in antibody levels relative to serum PFAS levels) in Figures 2-19 (PFOA), 2-21 (PFOS), 2-23 (PFHxS), 2-25 (PFNA) and 2-27 (PFDeA). These figures clearly illustrate the heterogeneity in results both within and across the 8 studies reviewed by ATSDR. For example, Figure 2-19 (below), shows that of the 5 studies that examined antibody responses to the tetanus vaccine relative to serum PFOA levels, only one study reported a significant decrease in antibody levels (Grandjean et al., 2012). The other 4 studies, including a follow-up study of Grandjean et al., 2012, did not observe a significant decrease in tetanus antibody levels (Grandjean et al., 2017).

Figure 2-19. Antibody Responses Relative to Serum PFOA Levels in Epidemiology Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFOA)



(Note: Not included in Figure 2-19 are results from two influenza studies with mostly null findings (Looker et al. 2014; Stein et al. 2016b). While both studies are cited in the draft profile, ATSDR should acknowledge that results from these two studies were omitted from the Figure and provide reasons for their omission.)

Similar to the results observed for PFOA, inconsistent results were also observed for PFOS, PFHxS and PFDeA. None of the 5 studies reported a significant association between tetanus antibody levels and PFNA. In addition, findings across all vaccine types were also inconsistent. As presented in Figure 2-19, for example, only 5 of the 18 associations between PFOA and a change in antibody levels were statistically significant. Similar inconsistencies across all vaccine types are also apparent for PFOS, PFHxS, PFNA, and PFDeA. Considering the inconsistent (and mostly non-significant) findings

across the 8 published studies, the available epidemiologic evidence of an effect of PFOA, PFOS, PFHxS and PFDeA on antibody response to vaccines is weak at best. Moreover, ATSDR failed to recognize that small changes in antibody response do not necessarily translate to an increased risk of infectious disease. Six epidemiologic studies ((Dalsager et al. 2016; Fei et al. 2010a; Leonard et al. 2008; Looker et al. 2014; Okada et al. 2014) have examined PFAS levels and infectious disease outcomes (i.e., occurrence of common colds and otitis media, mortality from infectious and parasitic diseases, and hospitalizations from infectious diseases). Most of these studies reported no association between PFAS levels and increased risk of infectious disease outcomes. As noted in the ATSDR draft profile (page 268), the NTP (2016) concluded that there is low confidence that exposure to PFOA and PFOS is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease). Other regulatory bodies have reached similar conclusions (FSANZ 2017; USEPA 2016a, b). Given the absence of increased infectious disease susceptibility, it is questionable whether the observed decreases in antibody response are clinically relevant.

Finally, the ATSDR did not provide an interpretation of the epidemiologic evidence or a conclusion regarding the potential association between PFAS levels and decreased antibody response to vaccines. Instead, ATSDR quoted the 2016 NTP conclusion (page 268) that "*exposure to PFOA or PFOS is presumed to be an immune hazard to humans*" while ignoring conclusions from other regulatory bodies and expert health panels. These conclusions (provided below) should be included in the ATSDR draft profile to provide readers with a more balanced and thorough interpretation of the epidemiologic evidence. It is inappropriate for ATSDR to cite a single conclusion from one regulatory body and not cite others with divergent conclusions.

Other regulatory have made the following conclusions regarding PFAS and immunotoxicity:

<u>Australia Expert Health Panel (2018):</u>
"*The strongest evidence for a link between PFAS and clinically important immunological effects is for impaired vaccine response. However, the human dose-response/threshold for potential immune effects is very poorly characterized, and the overall human evidence is weak.*"

<u>Food Standards Australia New Zealand, FSANZ (2016):</u>
A literature review commissioned by FSANZ concluded that *"there are both positive and negative studies showing associations for increasing PFOS and PFOA concentrations to compromise antibody production in humans. However, to date there is no convincing evidence for increased incidence of infective disease associated with PFOS or PFOA effects on human immune function".*

<u>Health Canada (2017a):</u>
"Studies in environmentally-exposed populations have identified associations between PFOS levels and decreased antibodies against various illnesses, but the influence of PFOS exposure on clinical immunosuppression (i.e., incidence of illnesses) appears to be

more tenuous." Health Canada further commented that "*a low level of consistency was observed across studies, with variations between genders, specific microbial immunoglobins, infections, mother vs. child exposure, and child years, amongst other characteristics. Moreover, the risk of residual confounding, bias, and chance cannot be discarded. These flaws impede concluding on a causative mechanism, and the nature of the association remains unclear*." Health Canada reached similar conclusions regarding PFOA (Health Canada, 2017b).

National Institute for Public Health and the Environment (RIVM, 2016):
RIVM concluded that "*associations have been found between exposure to PFOA and a decreased vaccination response*", but the "*evidence is unclear*".

New Jersey Drinking Water Quality Institute (DWQI, 2017):
"Review of epidemiologic studies provides evidence of consistent findings among studies of decreased antibody concentrations following vaccination and PFOA. There is epidemiologic evidence of temporality. However, there are a limited number of comparisons across the same vaccination types, making consistency/specificity difficult to evaluate."

3M Conclusion on decreased antibody responses to vaccines

The inconsistent findings both within and across studies, along with the absence of clinical immunosuppression, do not support the ATSDR conclusion "s*uggestive of a link between serum PFOA, PFOS, PfHxS, and PFDeA levels and decreased antibody responses to vaccines".*

Detailed Comments on Increased Risk of Asthma Diagnosis (PFOA)

ATSDR Position

The ATSDR draft profile concluded there is a "*possible link between serum PFOA levels and an increased risk of asthma diagnosis*". The draft profile cites 8 epidemiologic studies (2 prospective cohort studies, 2 case-control studies and 4 cross-sectional studies) that examined the relationship between PFOA exposure and self-reported asthma. ATSDR provided no interpretation of the epidemiologic evidence or rationale for their conclusion of a "possible link". In fact, the only conclusion ATSDR provided in the document is the following statement: "*In tests of hypersensitivity, there is some evidence of an association between serum PFOA and asthma diagnosis in children and adults, although this finding was not consistent across studies; increased risk of allergy or allergic sensitization does not appear to be associated with serum PFOA (page 276).*"

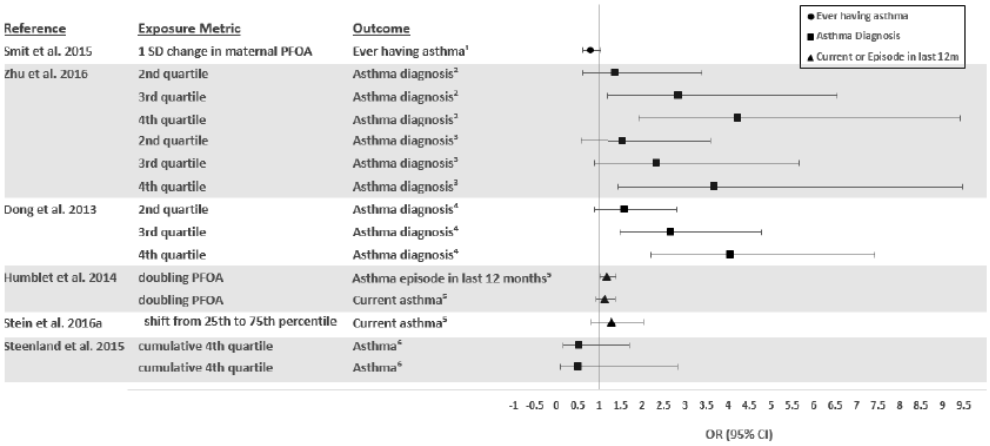
3M Comments

The ATSDR draft profile cited the NTP (2016) conclusion that "*there is low confidence that exposure to PFOA during childhood is associated with increased hypersensitivity responses based on the available studies*" (page 279). The ATSDR draft profile, however, does not include NTP's stated rationale for the conclusion of "low confidence" which was "*primarily due to the cross-sectional nature of the studies and uncertainty as to whether exposure levels reflect exposure prior to the development of hypersensitivity*. (NTP, 2016)". The ATSDR failed to recognize these important limitations or other methodological issues in the draft document. The following comments are provided to offer this insight.

Five of the 8 referenced epidemiologic studies used self-reported asthma (Anderson-Mahoney et al. 2008; Granum et al. 2013; Humblet et al. 2014; Smit et al. 2015; Stein et al. 2016b). The validity of self-reported asthma is largely unknown. However, a review of asthma questionnaires reported a mean sensitivity of 68% and specificity of 94% for self-reported asthma when compared with a clinical diagnosis of asthma (Toren et al. 1993). Consequently, studies using self-reported asthma diagnosis are subject to some degree of measurement error, which may bias the study results.

Asthma diagnosis was medically validated in 3 studies ((Dong et al. 2013); (Steenland et al. 2015); (Zhu et al. 2016)). It is important to note that 2 of these studies (Dong et al. 2013; Zhu et al. 2016) each reported on results from a single case-control study of the same population (456 Taiwanese children enrolled in the Genetic and Biomarkers study of Childhood Asthma (GBCA) study). While, the ATSDR document acknowledged in Table 2-16 that the same group of children (231 asthmatic and 225 non-asthmatic) were evaluated by both authors, the ATSDR did not address this in the text or in Figure 2-20 (below). This gives readers the false impression that these are two distinct studies with consistent findings.

Figure 2-20. Risk of Asthma Diagnosis Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



Dong et al. (2013) reported a significant association and exposure trend between serum PFOA levels and asthma diagnosed in the last 12 months among children aged 10-15 years (OR for highest versus lowest quartile of serum PFOA = 4.05, 95% CI: 2.21, 7.42, P_{trend} = <0.001). However, no significant association between serum PFOA levels and asthma severity score was reported (p=0.119). Zhu et al. (2016), observed significant associations and exposure trends in both males and females in a stratified analysis of the same study population. An important limitation in the study by Dong et al (2013) and Zhu et al (2016), not mentioned in the ATSDR draft profile, is that asthma diagnosis preceded serum PFOA measurements. The third study (Steenland et al. 2015), examined the potential association between occupational exposure to PFOA and validated asthma with reported current medication. However, only study participants who self-reported having asthma were asked to give consent for medical records review to validate cases. Of the 138 self-reported asthma cases, 108 (78%) provided consent for medical records review; 82 cases were validated and included in the statistical analysis. Therefore, asthma diagnosis was validated only among study participants who self-reported having asthma and not for participants whose medical records were not reviewed. In contrast to findings reported by Dong et al (2013) and Zhu et al (2016), Steenland et al.(2015) observed no significant association between PFOA exposure and risk of medicated asthma..

Two additional studies, published since 2016, should be included in the ATSDR draft profile ((Impinen et al. 2018; Timmermann et al. 2017). Study by Timmerman et al. used a cross-sectional design to examine the potential association between pre- and postnatal PFAS exposure and self-reported childhood asthma in a cohort of Faroese children. Among 22 MMR-unvaccinated children, a doubling of serum PFOA levels (measured at age 5) was significantly associated with increased odds of asthma at age 5 (OR = 10.37, 95%CI: 1.06, 101.93) and 13 (OR = 9.92, 95%CI: 1.06, 93.22). No significant associations were observed among MMR-vaccinated children. Additionally, no associations were observed between maternal PFOA exposure and childhood asthma at age 5 and 13 years. Due to the small sample size, precision of the estimates was poor as evident by the wide confidence intervals. Study by Impinen et al. was a well-designed prospective cohort study of 641 children enrolled in the Norwegian Environment and

Childhood Asthma (ECA) birth cohort which examined the association between PFAS measurement from cord blood and medically validated asthma diagnosis in children 2 and 10 years of age. Investigators found no significant associations between prenatal exposure to PFOA and asthma related outcomes. This study was strengthened by its prospective exposure assessment and validated asthma diagnosis.

3M Conclusion on increased risk of asthma diagnosis

Prospective cohort studies have consistently reported no association between PFOA and asthma. Conversely, cross-sectional and case-cohort studies are limited by temporal ambiguity, lack of consistent findings, and unvalidated outcome assessment. Collectively, the existing epidemiologic evidence does not support an association between PFOA exposure and asthma risk.

Detailed Comments on Increased Risk of Decreased Fertility

ATSDR position

On page 5 and 6, ATSDR wrote, "Although a large number of epidemiology studies have examined the potential of perfluoroalkyl compounds to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causation. Based on a number of factors including the consistency of findings across studies, the available epidemiology studies suggest associations between perfluoroalkyl exposure and several health outcomes." According to the ATSDR, this included increased risk of decreased fertility (PFOA, PFOS). This was reiterated on page 24 where ATSDR wrote, "A suggestive link between serum PFOA and PFOS levels and an increased risk of decreased fertility has been found." Table 2-21 (pages 318-320) provided point estimates for selected categorically-defined PFOA or PFOS serum concentrations that are sometimes stratified by the subgroups parous or nulliparous. Page 325-326 is ATSDR's written description of the epidemiology studies that describe effects on fertility as related to PFOA. On page 326 is Figure 2-29. This figure provides adjusted fecundability ratios (95% CI) form PFOA for 13 references. These ratios were stratified by parity status. On page 327 is Figure 2-30. This figure provides infertility (95% CI) relative to PFOA for 16 references. This was stratified by parity status. On page 332, paragraph 3. ATSDR provides its written description of the epidemiology studies that describe effects on fertility as related to PFOS. On page 333 is Figure 2-31. This figure provides adjusted fecundability ratios (95% CI) from PFOS for 13 references. These ratios were stratified by parity status. On page 334 is Figure 2-32. This figure provides infertility (95% CI) relative to PFOS for 16 references. This figure was stratified by parity status. Within the framework of the text on pages 325-326 for PFOA or page 332 for PFOS, there is no discussion on how ATSDR evaluated the weight of the evidence to arrive at its conclusion that there was an association with "increased risk of decreased fertility (PFOA, PFOS)."

3M Comments

ATSDR failed to offer a critical assessment of the epidemiology literature and the study methods used related to fertility and exposure to PFOA and PFOS. ATSDR neglected to discuss the very important methodological issues surrounding the metric time to pregnancy and when measured serum perfluoroalkyl concentrations are taken in nulliparous and parous women. This has been a topic of considerable interest and controversy as extensively discussed in the perfluoroalkyl literature since 2009. In this regard, ATSDR never explained why the studies discussed on pages 325-326 (PFOA), page 332 (PFOS), and their associated figures and tables, are stratified by nulliparous or parous status. This reflects ATSDR's failure to properly assess the reproductive epidemiology literature and its methods regarding PFOA and PFOS, which preclude a conclusion for finding an association between an increased risk of decreased fertility with exposures to PFOA and PFOS.

While Fei et al. (2009) reported an association (the first to do so) between PFOA and a decrease in fecundability and an increase in infertility with women in the Danish National Birth Cohort (page 330), they did not stratify their data by parity. This stratified analysis was published 3 years later (see (Fei et al. 2012). Commentary. Perfluorinated chemicals and time to pregnancy: A link based on reverse causation? Epidemiology 23:264-266). This stratified analysis was prompted by a review of the original Fei et al. 2009 publication by (Olsen et al. 2009) (Note: Olsen et al. 2009 was never cited by ATSDR. For Olsen et al. 2009 see Perfluoroalkyl chemicals and human fetal development: An epidemiologic review with clinical and toxicological perspectives. Reprod Toxicol 27:212-230). Olsen et al. wrote (see page 228 of their paper.) the following describing their suspected methodological question of Fei et al. 2009:

> *"Another troubling issue depicted in Fig. 6 (see obtained copyright figure below) is that parity is both an outcome of fecundity and a cause of PFC concentration: this induces a cyclic change that violates the conditions of causal inference. Although this is an artificial cycle that arises from not explicitly representing the variation of PFC level over time, it highlights the conundrum of trying to make do with a current PFC level, when the actual level may be an earlier and somewhat different level, even with compounds that may have long serum elimination half-lives such as PFOS or PFOA. For example, under the reasonable assumption that PFC levels will be lower after a pregnancy, a longer interval between births would result in more time for a woman to absorb PFCs that could replace the loss incurred from the birth. Women who begin with comparable PFC concentrations and equal parity may have different PFC concentrations at their next birth based on the time that passed between births. All else being equal, those women with longer TTP will have longer intervals of time between births and so may have higher PFC levels prior to the next pregnancy. This would result in longer TTP measurements associated with PFC levels, but the direction of the causality would be backwards: it would be the longer time between births (including the TP) that resulted in higher PFC concentrations. This illustrates the complexity of situation that could be encountered when a causal model (Fig 6) has an unelaborated time-dependent cyclic chain."*

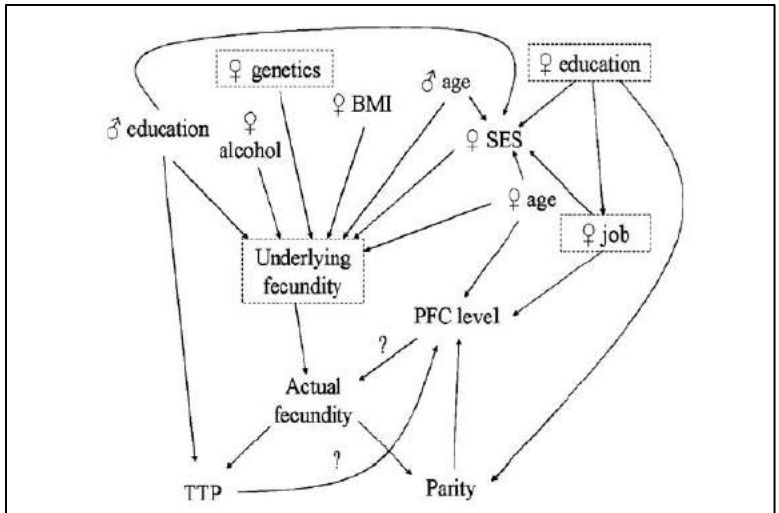


Fig. 6. Suggested causal diagram of maternal perfluorochemical concentrations (PFC) and time to pregnancy.

From Olsen et al. 2009. Reprod Toxicol 27:212-230.

Given this methodological interpretation and question raised by Olsen et al. (2009), Whitworth et al. (2012) examined this issue on fecundability and infertility with their use of the Norwegian Mother and Child Cohort Study (MOBA) database. While Whitworth et al. also found an association with decrease fecundability and exposure to PFOA and PFOS; however, when they stratified their data by parity (nulliparous, parous), the association was only observed among parous women. Whitworth et al. wrote the following in their discussion:

> *"The discrepant results we observed among parous and nulliparous women may be explained by factors related to pregnancy history. As noted earlier, there is a complex relation between a woman's pregnancy history and current levels of environmental toxicants, particularly when exposures to the toxicant vary over time. Due to the pharmacokinetics of PFCs during pregnancy and lactation, an apparent association between PFCs and subfecundity may be produced even when a causal association does not exist. It is possible that following the decrease in maternal PFC levels observed during pregnancy, deliver, and lactation, the levels again increase to baseline. Therefore, as mentioned earlier, a long interval between the birth of the previous child and the start of the next pregnancy attempt will allow for a longer time during which levels can increase-potential resulting in a noncausal association between subfecundity and PFC levels. Results from women with no previous pregnancies may be more informative regarding toxic effects of these compounds. Based on the nulliparous women in our study, we found no evidence of an adverse effect on subfecundity at the PFC levels in our population."*

In 2012, Fei et al. published their stratified analysis by pregnancy history of their 2009 paper because of the question raised by Olsen et al. 2009) and regarding the timing of the

measurement of perfluorinated compounds. Fei et al. (2012) wrote in their Introduction the following:

> *"In 2008, we reported that high maternal levels of perfluorooctatnoate (POFA) and perfluorooctane sulfonate (PFOS) were associated with longer time to pregnancy (TTP) in the Danish National Birth Cohort. Reverse causality is a possible explanation for the association, as has been pointed out by Olsen and colleagues. Even with age adjustment, past pregnancies and deliveries may serve to lower stored levels of PFOA and PFOS. On average, women with longer TTP will have had more time to reaccumulate perfluorinated chemicals (PFCs). "*

Furthermore, Fei et al. (2012) wrote,

> *"A directed acylic graph (DAG) representing the relationships among these factors is shown in the Figure. (provided by Fei et. al 2012). Present and past fecundability share common determinants, and those determinants confound the relationship between PFOA/PFOS and present fecundability. Adjusting for parity should serve to block that pathway and hence control confounding. However, a subtlety not capture by the DAG is that PFOA/PFOS were not measured at the beginning of the attempt at conception (which would have been ideal), but at the end, after a pregnancy had been achieved. Thus, in the available data, the measurement of PFOA/PFOS can potentially be influenced by TTP for parous women through reaccumulation of the chemicals. Such influence produces a cycle in the graph through the arrow from TTP to the measured PFOA/PFOS. However, for nulliparous women, that arrow does not exist in a model that adjusts for age."*

As the ATSDR (page 325) displayed in their subsequent figures, when the women were then categorized by parity, decreased fecundability OR and increased infertility ORs were more often found in the parous women and these risks attenuated more towards the null among nulliparous women. [Note: the association remained after stratification for parity with PFOS in the Fei et al. 2012 study.] Fei et al. surmised their study showed limited evidence for reverse causation as an explanation for their results and welcomed further studies.

ATSDR was correct that there were additional analyses of this particular Danish National Birth Cohort by Bach et al. (2015). There was an updated analysis of the original sample n = 1161 as well as an additional 440 women included. Bach et al. wrote "*the pooled analyses (both samples) were driven by the larger old sample, but we did not corroborate our previous finding of an association between high PFOS and longer TTP in the new sample. The tendency towards an association for PFOA and TTP in parous women may be due to reverse causation.*" In ATDSR's discussion (see page 325), ATSDR failed to recognize this issue of 'reverse causation' among parous women with TTP and PFOA.

Additional studies were forthcoming including, as ATSDR notes (page 328), studies by, (Jorgensen et al. 2014) and (Vestergaard et al. 2012)that reported no associations.

ATSDR did not include the preplanner study by Buck Louis et al (2013) which showed no association with fecundability for PFOA (adjusted odds ratio 0.94, 95% CI 0.81 – 1.10) or PFOS (adjusted odds ratio 0.99 (95 CI 0.85 – 1.17). Buck Louis et al. did show an association with PFOSA (the primary amide of PFOS) but this finding was difficult to interpret because 90% of the measurements for PFOSA were below the limit of detection. Another study by Whitworth (2016) only reported a weak decreased fecundability odds ratio with PFOSA (interquartile distance was 0.91 (95% CI 0.71 – 1.17) among primiparous women. Neither of these studies (Buck Louis 2013 or Whitmore 2016) were cited in the draft ATSDR 2018 document.

Finally, Vélez et al. (2015) concluded there was reduced fecundity with PFOA (not PFOS) in the MIREC study. Unlike many other studies discussed above, however, Vélez et al. chose not to adjust or stratify their analyses for parity when studying the potential adverse reproductive effects (decreased fecundability, infertility) as they reasoned that conditioning on parity would introduce over adjustment through collider stratification bias. Vélez et al. maintained this argument in a letter to the editor (not cited by ATSDR) when they criticized Bach et al. (2015) by having restricted their analyses of serum perfluoroalkyl acids and TTP to 1,372 women from the Aarhus Birth Cohort. In this study, Bach et al. reported there was no evidence of an association between TTP and serum levels of PFOA (odds ratio 1.10; 95% CI 0.93-1.30) and PFOS (odds ratio 1.09; 95% CI -0.95-1.29). Bach et al. (2016) (not cited by ATSDR) argued that if parity is not conditioned on, reverse causality may still be a spurious association between PFAS levels and TTP in parous women due to reaccumulation issues addressed above. Subsequently, Bach et al. (2016b) (not cited by ATSDR) conducted a systematic review of PFAS and measures of human fertility, including fecundability and infertility. They reported 8 studies that examined the association between PFAS and TTP. Only one study found an association when restricted to nulliparous women; 4 studies reported an association with parous women. Bach et al. concluded the latter was likely not causal but a result of reverse causation and unmeasured confounding related to prior pregnancies and childbirths that could influence the measurement of PFAS.

Given the above discussion in the literature and the omission by ATSDR of discussion of these above methodological issues, ATSDR does not appear to have documented or conducted an appropriate weight-of-the-evidence assessment. These methodological issues, analyses and insights have been extensively discussed since 2009. ATSDR should reconsider its assessment as there is an insufficient basis to conclude that there is an "increased risk of decreased fertility (PFOA, PFOS)" based on a thorough examination of this published epidemiology literature.

3M Conclusion on increased risk of decreased fertility

There is no association of an increase in decreased fertility, when analyzed as the metric time to pregnancy, in nulliparous women for PFOA or PFOS exposure. A longer time period between the birth of the previous child and the start of the next pregnancy attempt will allow for a greater potential for reaccumulation of PFOA or PFOS. This could

potentially result in noncausal associations observed in parous women when assessing subfecundity by the metric of time to pregnancy with PFOA or PFOS.

Detailed Comments on Lower Birth Weight

ATSDR position

On page 5 and 6, ATSDR wrote, "Although a large number of epidemiology studies have examined the potential of perfluoroalkyl compounds to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causation. Based on a number of factors including the consistency of findings across studies, the available epidemiology studies suggest associations between perfluoroalkyl exposure and several health outcomes." According to the ATSDR, this includes "small (<20 g or 0.7 ounces per 1 ng/mL increase in blood perfluoroalkyl level) decreases in birth weight (PFOA, PFOS)." Similar statement was provided on page 25. Table 2-23 provides a summary of epidemiologic studies that evaluated birth outcomes in humans. On page 377, ATSDR states, "mixed results have been found for birth outcomes, particularly birth weight. Some epidemiology studies have found associations between maternal PFOA or PFOS exposure and decreases in birth weight, and meta-analyses of these data have found that increases in maternal PFOA or PFOS were associated with 15-19 g or 5 g decreases in birth weight, respectively; accounting for maternal glomerular filtration rate attenuated these results by about 50%." On page 381, ATSDR briefly discussed the meta-analyses of Johnson et al. (2014) for PFOA and Verner et al. (2015) for PFOA and PFOS. In the Johnson et al. meta-analysis, they reported an estimate of -18.9 g (95% CC -29.8, -7.9) change in birth weight per 1 ng/mL increase in serum or plasma PFOA. Using not quite the same number of studies, Verner et al. provided an estimate of a -14.72 g change in birth weight (95% CI -21.66, - 7.78) per ng/mL PFOA. Through PBPK model simulations, they estimated that taking into account the maternal glomerular filtration rate would reduce this estimate to -7.92 g change (95% CI -9.42, -6.43) per ng/mL PFOA measured at delivery and -7.13 g change (95% CI -8.46, -5.80) per ng/mL PFOA measured in cord blood. For PFOS, Johnson did not provide a meta-analysis estimate but Verner et al. did at -5.00 g change (95% CI -8.92, -1.09) per ng/mL PFOS that would attenuate to -1.46 g change (-181, -1.11) per ng/mL PFOS measured at delivery and -2.72 g change (95% CI -3.40, -2.04) per ng/mL PFOS measured in cord blood.

3M Comments

ATSDR briefly discussed two meta-analyses conducted by Johnson et al. (2014) and Verner et al. (2015). ATSDR provided no historical context to these two studies. Unfortunately, several important issues were not discussed by ATSDR that are critical to deciding whether sufficient information exists to even describe whether an association exists. In addition, two additional meta-analyses were not considered by ATSDR ((Negri et al. 2017; Steenland et al. 2018). The latter was recently released in abstract form in the journal *Epidemiology* and is critical to understanding whether an association between lower birth weight and PFOA is likely to even exist, let alone be biologically relevant (see ATSDR Toxicological Profile, page 573.

First, as a minor point, ATSDR stated there were 7 papers included in the meta-analysis by Johnson et al. (2014) whereas there were 9 papers. Not cited by ATSDR were the

Washino et al. (2009) and Whitworth et al. (2012) publications considered by Johnson et al. Thus, the only difference between Johnson et al. (2014) and Verner et al. (2015) meta analyses were the inclusion of the Fromme et al. (2010) and Kim et al. (2011) papers by Johnson but not by Verner et al. (2015). Fromme et al. (2010) and Kim et al. (2011) were small studies whose point estimates for reported birth weights were large but highly imprecise (see Figure 5 in Johnson et al.). Verner et al. did not consider these two papers and subsequently Verner reported a lower meta-analysis point estimate of 14.7 gm (95% CI -21.66, -7.78) birth per ng/mL PFOA in their meta-analysis than did Johnson et al. who reported -18.91 (95% CI -29.8 to -7.9) birth per ng/mL PFOA.

A more critically important difference between the Johnson et al. and Verner et al. papers was the fact that Johnson et al. (see also (Lam et al. 2014)) stated they found "limited and inconsistent data that were inadequate to draw conclusions on the association between fetal growth and glomerular filtration rate (GFR)." ATSDR should also include the Lam et al. (2014) paper for the background that led to this conclusion as well as their systematic review of fetal growth and maternal GFR by Vesterinen et al. (2015) (which included most of the authors of Johnson et al (2014) and Lam et al. (2014). The hypothesis (discussed by both Johnson et al. and Verner et al.) was that the increase in plasma volume expansion that occurs in early to first trimester will result in an increase in the maternal glomerular filtration rate, but less so in mothers of lower weight births (compared to mothers of higher weight births during their pregnancy). As a result, the former would have higher PFAS concentrations retained due to less PFAS eliminated via the kidney because of the comparably lower maternal GFR.

Thus, GFR would be an important confounder that could influence the association between birth weight and measured PFOA or PFOS in maternal or cord blood. In their systematic review of fetal growth and maternal GFR, Vesterinen et al. did not include the largest published study (Morken et al. 2014) to examine this relationship because it was published after their review. Morken et al. examined a subcohort of 953 selected women (470 women with and 483 women without preeclampsia in the Norwegian Mother Child Cohort study) and reported an association between maternal GFR during pregnancy and infant birth weight thus showing GFR could, indeed, confound selected epidemiologic associations. [Note: this one study by Morken et al. equaled the entire size of the database that Vesterinen et al. reviewed in their meta-analysis of 16 very small studies that were published in the scientific literature on fetal growth and maternal GFR. As with very small studies, they lacked statistical power.]

Because the association between fetal growth and maternal GFR was shown in Morken et al., Verner et al. then utilized an established PBPK model to examine the influence that GFR may have on simulated maternal serum concentrations based on the epidemiologic data. They subsequently reported that the association between simulated maternal and cord plasma PFOA levels and birth weight was dependent on the time elapsed after conception. This critical issue was not mentioned by the ATSDR. The association was not seen with PFOA measured in the first trimester and strongest at term where they reported an -7.92 g (95% CI -9.42, -6.43) reduction in birthweight per ng/mL PFOA measured at delivery. As stated above, simulation of measured cord blood PFAS resulted

in a -7.13 g birth weight per ng/ml PFOA. Verner et al. concluded a "substantial proportion of the association between prenatal PFAS and birth weight may be attributable to confounding by GFR which would be more important to examine in those studies with sample collection later in pregnancy".

Based on the analyses by Verner et al. showing maternal GFR may substantially confound any association between PFOA or PFOS and fetal growth (measured as birth weight), the available data do not permit ATSDR to conclude that there is an association between PFOA or PFOS and lower birth weight in this regard, especially without listing the caveats (confounding) known to date, let alone the unknown multitude of other physiologic changes occurring during the course of a pregnancy that have yet to be accounted for in any epidemiologic analyses.

The next most recent meta-analysis performed was published in 2017 by Negri et al. They included 16 studies in their meta-analysis. The additional studies not considered by Johnson et al. (2014) included the publications by Wu et al. (2012), Darrow et al. (2013), Bach et al. (2016a)), Lenters et al. (2016), Robledo et al. (2015)), and Lee et al. (2016).

The Negri et al meta-analyses used both the untransformed and natural log transformations of PFOA and PFOS. For PFOA, they reported a -12.8 g untransformed birthweight (95% CI -23.21, -2.38) and -27.12 (95 % CI -50.64, -3.6) g (natural log transformed) change per ng/mL PFOA. For PFOS, they reported a -0.92 g untransformed birthweight (95% CI -3.43, 1.60) and -46.09 g (natural log transformed) (95% CI -80.33, -11.85) per ng/mL PFOS. Based on their sensitivity analyses, there were stronger associations from studies conducted in Asia and significant heterogeneity was observed when the measurement of PFOA/PFOS was done later in the pregnancy or using cord blood. The latter is consistent with the simulation PBPK modeling done by Verner et al. (2015) as it relates to the potential confounding influence of maternal GFR with the timing of when PFOA is measured during pregnancy. Negri et al. also examined the laboratory animal data (results not reported here) and concluded the animal data showed similar dose-response trends but the effective serum concentrations in rodents were 100 to 1000 times higher than in humans based on the epidemiological evidence. This led Negri et al. to increase their degree of uncertainty as to the biological plausibility of a causal relationship between PFOA or PFOS exposure and lower birthweight in humans. This doubt led these authors to suggest there might be some, not yet identified, confounding factors that lead to this spurious association of lower birth weight and perfluoroalkyl measurements in humans. For reasons not explained, Negri et al. chose not to reference the Verner et al. (2015) PBPK simulation study who aptly demonstrated the potential confounding of maternal GFR, the timing of measurement of PFOA/PFOS during and through pregnancy, and reported birth weight.

Published in abstract form in August 2018 is a fourth meta-analysis authored by Steenland et al. (Epidemiology 2018). It is anticipated the full study will be available on-line in 60 to 90 days. These investigators conducted a meta-analysis of 24 studies, which

examined the association between lower birth weight and PFOA. (PFOS was not part of this meta-analysis.) The additional nine new studies (not identified in the abstract) added 6019 births to the 6937 births examined by Negri et al. in their meta-analysis. They included another large study (not identified in abstract) that was excluded from previous analyses, in a sensitivity analysis. Overall, they found a change of birthweight of -10.5 grams (95% CI -16.7, -4.4) per ng/ml PFOA in maternal or cord blood. After adding the one previously excluded large study, Steenland et al. found "little" evidence of an association (-1.0 grams, 95% CI -2.4, 0.4) per ng/mL PFOA. Restricting to the studies where blood was sampled from mothers early in the pregnancy or shortly before conception (5393 births), they reported "little" association of PFOA with birthweight (-3.3 grams (95% CI -9.6, 3.0)). In studies where blood was sampled late in the pregnancy (7563 pregnancies), lower birthweight was associated with PFOA (-17.8 g (95% CI -25.0, -10.6)/ ng/mL PFOA. Steenland et al. concluded the present human evidence provides only modest support for decreased birthweight with increasing PFOA. Critically important to understand is the time interval when perfluoroalkyls were measured.

Steenland et al. concluded "*studies with a wide range of exposure and studies with blood sampled early in pregnancy showed little or no association of PFOA with birthweight. These are the studies in which confounding and reverse causality would be of less concern."* This conclusion is consistent with the findings from Verner et al. [Note: ATSDR also concluded in its draft Toxicological Profile on page 517 (without citing Negri et al. or Steenland et al. meta-analyses) that "the decreases in birth weight were small and not likely biologically relevant."]

3M Conclusion on lower birth weight

There is no association between low birth weight (<2500 g) in humans and exposure to PFOA or PFOS. Taking into account 1) confounding by the increased maternal glomerular filtration rate that increases during early pregnancy, 2) the time period when PFOA/PFOS are measured before, during or after pregnancy, and 3) the possibility of reverse causation, there is insufficient epidemiologic evidence to conclude an association exists between lower birth weight (i.e., several grams) and PFOA or PFOS concentration (per ng/ml).

Additional Comments

General note:

There is no authorship by chapters or sections within chapters.

Page v.

- The role of SRC, Inc. as it relates to this Toxicological Profile needs to be described on this page under Chemical Manager Team.

- Dr. Emmett has served as a peer reviewer selected by ATSDR on the 2009, 2015, and now 2018 draft Toxicological Profiles for Perfluoroalkyls. Dr. David Savitz's role as a peer reviewer on the draft 2009 Toxicological Profile should be acknowledged as well as ATSDR's request that Dr. Savitz provide publicly available comments on the draft 2015 ATSDR Toxicological Profile. Dr. Cory-Slechta has served as: 1) the chairperson of the 2005 U.S. Environmental Protection Agency Science Advisory Board Perfluorooctanoic Acid (PFOA) Risk Assessment Review Panel; 2) a peer reviewer (and the chairperson) on the U.S. EPA draft 2014 health effects document for PFOA; 3) a peer reviewer (and the chairperson) on the U.S. EPA draft 2014 health effects document for PFOS; 4) a peer-reviewer of the draft 2015 ATSDR Toxicological Profiles on Perfluoroalkyls; and 5) a peer-reviewer of the draft 2018 ATSDR Toxicological Profiles on Perfluoroalkyls. Dr. DeWitt was one of 20 members of the 2014 IARC Workshop that reviewed PFOA; a peer reviewer on the U.S. EPA draft 2014 health effects document for PFOA; and a peer reviewer on the U.S. EPA draft 2014 health effects document for PFOS.

  To have repeatedly selected these reviewers minimizes the peer-review process of receiving comments that could have been made available to ATSDR.

- Dr. Jamie DeWitt was paid by plaintiff attorneys in the case of State of Minnesota vs. 3M. This financial conflict of interest with another governmental agency should be noted in this draft 2018 ATSDR Toxicological Profile. Dr. DeWitt should not have been chosen as a peer reviewer to a federal government agency given this paid financial conflict of interest regarding another governmental agency. Any other financial conflicts of interest by Dr. DeWitt should also be listed as to her funded role in any litigation effort, to the present date, regarding perfluoroalkyls.

Page 1:

- ATSDR used the term "perfluoroalkyls" for the 14 compounds that it has evaluated. While it is acceptable to use this general nomenclature in some parts of the discussion, it is not applicable for topics such as major applications listed under section 1.1.

- For clarity most of the 14 perfluoroalkyl substances that are the focus of this report have limited commercial utility. PFOS, PFOA and PFOA pre-cursors have been used extensively.
- On a technical definition, ATSDR should make note to differentiate that the following two compounds (among the 14 evaluated) are polyfluoroalkyls, not perfluoroalkyls.
    - 2-(N-Methyl-perfluorooctane sulfonamide) acetic acid (Me-PFOSA-AcOH)
    - 2-(N-Ethyl-perfluorooctane sulfonamide) acetic acid (Et-PFOSA-AcOH)
- The ATSDR draft profile cites a 2003-2004 NHANES study (Calafat et al, 2007). More recent NHANES biomonitoring data was published in the CDC's "Fourth National Report on Human Exposure to Environmental Chemicals" in 2018.

Page 2:

- The ATSDR draft profile recognized that serum levels of PFOA and PFOS in the U.S. general population have "decreased dramatically in recent years". For further clarification, from 1999-2000 to 2013-2014 mean blood levels of PFOS and PFOA have decreased by approximately 84% and 63%, respectively, based on NHANES data. A more recent study, using data from the American Red Cross, reported an 88% and 77% decline in serum PFOS and PFOA levels, respectively, from 2000-2001 to 2015 (Olsen et al., 2017). These reductions are largely attributed to the concerted efforts by industry and the U.S. EPA to decrease the use of these chemicals in manufacturing and releases to the environment.
- ATSDR should revise the last paragraph on this page. Contaminated drinking water near fluoropolymer manufacturing facility in southeastern Ohio and West Virginia did not have high levels of exposure to PFOS.
- Page 2, Paragraph 1. The statement that PFOS and PFOA are no longer imported is not entirely accurate. PFOS, FC-98 and a few other PFOS-precursor substances are not TSCA prohibited, and may be imported.
- ATSDR stated: "Volatile ***fluorotelomer*** alcohols may be ***broken down*** into substances like PFOA, and atmospheric deposition can lead to contamination of soils and leaching into groundwater away from point sources." There is no description of what fluorotelomers are. "Broken down" is inappropriate scientific terminology.
- There is no definition of the word "high". "High" is relative to some other value and is subjective language The ATSDR should substitute this word "high" throughout this document for the specific concentrations referred to when "high" or "low" are used and be specific whether these values are arithmetic means, geometric means, or medians, as well as offer a measure of variation to the point estimates (e.g., standard deviation, standard error, 95% confidence interval, or a range minimum/maximum). Also, it is important to refer to the year in which these perfluoroalkyl values were actually measured (not just the author and reference year) because of the declining trends over the past 15+ years in most general populations not exposed to an environmental point source of exposure.

Page 3:

- ATSDR should provide the actual median value and corresponding year-dependent NHANES median value. ATSDR should provide the percentage decline as well in these geometric mean values for PFOS (decline of 83.6%) and PFOA (decline of 62.7%) between 1999-2000 and 2013-2014.
- In the last paragraph, ATSDR reported breast milk concentrations, but does not indicate when such concentrations were measured. This is important because breast milk concentrations have declined similar to serum concentrations in adults. See above comment on incomplete paragraph 1 on page 3. Concentrations have also declined in children. See Olsen et al. (2005) who reported on children (2 – 12) serum measurements made in 1994-1995 to those measurements recently reported by Ye et al (2018) who reported, in a nationally representative sample of children age 3-11, that their concentrations were comparable to adults measured also in 2013-2014. The measured concentrations in these children were substantially lower in other non-representative samples of 597 children reported by Olsen et al. (measured in 1994-1995). Therefore, breast milk concentrations have also likely declined over time.
- There are additional studies on human breast milk biomonitoring studies, ATSDR should reference and summarize studies by: Sundstrom et al. 2011 Environ Int 37 178-183; Karrman et al 2009 Environ Int 35 712-17; Llorca et al 2010 Environ Int 36 584-592; Mosch et al. 2010 J Chromatog B 878 2652-2658; Kang et al. 2016 Environ Res 148 351-359; Cariou et al. 2015 Environ Int 84 71-81; Al-sheyab et al. 2015 Environ Sci Pollut Res 22 12415-12423; Lankova et al. 2013 Talanta 117 318-25; Pratt et al. 2013 Food Addit Contam A 30 1788-1798; Guerranti et al. 2013 Food Chem 140 197-203; Antignac et al. 2013 Chermosphere 91 802-808; Barbarossa et al. 2013 Environ Int 51 27-30; Croes et al. 2012 Chemosphere 89 988-994; Domingo et al. 2012 Food Chem 135 1575-1582; Thomsen et al. 2010 Environ Sci Technol 44 9550-9556.

Page 4:

- ATSDR used the term “perfluoroalkyls” to describe the 14 compounds that are listed on page 1 (including Perfluorooctane sulfonamide (PFOSA), 2-(N-Methyl-perfluorooctane sulfonamide) acetic acid (Me-PFOSA-AcOH), and 2-(N-Ethyl-perfluorooctane sulfonamide) acetic acid (Et-PFOSA-AcOH)). Accordingly, ATSDR cannot make the blanket statement that perfluoroalkyls “are not metabolized in humans or laboratory animals” because these 3 compounds can and do metabolize in laboratory animals.
- Table 1-1. The estimated elimination half-life of PFOA in humans is clearly not 8 years. This estimate is not found in the Olsen et al. 2007a paper. More importantly, similar to the data reported in rats and mice, there are available ranges of the estimated elimination half-lives of PFOA, PFOS, and PFHxS. There are several high-quality and more recent studies of populations whose exposure was mitigated by installation of GAC filters that have shown the serum elimination half-life of PFOA to be between 2.3 years (95% CI) (Bartell 2013) and 2.8 years (95% CI) (Li et al. 2018). Similarly, the serum elimination half-life for PFOS of 5.4 years is the highest estimate of 6 studies.

Page 5:

- It is incorrect for ATSDR to state that "In general, epidemiology studies use serum perfluoroalkyl levels as a biomarker of exposure, which contrasts experimental studies that utilize dose, expressed in mg/kg/body weight/day units". As difference in toxicokinetics have been well-recognized, it is the serum levels in the animals (resulted from doses given) that should be used for data interpretation; and many toxicological studies have been measuring and reporting serum levels in the laboratory animals as internal dose metrics (ng/mL) as well as benchmark lower bound internal serum concentrations.
- ATSDR relied on animal PBPK model to predict subsequent POD of MRL derivation, but on the other hand, it has also explicitly stated that "Although physiologically based pharmacokinetic (PBPK) models have been developed for rodents and humans, these models are not sufficient to allow for comparisons between administered doses in laboratory animals and serum concentrations in humans". This statement indicated a great amount of uncertainty associated with the PBPK model used hence ATSDR needs to reflect and acknowledge this fact in its summary.
- It is inappropriate to solely consider the Emmett et al. (2006a) mean PFOA estimate of 423 ng/mL as the mean estimate of PFOA level in highly exposed residents for the community surrounding the DuPont Washington Works facility in west Virginia because other data are available. Furthermore, Sakr et al. 2007a did not provide the most appropriate estimate for the average PFOA concentration for the workers (Woskie et al. 2012 Ann Occup Hyg 56 1025-1037).
- Throughout this draft toxicological profile, ATSDR stated that most epidemiology studies were of the cross-sectional design. However, nowhere does ATSDR provide the actual quantitative number of epidemiological studies by the type of study design. Furthermore, in most tables reported in Chapter 2, ATSDR never provides the type of study design of the author. It assumes the reader will look at more detail in the abridged abstracts of these studies presented in the Supporting Document. This is highly unfortunate and a major shortcoming of the ATSDR report. All studies listed in tables should be listed as to their study design.
- It is highly misleading for ATSDR to state on page 5, paragraph 2, prior to identifying associations between PFAS exposure and eight health outcomes, that "Based on a number of factors including the consistency of findings across studies, the available epidemiology studies suggest associations between perfluoroalkyl exposure and several health outcomes" because on page 635/636 (chapter on the adequacy of the database), it makes the following contradictory statement: "The available human studies have identified some potential targets of toxicity; however, cause-and-effect relationships have not been established for any of the effects, and the effects have not been consistently found in all studies." Indeed, there is not consistency of findings in the epidemiology data across these 8 associations. Moreover, ATSDR does a disservice to the scientific literature to suggest that there is consistency. Therefore, it is imperative that the statement found on page 635/636 be placed either in front of or immediately after the

listing of the 8 associations provided on page 5/6 in Chapter 1. Otherwise, these "associations" may be misperceived to reflect causality by scientists as well as the public reading this Toxicological Profile.

Pages 6 – 9:

Figures 1-1, 1-2, and 1-3 are misleading. The studies compiled in each figure have different study designs with different animal models used and different dosing regimens; they simply do not reflect final body burden achieved. These figures should either be removed or revised by taking toxicokinetic into consideration.

Page 10:

Under liver effects: ATSDR should also cite other key studies such as Elcombe et al 2010 Arch Toxicol 84 787-798; Albrecht et al. 2013 Toxicol Sci 131 568-582; and Butenhoff et al. 2012 Reprod Toxicol 33 513-530.

Page 11:

- ATSDR should also include other nuclear receptors in its discussion, such as CAR/PXR. It should include studies by Elcombe et al 2010 Arch Toxicol 84 787-798; Vanden Heuvel et al. 2006 Toxicol Sci 92 476-489; Albrecht et al. 2013 Toxicol Sci 131 568-582; Bjork & Wallace 2009 Toxicol Sci 111 89-99; and Bjork et al. 2011 Toxicology 288 8-17.
- ATSDR is incorrect stating that increased hepatic palmitoyl CoA oxidase activity was increased in PFOS-treated monkeys in Seacat et al. (2002) study (see Table 6 of Seacat et al. manuscript).
- ATSDR should also cite another relevant study for the serum lipid change in monkeys (Chang et al. 2017 Toxicol Sci 156 387-401), which followed a cohort of monkeys for 400+ days and their serum lipid profiles were characterized before and after PFOS treatments. The lower benchmark concentration was around 75 µg/mL (75000 ng/mL) in the serum where a decrease in serum cholesterol occurred in these monkeys.

Page 12:

- ATSDR should provide compelling scientific data to explain why they concluded the following:

> "*Specific effects reported include prenatal loss, reduced neonate weight and viability, neurodevelopment toxicity, and delays in mammary gland differentiation, eye opening, vaginal opening, and first estrus (Abbott et al. 2007; Albrecht et al. 2013; Cheng et al. 2013; Johansson et al. 2008; Koskela et al.*

2016; Lau et al. 2006; Macon et al. 2011; Ngo et al. 2014; Onishchenko et al. 2011; Sobolewski et al. 2014; White et al. 2007, 2009, 2011; Wolf et al. 2007; Yahia et al. 2010). These effects occurred generally in the absence of overt maternal toxicity."

In the studies cited by ATSDR above, there were compelling supporting data to illustrate developmental toxicity with PFOA exposure under maternal influences. In addition, there was no standardized method evaluating mammary gland during pup developments and the delayed mammary gland conclusions reported by White et al. (2007, 2009, 2011) and Macon et al. (2011) contradicted with the conclusions reported by others (Albrecht et al. 2014, Yang et al. 2009 Reproduct Toxicol 27 299-306; Hardisty et al 2010 Drug Chem Toxicol 33 131-137) where strain-specific responses cannot be ruled out.

- Study outcomes reported by Onishchenko et al. (2011) had many technical issues and its data lacked scientific rigors necessary for it to be used in any meaningful human risk assessment.

- Brain and nervous system have not been identified as target organs in long-term toxicological studies, including 2-year bioassays in rats (Butenhoff et al. 2012 Toxicology 298 1-13; Biegel et al 2001 ToxSci 60 44-55), 13-week study in rats (Perkins et al. 2004 Drug Chem Toxicol 27 361-378), 2-generation in rats (Butenhoff et al 2004 Toxicology 196 95-116), or 6-month study in monkeys (Butenhoff et al 2002 ToxSci 69 244-257).

Pages 13 and 14:

- Similar to comments provided on PFOA, there were compelling supporting data to illustrate developmental toxicity with PFOS exposure was mediated by maternal toxicity. In addition, the neurodevelopmental alterations in mice cited by ATSDR were confounded by poor study design (Onishchenko et al. 2011, where only a single PFOS dose was used) or unexplained non-PFOS-related stress such as restraining during pregnancy (Fuentes et al. 2007a). Evaluation of immune parameters based on the results reported by Keil et al. (2008) was not comprehensive in that normal response to immunization is based on IgG titer, not IgM; and that Keil et al. did not evaluate the subpopulation in other key immune organs such as bone marrow and blood.

- Study by Dong et al. (2009) also had numerous deficiencies which precluded its data to be used in a proper human risk assessment. The data presented by Dong et al. lacked scientific validity to support the conclusion that PFOS suppresses immune responses. There should be concordance between several key immune parameters (as discussed below) and the study by Dong et al. failed to demonstrate such many important aspects of immunotoxicity study. Briefly, antibody response is IgG isotype, not IgM, and as an immunosuppressing agent, one would expect similar suppressive immune responses to be

observed in major key organs such as decreased IgM and IgG in spleen, thymus, and serum. Dong et al. evaluated IgM in spleen only but did not provide any concurrent IgM status in other key organs such as thymus or serum. As an immunosuppressing agent, one would expect decreased immune cell populations in spleen, thymus, blood, and bone marrow and Dong et al. only looked at spleen and thymus. As an immunosuppressing agent, one would expect decreased proliferation in immune cells and Dong et al. did not use the correct methods to evaluate these responses and improperly reported their data. Collectively, the study by Dong et al. did not provide any robust or compelling scientific evidence to support the claim that PFOS is associated with immune suppression in mice.

Page 21:

As stated previously, the ATSDR draft profile cited a 2003-2004 NHANES study (Calafat et al, 2007). More recent NHANES biomonitoring data was published in the CDC's "Fourth National Report on Human Exposure to Environmental Chemicals" in 2018.

Page 22:

ATSDR stated that "*For studies in which the population was divided into perfluoroalkyl exposure categories, such as quartiles, the risk ratio reported in the summary table is for the lowest exposure category with a statistically significant association; risk ratios for higher exposure categories are presented in the Supporting Document for Epidemiological Studies for Perfluoroalkyls*". This approach is problematic for several reasons. First, readers will likely refer only to the ATSDR draft profile and not the Supporting Document. As such, readers will not be informed of all findings including those exposure categories with non-significant findings and evidence (or lack thereof) of a dose-response. Second, results from continuous exposure metrics and other statistical measures are not reported in Summary tables or in the Supporting Document. It is inappropriate for ATSDR to include only categorical results and not present all the available evidence (both significant and non-significant findings).

Page 23:

ATSDR stated that "*The discussion of the available data for each health effect is divided into several subsections. Each health effect section begins with an overview, which contains a brief discussion of the available data and conclusions that can be drawn from the data*". However, the section overview, for most health effects, failed to provide any conclusions that can be drawn from the data or any discussion beyond presenting overall study findings. Of the 18 health effects reviewed in draft profile, ATSDR did not provide their overall conclusion for 10 health effects, including death (page 106), body weight (page 109), respiratory (page 121), cardiovascular (page 123), gastrointestinal (page 135),

hematological (page 137), dermal (page 219), ocular (page 220), neurological (page 293) and cancer (page 418).

Page 24:

ATSDR reported that a "weight-of-evidence" approach was used to evaluate whether the available data support a link between perfluoroalkyl exposure and a particular health outcome. Further, ATSDR stated that "*this weight-of-evidence approach takes into consideration the consistency of the findings across studies, the quality of the studies, dose-response and plausibility*". However, ATSDR failed to 1) cite the "weight-of-evidence" approach that was used, and 2) provide scientific justification or documentation of the underlying evidence used to reach a conclusion. Given that a "weight-of-evidence approach" requires use of scientific judgment, the ATSDR must be transparent in all steps of the evaluation process and all conclusions drawn. For example, on the 8 associations listed on page 25, the ATSDR has failed to explain to the reader how it reached such a collective conclusion for each one given the quality (often cross sectional) of the studies reviewed, the lack of dose-responses, and lack of any known biological plausibility in the human, especially when such plausibility was either not shown or known to result in contradictory findings in the human.

Page 25:

- The term "links" does not have a precise scientific meaning. This word is not standard scientific language taught in epidemiology courses in Schools of Public Health. Therefore, the ATSDR should delete throughout this document the word "link or links" and replace with the word "association or associations."
- See comments for Page 5, Paragraph 2. It is not possible to discuss associations without explicitly stating the admission by ATSDR, found on page 635/636 of the chapter on the adequacy of the database, the following statement (see section on Epidemiology and Human Dosimetry Studies): "The available human studies have identified some potential targets of toxicity; however, cause-and-effect relationships have not been established for any of the effects, and the effects have not been consistently found in all studies." This statement should immediately precede or follow the associations whenever the associations are listed; otherwise these "associations" may be erroneously assumed to reflect causality by non-epidemiologists as well as the public-at-large or others that may read this Toxicological Profile or parts therein.

Page 108:

OECD (2002) document cited on this page is public information and can be found on the following web link:

https://www.oecd.org/env/ehs/risk-assessment/2382880.pdf

Pages 109 – 433:

For each of the endpoints listed here, ATSDR reported the study findings for each compound under each effect but did not provide its overall assessment. The data presentation (spanning 300+ pages) was on the who/how/what of the selected epidemiological and toxicological studies. It lacked overall conclusion and there was no "synthesis" on the selected data presented by ATSDR in this section. A conclusion or position statement by ATSDR at the end of each endpoint will be helpful to the readers.

Page 131:

ATSDR incorrectly stated that *"Another"* study (Darrow et al, 2013) found significant increases in odds ratios for pregnancy-induced hypertension. This study is the same study that is cited in the previous sentence.

Pages 244-300 (Section 2.14):

Two additional studies (Timmermann et al. 2017; Impinen et al. 2018) have been published since 2016 and should be included in the ATSDR draft profile.

Pages 245-250, Table 2-16:

- ATSDR did not cite the study by Anderson-Mahoney et al (2008). It is, however, cited in the Supporting Document (page 105, Table 10).
- ATSDR did not cite a study (Leonard et al., 2008) of PFOA/PFOS exposure and mortality from infectious and parasitic diseases. While this study was cited in Section 2.2, it should also be included in Section 2.14 (as other studies have been cited in more than one section).

Pages 268 - 281:

ATSDR cited several National Toxicology Program (NTP 2016) conclusions on immunosuppression outcomes without providing the NTP rationale for reaching such conclusions. For example, on page 269, in a separate paragraph, ATSDR states "*NTP (2016b) concluded that there is moderate confidence that exposure to PFOA is associated with suppression of the antibody response based on the available human studies. NTP (2016b) also concluded that there is low confidence that exposure to PFOA is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease.*" ATSDR should describe NTPs confidence ratings in more detail (i.e. inadequate, low, moderate, high) and provide the rationale for reaching each conclusion.

Pages 270, Figure 2-19:

The "percent difference in antibody concentration per 2-fold increase in serum PFOA" is presented in Figure 2-19. However, findings from two influenza studies (Looker et al. 2014; Stein et al. 2016b) that used other measures of association, and reported null findings, were not included. Although both studies were cited in the draft profile (page 269), the ATSDR should acknowledge that results from these two studies were omitted from Figure 2-19 and provide reasons for their omission.

Pages 272, Figure 2-20:

Results from asthma studies reporting adjusted odds ratios are presented in Figure 2-20. Similar to the previous comment, results from two studies (Anderson-Mahoney et al 2008; Granum et al 2013) which reported different measures of association were not included in the Figure. The ATSDR should acknowledge that results from these two studies were omitted from Figure 2-20 and provide reasons for their omission.

Pages 272 (Figure 2-20), 280 (Figure 2-22), 285 (Figure 2-24), 288 (Figure 2-26), and 292 (Figure 2-28):

The ATSDR should clearly acknowledge that results from Zhu et al (2016) and Dong et al (2013) were from a single case-control study of the same population (456 Taiwanese children). As currently presented, it gives readers a false impression that these are two distinct studies with consistent findings, which they are not.

Pages 277, Figure 2-21:

The "percent difference in antibody concentration per 2-fold increase in serum PFOS" is presented in Figure 2-21. However, findings from two influenza studies (Looker et al. 2014; Stein et al. 2016b), which used different measures of association, and reported null findings, were not included. The results by Looker et al (2014) were cited in the draft profile (page 277), but not the results from Stein et al (2016b). The ATSDR should acknowledge that results from these two studies were omitted from Figure 2-21 and provide reasons for their omission.

Pages 289-291 and Figure 2-27:

ATSDR offered no explanation for how it concluded that there is an association between PFDeA and decreased antibody responses to vaccines given that only 3 studies have examined this potential association and have reported mixed results. This conclusion is not scientifically supported given the limited and inconsistent evidence.

Pages 433 – 449:

Among all the mechanisms listed here, ATSDR failed to highlight the lipid mechanism. Albeit it was discussed under hepatic toxicity mechanism, it should be emphasized because lipid-lowering is a hallmark biological event with exposures to many of the perfluoroalkyls (at relatively high doses). The lipid-lowering mechanism has been elucidated for PFBS, PFHxS, and PFOS using ApoE3*Leiden.CETP mice (Bijland et al. 2011 Tox Sci 123 290-303). The hypolipidemia has been extensively discussed with PFOA by others (which are cited by ATSDR on page 11).

Pages 434 – 438:

For PPARalpha-dependent mechanism, ATSDR should offer a summary or a position statement on PPARalpha-mediated effects reported in animals and their lack of relevance to humans.

Pages 438 – 441:

Similarly, ATSDR should offer a summary or a position statement on PPARalpha-independent effects reported in animals and their relevance to humans.

Pages 441 - 443:

The liver toxicity mechanism in rodents, in part, has been well-documented and ATSDR should offer a summary or a position statement on the rodent liver effects and their relevance to humans.

Pages 443-444:

Research on immunotoxicity has produced only inconclusive evidence, as acknowledged by EPA in its 2016 Health Effects Document for PFOS, where it stated that:

> *"Both human and animal studies have demonstrated the potential impact of PFOS on the immune system; however, uncertainties exist related to MOA and the level, duration, and/or timing of exposure that are not yet clearly delineated. The animal immunotoxicity studies support the association between PFOS and effects on the response to sheep red blood cells as foreign material and on the natural killer cell populations; however, the doses with effects are inconsistent across studies for comparable endpoints. When both males and females were evaluated, the males responded at a lower dose than the females. Because of these uncertainties, EPA did not quantitatively assess this endpoint."*

Page 445:

Although many toxicological studies had reported endocrine disturbance potential with PFOA and PFOS exposures, specifically on the thyroid hormones, it is important to realize that most of these studies were done either under *in vitro* conditions (to which high concentrations of PFOA or PFOS were employed) or *in vivo* but only with a limited set of endpoints evaluated such as selected gene expressions (D'Orazio et al. 2014; Dankers et al. 2013; Dixon et al. 2012; Du et al. 2012; Du et al. 2013; Gao et al. 2013; Kraugerud et al. 2011; Sales et al. 2013; Sonthithai et al. 2015; Wens et al. 2013; White et al. 2011a; Feng et al. 2015; Lopez-Doval et al. 2015; Lopez-Doval et al. 2014; Pereiro et al. 2014; Wang et al. 2011).

In the study cited by ATSDR, Ren et al. (2015) evaluated perfluoroalkyl bindings using a computer software model to simulate thyroid hormone binding; and their in vivo portion of the study was on tadpoles, not in mammalian species. The endocrine system is very complicated and evaluation of endocrine functions is a very highly specialized field (this is especially true in human clinical medicine). Given that PFOA and PFOS are strong surfactants, the toxicity effects reported from the typical mono-layered *in vitro* tissue culture system offered very little insight and scientific value because the data were often comprised by the surfactant-induced toxicity. Similarly, gene expressions do not represent functionality and endocrine function is an intricate network.

Based on data from the large scale 2-generation reproductive and developmental studies (which are considered as the most comprehensive test by various agencies for evaluating endocrine functions), PFOA and PFOS clearly did not alter the reproductive functions as the reproductive performances in both males and females were normal (*vide supra*). If they were indeed endocrine disrupting compounds, then one would expect it to directly activate endocrine receptors such as estrogen receptors or thyroid receptors.

Ishibashi et al. (2007) reported that PFOA or PFOS did not activate human estrogen receptor α or β. Likewise, Yao et al. (2014) did not report that PFOA can activate mouse or human estrogen receptors. Yao et al. also showed a lack of change in the histomorphology of uterine/cervix and vaginal tissues in female mice after receiving oral ammonium PFOA treatments. Furthermore, while triiodothyronine (T3, the active form of thyroid hormone) elicits a dose-response activation of human thyroid receptor α from 0.000001 – 0.01 uM, under the same study condition, there was no activation of human thyroid receptor α when exposed to ammonium PFOA or PFOS up to 100 uM (Ehresman et al. 2014 The Toxicologist (abstract 1135) 138 302).

Under in vitro condition, Chang et al. had extensively evaluated the effects of PFOS and thyroid hormone status in rodents (Chang et al 2007 Toxicology 234 21-33; Chang et al 2008 Toxicology 243 330-339; Chang et al 2009 Reproduct Toxicol 27 387-399) and

monkeys (Chang et al. 2017 Toxicol Sci 156 387-401) and did not observe any toxicological relevant alterations in functional aspects of thyroid hormone homeostasis. Furthermore, Convertino et al. (2018) reported that, in a phase 1 clinical trial study with 49 human subjects that received large doses of PFOA where serum PFOA level was up to 600,000 ng/mL (5 orders of magnitude higher than general population in the US), there was no alteration in serum TSH level in these human subjects (TSH is the key serum diagnostic parameter for thyroid hormone status used by the physicians).

Overall, the weight-of-evidence does not support that PFOS or PFOA can cause endocrine disruption and ATSDR should recognize and acknowledge this conclusion.

Pages 447 – 449:

The genotoxicity summary by Butenhoff et al. (2014 Toxicology Reports 1 252-270) should be included in the discussion.

Page 450:

Given that the perfluoroalkyls are highly bound to serum albumins, ATSDR should recognize that the distribution patterns in tissues are bloodborne-based.

Page 450:

- As stated earlier, because ATSDR used the term "perfluoralkyls" that included Perfluorooctane sulfonamide (PFOSA), 2-(N-Methyl-perfluorooctane sulfonamide) acetic acid (Me-PFOSA-AcOH), and 2-(N-Ethyl-perfluorooctane sulfonamide) acetic acid (Et-PFOSA-AcOH)), it cannot state that perfluoroalkyls "are not metabolized in humans or laboratory animals" because these 3 compounds listed above can and do metabolize in laboratory animals.

- An inhalation study for 2-(N-Ethyl-perfluorooctane sulfonamide) acetic acid (Et-PFOSA-AcOH) is available in rats and the study data indicated that Et-PFOSA-AcOH can be metabolized to form PFOS via inhalation (see Chang et al. 2017 Environ Res 155 307-313)

Page 514:

ATSDR wrote: 'Assuming a terminal elimination t1/2 of 1,400 days for PFOA in humans (Olsen et al. 2007a), a constant rate of intake for 17 years would be required to achieve 95% of steady state.' This is only applicable with a constant rate of daily (PFOA) intake for 17 years, which is an untenable assumption for any population whether occupational

(inhalation, oral, dermal) or affected communities (primarily oral via drinking water) or general population (primarily oral via diet).

Page 518:

- Given the findings reported by Convertino et al. (2018), the following statement is highly speculative and has no basis of fact, and should be deleted.

> *"Increase in serum cholesterol may result in a greater health impact in individuals with high levels of cholesterol or with other existing cardiovascular risk factors."*

- Given the fact that ATSDR did not find perfluoroalkyl associated with uric acid, the following statement is highly speculative and has no basis of fact. It should be deleted.

> *"Increases in uric levels have been observed in individuals with higher perfluoroalkyl levels. Increased uric acid may be associated with an increased risk in high blood pressure and individuals with hypertension may be at greater risk."*

Page 539, Figure 5-2:

Title of Figure 5-2: Timeline of Important Events in the History of Polyfluorinated Compounds

This figure, taken from the copyrighted paper of Lindstrom et al., is factually inaccurate as to what was stated in a 1976 publication of an abstract by Taves et al. (1976). In the figure that ATSDR secured copyright permission to display from a journal, the figure states "1976 - Taves et al. tentatively identified PFOA in pooled blood." This is not true and does not reflect what was stated in the study abstract by Taves et el. Furthermore, it ignores the limitations of the analytical procedures used, including the complex analytical processes and biases that were employed at the time (See Guy WS. 1979. Inorganic and organic fluorine in human blood. In (eds) Johansen E, Taves DR, Olsen TO. AAAS Selected Symposium 11. Pages 125-14. Westview Press; Boulder, Colorado). Thus, ATSDR needs to change this figure accordingly to reflect the technical details of the abstract.

Page 541:

The statement "Similarly, 3M and other manufacturers are using various perfluoropolyethers in fluoropolymer manufacturing and have reformulated surface treatment products to employ short-chain substances that are not as bioaccumulative as the long-chain perfluoroalkyls." Should be revised to state "3M and other manufacturers

are using various poly and ~~perfluoropolyethers~~ perfluoroether acid salts fluoropolymer manufacturing …"

Page 581:

The µg/L concentration discussed by Chang et al (2008) was only based on one sample. This should be so noted in this sentence.

Page 596:

Percentage declines should be provided in addition to modifiers such as "dramatic" or "clear" trend.

Page 633:

ATSDR should identify how many of the 400 epidemiological studies were cross-sectional.

Page 636:

As discussed elsewhere, the statement – "The available human studies have identified some potential targets of toxicity; however, cause-and-effect relationships have not been established for any of the effects, and the effects have not been consistently found in all studies" should be included up front on page 5 before the potential associations are discussed.

Additional comments:

- **Consolidate Epidemiological Study Information into Chapter 2.** ATSDR included a 277-page draft Supporting Document for Epidemiological Studies on Perfluoroalkyls. This provided the references, study populations, exposures, and outcomes for these epidemiological studies. While this information is helpful, it was burdensome to go from the figures and tables in Chapter 2 to this draft supporting document to identify the study designs identified in figures and tables in Chapter 2. Therefore, the study designs must be provided in tables and figures in Chapter 2 because the vast majority of the studies cited are cross-sectional where temporality cannot be determined.

- The draft Toxicological Profile mischaracterized the C8 Science Panel studies as having reported "cumulative PFOA exposure" when these estimates were based on an exposure model and not actually measured cumulative PFOA concentrations since they are reported as ng/mL-year. Therefore, ATSDR should consistently insert the word 'estimated' or 'modeled' in front of the word 'cumulative' throughout this document when referring to their data. Provided below are the references and page numbers where

these corrections must be made. This may not be exhaustive so ATSDR should do its own assessment of this mischaracterization. This issue also has to be addressed in the Draft Supporting Information for Epidemiologic Studies for Perfluoroalkyls (see below) where ATSDR usually acknowledges the word ‘estimate’ or ‘modeled’ in the Exposure Column of the C8 Science Panel references but rarely does the ATSDR use the words ‘estimated’ or ‘modeled’ in the Outcomes column.

	Study	**Page**
Reference	Steenland et al. 2015	10
	Steenland et al. 2015	14
	Simpson et al. 2015	18
	Winquist et al. 2014	19
	Steenland et al. 2015	31
	Steenland et al 2015	42
	Darrow et al. 2016	43
	Darrow et al. 2016	44
	Winquist et al. 2014a	46
	Steenland et al. 2015	71
	Steenland et al. 2015	84
	Winquist and Steenland 2014b	86
	Winquist and Steenland 2014b	87
	Steenland et al. 2015	105
	Steenland et al. 2015	106
	Steenland et al. 2015	237
	Karnes et al. 2014	239
	Steenland et al. 2015	253

Draft Supporting Document for Epidemiological Studies for Perfluoroalkyls	Study	Additional Note	Page
	Steenland et al. 2015		10
	Steenland et al. 2015		14
	Simpson et al. 2013		18
	Winquist and Steenland 2014a		19
	Olsen et al. 1998a.	Should be cross-sectional study	29
	Steenland et al. 2015		31
	Gilliland and Mandel 1996	Should be cross-sectional study	38
	Olsen et al. 2000	Should be cross-sectional study	39
	Olsen and Zobel 2007	Should be cross-sectional study	40
	Steenland et al. 2015		42
	Darrow et al. 2016		43
	Winquist and Steenland 2014a		46
	Olsen et al. 1999	Should be cross-sectional study	52
	Olsen et al. 2003	Should be cross-sectional study	53
	Mundt et al. 2007	Should be cross-sectional study	63
	Lundin et al. 2009	Should be cross-sectional study	69
	Steenland et al. 2015		71
	Olsen et al. 1998a	Should be cross-sectional study	76
	Olsen et al. 1998b	Should be cross-sectional study	83
	Olsen and Zobel 2007	Should be cross-sectional study	83
	Steenland et al. 2015		84
	Steenland and Winquist 2014b		86
	Olsen et al. 1998a	Should be cross-sectional study	90
	Mundt et al. 2007	Should be cross-sectional study	98
	Steenland et al. 2015		105
	Olsen et al. 1998b	Should be cross-sectional study	140
	Dhingra et al. 2016a		141
	Dhingra et al. 2016a		142
	Bach et al. 2016	Should be cohort study	143
	Olsen et al. 1998a	Should be cross-sectional study	152
	Bach et al. 2016	Should be cohort study	152
	Bach et al. 2016	Should be cohort study	168
	Whitworth et al. 2012a.	Should be cohort study	182
	Bach et al. 2016	Should be cohort study	225
	Bach et al. 2016	Should be cohort study	229
	Steenland et al 2015		237
	Steenland and Woskie et al. 2012		238
	Lundin et al. 2009.	Should be retrospective cohort study	251
	Steenland et al. 2015		253
	Steenland and Woskie et al. 2012		253

Citations:

Abbott, B.D., Wolf, C.J., Schmid, J.E., Das, K.P., Zehr, R.D., Helfant, L., Nakayama, S., Lindstrom, A.B., Strynar, M.J., Lau, C.S., 2007. Perfluorooctanoic Acid (PFOA)-induced Developmental Toxicity in the Mouse is Dependent on Expression of Peroxisome Proliferator Activated Receptor-alpha (PPAR{alpha}). Toxicol Sci 98, 571-581.

Abbott, B.D., 2009. Review of the expression of Peroxisome Proliferator Activated Receptors alpha (PPARα), beta (PPARβ), and gamma (PPARγ) in rodent and human development. Reproductive Toxicology 27.

Abbott, B.D., Wolf, C.J., Das, K., Zehr, R.D., Schmid, J.E., Lindstrom, A.B., Strynar, M.J., Lau, C., 2009. Developmental Toxicity of Perfluorooctane Sulfonate (PFOS) is not dependent on expression of Peroxisome Prooliferator activated Receptor-alpha (PPARα) in the mouse
Reproductive Toxicology 27, 258-265.

Abbott, B.D., 2015. Developmental Toxicity. In: J. DeWitt (Ed), Toxicological Effects of Perfluoroalkyl and polyfluoroalkyl Substances, Springer International Publishing, Switzerland, pp. 203-218.

Aerssens, J., Boonen, S., Lowet, G., Dequeker, J., 1998. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. Endocrinology 139, 663-670.

Albrecht, P.P., Torsell, N.E., Krishnan, P., Ehresman, D.J., Frame, S.R., Chang, S.C., Butenhoff, J.L., Kennedy, G.L., Gonzalez, F.J., Peters, J.M., 2013. A Species Difference in the Peroxisome Proliferator-Activated Receptor alpha-Dependent Response to the Developmental Effects of Perfluorooctanoic Acid. Toxicol Sci 131, 568-582.

American College of Obstetricians and Gynecologists. 2013. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstetrics and gynecology 122, 1122-1131.

Andersen, M.E., Butenhoff, J.L., Chang, S.C., Farrar, D.G., Kennedy, G.L., Jr., Lau, C., Olsen, G.W., Seed, J., Wallace, K.B., 2008. Perfluoroalkyl acids and related chemistries--toxicokinetics and modes of action. Toxicol Sci 102, 3-14.

Anderson-Mahoney, P., Kotlerman, J., Takhar, H., Gray, D., Dahlgren, J., 2008. Self-reported health effects among community residents exposed to perfluorooctanoate New Solutions 18, 129-143.

Bach, C.C., Liew, Z., Bech, B.H., Nohr, E.A., Fei, C., Bonefeld-Jorgensen, E.C., Henriksen, T.B., Olsen, J., 2015. Perfluoroalkyl acids and time to pregnancy revisited: An update from the Danish National Birth Cohort. Environ Health 14, 59.

Bach, C.C., Bech, B.H., Nohr, E.A., Olsen, J., Matthiesen, N.B., Bonefeld-Jorgensen, E.C., Bossi, R., Henriksen, T.B., 2016a. Perfluoroalkyl Acids in Maternal Serum and Indices of Fetal Growth: The Aarhus Birth Cohort. Environ Health Perspect 124, 848-854.

Bach, C.C., Vested, A., Jørgensen, K.T., Bonde, J.P.E., Henriksen, T.B., Toft, G., 2016b. Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: a systematic review. Critical reviews in toxicology 46, 735-755.

Bartell, S.M., Calafat, A.M., Lyu, C., Kato, K., Ryan, P.B., Steenland, K., 2010. Rate of Decline in Serum PFOA Concentrations After Granular Activated Carbon Filtration at Two Public Water Systems in Ohio and West Virginia. Environmental Health Perspectives 118, 222-228.

Baxter, D., 2007. Active and passive immunity, vaccine types, excipients and licensing. Occup Med (Lond) 57, 552-556.

Biegel, L.B., Hurtt, M.E., Frame, S.R., O'Connor, J.C., Cook, J.C., 2001. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicol Sci 60, 44-55.

Bijland, S., Rensen, P.C.N., Pieterman, E.J., Maas, A.C.E., van der Hoorn, J.W., Van Erk, M.J., Havekes, L.M., van Dijk, K.W., Chang, S.-C., Ehresman, D.J., Butenhoff, J.L., Princen, H.M.G., 2011. Perfluoroalkyl Sulfonates Cause Alkyl Chain Length-Dependent Hepatic Steatosis and Hypolipidemia Mainly by Impairing Lipoprotein Production in APOE*3-Leiden CETP Mice. Toxicol Sci 123, 290-303.

Bjork, J.A., Wallace, K.B., 2009. Structure-activity relationships and human relevance for perfluoroalkyl acid-induced transcriptional activation of peroxisome proliferation in liver cell cultures. Toxicol Sci 111, 89-99.

Bjork, J.A., Butenhoff, J.L., Wallace, K.B., 2011. Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. Toxicology 288, 8-17.

Boschen, K.E., Klintsova, A.Y., 2017. Neurotrophins in the Brain: Interaction With Alcohol Exposure During Development. Vitam Horm 104, 197-242.

Boskey, A.L., Coleman, R., 2010. Aging and bone. Journal of dental research 89, 1333-1348.

Buck Louis, G.M., Sundaram, R., Schisterman, E.F., Sweeney, A.M., Lynch, C.D., Gore-Langton, R.E., Maisog, J., Kim, S., Chen, Z., Barr, D.B., 2013. Persistent environmental pollutants and couple fecundity: the LIFE study. Environ Health Perspect 121, 231-236.

Butenhoff, J., Costa, G., Elcombe, C., Farrar, D., Hansen, K., Iwai, H., Jung, R., Kennedy, G., Jr., Lieder, P., Olsen, G., Thomford, P., 2002. Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. Toxicol Sci 69, 244-257.

Butenhoff, J.L., Kennedy, G.L., Jr., Frame, S.R., O'Connor, J.C., York, R.G., 2004. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology 196, 95-116.

Butenhoff, J.L., Chang, S.C., Ehresman, D.J., York, R.G., 2009a. Evaluation of Potential Reproductive and Developmental Toxicity of Potassium Perfluorohexanesulfonate in Sprague Dawley Rats. Reproductive Toxicology 27, 331-341.

Butenhoff, J.L., Ehresman, D.J., Chang, S.C., Parker, G.A., Stump, D.G., 2009b. Gestational and Lactational Exposure to Potassium Perfluoroctanesulfonate (K^+PFOS) in Rats: Developmental Neurotoxicity. Reproductive Toxicology 27, 319-330.

Butenhoff, J.L., Chang, S.C., Olsen, G.W., Thomford, P.J., 2012a. Chronic Dietary Toxicity and Carcinogenicity Study with Potassium Perfluorooctanesulfonate in Sprague Dawley Rats. Toxicology 293, 1-15.

Butenhoff, J.L., Kennedy Jr., G.L., Chang, S.-C., Olsen, G.W., 2012b. Chronic Dietary Toxicity and Carcinogenicity Study with Ammonium Perfluorooctanoate in Sprague Dawley Rats. Toxicology in press.

Butenhoff, J.L., Kennedy Jr., G.L., Chang, S.C., Olsen, G.W., 2012c. Chronic Dietary Toxicity and Carcinogenicity Study with Ammonium Perfluorooctanoate in Sprague Dawley Rats. Toxicology 298, 1-13.

Butenhoff, J.L., Pieterman, E., Ehresman, D.J., Gorman, G.S., Olsen, G.W., Chang, S.-C., Princen, H.M.G., 2012d. Distribution of Perfluorooctanesulfonate and Perfluorooctanoate into Human Plasma Lipoprotein Fractions. Toxicology Letters 210, 360-365.

Capen, C.C., 1997. Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicol Pathol 25, 39-48.

Case, M.C., York, R.G., Butenhoff, J.L., 2001. Oral (gavage) cross-fostering study of potassium perfluorooctanesulfonate (PFOS) in rats (abstract 1055). The Toxicologist 60 221-222.

Cattley, R.C., Cullen, J.M., 2013. Liver and gall bladder. In: W.M. Haschek, C.G. Rousseaux and M.A. Wallig (Eds), Toxicologic Pathology, Elsevier, New York, pp. 1509-1566.

Chang, S., Allen, B.C., Andres, K.L., Ehresman, D.J., Falvo, R., Provencher, A., Olsen, G.W., Butenhoff, J.L., 2017. Evaluation of Serum Lipid, Thyroid, and Hepatic Clinical Chemistries in Association With Serum Perfluorooctanesulfonate (PFOS) in Cynomolgus Monkeys After Oral Dosing With Potassium PFOS. Toxicol Sci 156, 387-401.

Chang, S., Butenhoff, J.L., Parker, G.A., Coder, P.S., Zitzow, J.D., Krisko, R.M., Bjork, J.A., Wallace, K.B., Seed, J.G., 2018. Reproductive and developmental toxicity of potassium perfluorohexanesulfonate in CD-1 mice. Reprod Toxicol 78, 150-168.

Chang, S.C., Ehresman, D.J., Bjork, J.A., Wallace, K., Parker, G.A., Stump, D.G., Butenhoff, J.L., 2009 Gestational and Lactational Exposure to Potassium Perfluorooctanesulfonate (K+PFOS) in Rats: toxicokinetics, thyroid hormone status, and related gene expression. Reproductive Toxicology 27, 387-399.

Chen, T., Zhang, L., Yue, J.Q., Lv, Z.Q., Xia, W., Wan, Y.J., Li, Y.Y., Xu, S.Q., 2012. Prenatal PFOS exposure induces oxidative stress and apoptosis in the lung of rat off-spring. Reprod Toxicol 33, 538-545.

Convertino, M., Church, T.R., Olsen, G.W., Liu, Y., Doyle, E., Elcomboe, C.R., Barnett, A.L., Macpherson, I.R., Evans, T.J., 2018. Stochastic Pharmacokinetic/Pharmacodynamic Modeling for Assessing the Systemic Health Risk of PFOA. Toxicol Sci 163, 293-306.

Cordoba, J., O'Riordan, K., Dupuis, J., Borensztajin, J., Blei, A., 1999. Diurnal variation of serum alanine transaminase activity in chronic liver disease. Hepatology 28, 1724-1725.

Corton, J.C., Cunningham, M.L., Hummer, B.T., Lau, C., Meek, B., Peters, J.M., Popp, J.A., Rhomberg, L., Seed, J., Klaunig, J.E., 2014. Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARalpha) as a case study. Critical reviews in toxicology 44, 1-49.

Curran, P.G., DeGroot, L.J., 1991. The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. Endocr Rev 12, 135-150.

Dalsager, L., Christensen, N., Husby, S., Kyhl, H., Nielsen, F., Høst, A., Grandjean, P., Jensen, T.K., 2016. Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1–4 years among 359 children in the Odense Child Cohort. Environment International 96, 58-64.

Darrow, L.A., Stein, C.R., Steenland, K., 2013. Serum Perfluorooctanoic Acid and Perfluorooctane Sulfonate Concentrations in Relation to Birth Outcomes in the Mid-Ohio Valley, 2005-2010. Environ Health Perspect 121, 1207-1213.

Darrow, L.A., Groth, A.C., Winquist, A., Shin, H.M., Bartell, S.M., Steenland, K., 2016. Modeled Perfluorooctanoic Acid (PFOA) Exposure and Liver Function in a Mid-Ohio Valley Community. Environ Health Perspect 124, 1227-1233.

Deb, S., Puthanveetil, P., Sakharkar, P., 2018. A Population-Based Cross-Sectional Study of the Association between Liver Enzymes and Lipid Levels. Int J Hepatol 2018, 1286170.

DeWitt, J.C., Copeland, C.B., Strynar, M.J., Luebke, R.W., 2008. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. Environ Health Perspect 116, 644-650.

DeWitt, J.C., Williams, W.C., Creech, N.J., Luebke, R.W., 2016. Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPARalpha and T- and B-cell targeting. J Immunotoxicol 13, 38-45.

Dong, G.-H., Liu, M.-M., Wang, D., Zheng, L., Liang, Z.-F., Jin, Y.-H., 2011. Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. Arch Toxicol 85, 1235-1244.

Dong, G.H., Zhang, Y.H., Zheng, L., Liu, W., Jin, Y.H., He, Q.C., 2009. Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. Arch Toxicol 83 805-815.

Dong, G.H., Tung, K.Y., Tsai, C.H., Liu, M.M., Wang, D., Liu, W., Jin, Y.H., Hsieh, W.S., Lee, Y.L., Chen, P.C., 2013. Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environ Health Perspect 121, 507-513, 513e501-508.

ECHA. 2015. Committee for Risk Assessment (RAC): Opinion on an Annex XV dossier proposing resctriction on Perfluorooctanoic acid (PFOA), its salts and PFOA-related substances. https://echa.europa.eu/documents/10162/3d13de3a-de0d-49ae-bfbd-749aea884966 ECHA/RAC/RES-O-0000006229-70-02/F.

EFSA. 2018. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Journal **16**.

Ehresman, D.J., Webb, P., Ayers, S.D., Vanden Heuvel, J., Olsen, G.W., Chang, S.C., Butenhoff, J.L., 2014. Effects of perfluoroalkyls on the activation of human CAR3, PXR, and TR receptors in vitro (Abstract 1135 occurring in The Toxicologist, supplement to Toxicological Sciences). Toxicological sciences : an official journal of the Society of Toxicology 138, 302.

Elcombe, C.R., Bell, D.R., Elias, E., Hasmall, S.C., Plant, N.J., 1996. Peroxisome proliferators: species differences in response of primary hepatocyte cultures. Annals of the New York Academy of Sciences 804, 628-635.

Elcombe, C.R., Elcombe, B.M., Foster, J.R., Farrar, D.G., Jung, R., Chang, S.C., Kennedy, G.L., Butenhoff, J.L., 2010. Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARα and CAR/PXR. Arch Toxicol 84, 787-798.

Elcombe, C.R., Elcombe, B.M., Foster, J.R., Chang, S.-C., Ehresman, D.J., Butenhoff, J.L., 2012a. Hepatocellular Hypertrophy and Cell Proliferation in Sprague-Dawley Rats from Dietary Exposure to Potassium Perfluorooctanesulfonate Results from Increased Expression of Xenosensor Nuclear Receptors PPARα and CAR/PXR. Toxicology 293, 16-29.

Elcombe, C.R., Elcombe, B.M., Foster, J.R., Chang, S.-C., Ehresman, D.J., Noker, P.E., Butenhoff, J.L., 2012b. Evaluation of Hepatic and Thyroid Responses in Male Sprague Dawley Rats for Up to Eighty-Four Days Following Seven Days of Dietary Exposure to Potassium Perfluorooctanesulfonate. Toxicology 293, 30-40.

Elcombe, C.R., Peffer, R.C., Wolf, D.C., Bailey, J., Bars, R., Bell, D., Cattley, R.C., Ferguson, S.S., Geter, D., Goetz, A., Goodman, J.I., Hester, S., Jacobs, A., Omiecinski, C.J., Schoeny, R., Xie, W., Lake, B.G., 2014. Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Critical reviews in toxicology 44, 64-82.

EMEA. 1999a. ICH Topic S 4: Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing) http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002800.pdf CPMP/ICH/300/95.

EMEA. 1999b. Committee for Veterinary Medicinal Products: Acetylsalicylic acid, sodium acetylsalicylate, acetylsalicylic acid DL-lysine, and carbasalate calcium - Summary Report (1). http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500011371.pdf EMEA/MRL/695/99-FINAL.

Fei, C., McLaughlin, J.K., Lipworth, L., Olsen, J., 2009. Maternal levels of perfluorinated chemicals and subfecundity. Human Reproduction 24, 1200-1205.

Fei, C., McLaughlin, J.K., Lipworth, L., Olsen, J., 2010a. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environmental Research 110, 773-777.

Fei, C., McLaughlin, J.K., Lipworth, L., Olsen, J., 2010b. Maternal concentrations of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) and duration of breastfeeding. Scand J Work Environ Health 36, 413-421.

Fei, C., Weinberg, C.R., Olsen, J., 2012. Commentary: perfluorinated chemicals and time to pregnancy: a link based on reverse causation? Epidemiology 23, 264-266.

Fisher, M., Arbuckle, T.E., Wade, M., Haines, D.A., 2013. Do perfluoroalkyl substances affect metabolic function and plasma lipids?-Analysis of the 2007-2009, Canadian Health Measures Survey (CHMS) Cycle 1. Environ Res 121, 95-103.

Francis, E.Z., Kimmel, C.A., Rees, D.C., 1990. Workshop on the qualitative and quantitative comparability of human and animal developmental neurotoxicity: summary and implications. Neurotoxicol Teratol 12, 285-292.

Frisbee, S.J., Shankar, A., Knox, S.S., Steenland, K., Savitz, D.A., Fletcher, T., Ducatman, A., 2010. Perfluorooctanoic Acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 health project. Archives of Pediatric and Adolescent Medicine 164, 860-869.

Fromme, H., Mosch, C., Morovitz, M., Alba-Alejandre, I., Boehmer, S., Kiranoglu, M., Faber, F., Hannibal, I., Genzel-Boroviczeny, O., Koletzko, B., Volkel, W., 2010. Pre- and Postnatal Exposure to Perfluorinated Compounds (PFCs). Environ Sci Technol 44, 7123-7129.

FSANZ. 2017. Perfluorinated chemicals in food. http://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE2086D480353CA482580C900817CDC/$File/Consoldiated-report-perflourianted-chemicals-food.pdf.

Gallo, V., Leonardi, G., Genser, B., Lopez-Espinosa, M.-J., Frisbee, S.J., Karlsson, L., Ducatman, A.M., Fletcher, J., 2012. Serum Perfluorooctanoate (PFOA) and Perfluorooctane Sulfonate (PFOS) Concentrations and Liver Function Biomarkers in a Population with Elevated PFOA Exposure. Environ Health Perspect 120, 655-660.

Giannini, E.G., Testa, R., Savarino, V., 2005. Liver enzyme alteration: a guide for clinicians. CMAJ 172, 367-379.

Gilliland, F.D., Mandel, J.S., 1996. Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins, and cholesterol: a study of occupationally exposed men. Am J Ind Med 29, 560-568.

Gleason, J.A., Post, G.B., Fagliano, J.A., 2015. Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population. Environ Res 136 8-14.

Goll, V., Alexandre, E., Viollon-Abadie, C., Nicod, L., Jaeck, D., Richert, L., 1999. Comparison of the effects of various peroxisome proliferators on peroxisomal enzyme activities, DNA synthesis, and apoptosis in rat and human hepatocyte cultures. Toxicology and applied pharmacology 160, 21-32.

Gonzalez, F.J., Shah, Y.M., 2008. PPARalpha: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. Toxicology 246, 2-8.

Gortner, E.G., Lamprecht, E.G., Case, M.T., 1980. Oral teratology study of FM-3422 in rats. Safety Evaluation Laboratory - Riker Laboratories, Inc St.Paul, MN, pp. 162-174.

Gortner, E.G., 1981. Oral teratology study of T-2998CoC in rats. Experiment Number 0681TR0110. Safety Evaluation Laboratory and Riker Laboratories, Inc., St. Paul, MN. USEPA Public Docket, AR-226-0463.

Gortner, E.G., 1982. Oral teratology study of T-3141CoC in rabbits. Experiment Number 0681TB0398. Safety Evaluation Laboratory and Riker Laboratories, Inc., St. Paul, MN. USEPA Public Docket AR-226-0465.

Grandjean, P., Andersen, E.W., Budtz-Jorgensen, E., Nielsen, F., Molbak, K., Weihe, P., Heilmann, C., 2012. Serum Vaccine Antibody Concentrations in Children Exposed to Perfluorinated Compounds. JAMA 307, 391-397.

Grandjean, P., Heilmann, C., Weihe, P., Nielsen, F., Mogensen, U.B., Timmermann, A., Budtz-Jorgensen, E., 2017. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. J Immunotoxicol 14, 188-195.

Granum, B., Haug, L.S., Namork, E., Stolevik, S.B., Thomsen, C., Aaberge, I.S., van Loveren, H., Lovik, M., Nygaard, U.C., 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J Immunotoxicol 10, 373-379.

Grasty, R.C., Bjork, J.A., Wallace, K.B., Wolf, D.C., Lau, C.S., Rogers, J.M., 2005. Effects of prenatal perfluorooctane sulfonate (PFOS) exposure on lung maturation in the perinatal rat. Birth Defects Res B Dev Reprod Toxicol 74, 405-416.

Guruge, K.S., Hikono, H., Shimada, N., Murakami, K., Hasegawa, J., Yeung, L.W., Yamanaka, N., Yamashita, N., 2009. Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice. J Toxicol Sci 34, 687-691.

Hall, A.P., Elcombe, C.R., Foster, J.R., Harada, T., Kaufmann, W., Knippel, A., Kuttler, K., Malarkey, D.E., Maronpot, R.R., Nishikawa, A., Nolte, T., Schulte, A., Strauss, V., York, M.J., 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop. Toxicol Pathol 40, 971-994.

Harrison, N.L., Skelly, M.J., Grosserode, E.K., Lowes, D.C., Zeric, T., Phister, S., Salling, M.C., 2017. Effects of acute alcohol on excitability in the CNS. Neuropharmacology 122, 36-45.

Hirose, Y., Nagahori, H., Yamada, T., Deguchi, Y., Tomigahara, Y., Nishioka, K., Uwagawa, S., Kawamura, S., Isobe, N., Lake, B.G., Okuno, Y., 2009. Comparison of the effects of the synthetic pyrethroid Metofluthrin and phenobarbital on CYP2B form induction and replicative DNA synthesis in cultured rat and human hepatocytes. Toxicology 258, 64-69.

Humblet, O., Diaz-Ramirez, L.G., Balmes, J.R., Pinney, S.M., Hiatt, R.A., 2014. Perfluoroalkyl Chemicals and Asthma among Children 12-19 Years of Age: NHANES (1999-2008). Environ Health Perspect 122, 1129-1133.

Impinen, A., Nygaard, U.C., Lodrup Carlsen, K.C., Mowinckel, P., Carlsen, K.H., Haug, L.S., Granum, B., 2018. Prenatal exposure to perfluoralkyl substances (PFASs) associated with

respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environ Res 160, 518-523.

Innocenzi, P., Malfatti, L., Costacurta, S., Kidchob, T., Piccinini, M., Marcelli, A., 2008. Evaporation of ethanol and ethanol-water mixtures studied by time-resolved infrared spectroscopy. J Phys Chem A 112, 6512-6516.

Iwaniec, U.T., Turner, R.T., 2016. Influence of body weight on bone mass, architecture and turnover. J Endocrinol 230, R115-130.

Jepsen, K.J., 2009. Systems analysis of bone. Wiley Interdiscip Rev Syst Biol Med 1, 73-88.

Johnson, P.I., Sutton, P., Atchley, D.S., Koustas, E., Lam, J., Sen, S., Robinson, K.A., Axelrad, D.A., Woodruff, T.J., 2014. The Navigation Guide-Evidence-Based Medicine Meets Environmental Health: Systematic Review of Human Evidence for PFOA Effects on Fetal Growth. Environ Health Perspect 122, 1028-1039.

Jorgensen, K.T., Specht, I.O., Lenters, V., Bach, C.C., Rylander, L., Jonsson, B.A., Lindh, C.H., Giwercman, A., Heederik, D., Toft, G., Bonde, J.P., 2014. Perfluoroalkyl substances and time to pregnancy in couples from Greenland, Poland and Ukraine. Environ Health 13, 116.

Kennedy, G.L., Jr., Butenhoff, J.L., Olsen, G.W., O'Connor, J.C., Seacat, A.M., Perkins, R.G., Biegel, L.B., Murphy, S.R., Farrar, D.G., 2004. The toxicology of perfluorooctanoate. Critical reviews in toxicology 34, 351-384.

Kielsen, K., Shamim, Z., Ryder, L.P., Nielsen, F., Grandjean, P., Budtz-Jorgensen, E., Heilmann, C., 2016. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. J Immunotoxicol 13, 270-273.

Kim, S., Choi, K., Ji, K., Seo, J., Kho, Y., Park, J., Kim, S., Park, S., Hwang, I., Jeon, J., Yang, H., Giesy, J.P., 2011. Trans-Placental Transfer of Thirteen Perfluorinated Compounds and Relations with Fetal Thyroid Hormones. Environ Sci Technol 45, 7465-7472.

Kim, W.R., Flamm, S.L., Di Bisceglie, A.M., Bodenheimer, H.C., Public Policy Committee of the American Association for the Study of Liver, D., 2008. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 47, 1363-1370.

Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A., 2003. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Critical reviews in toxicology 33, 655-780.

Klaunig, J.E., Hocevar, B.A., Kamendulis, L.M., 2012. Mode of Action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and Human Relevance. Reprod Toxicol 33, 410-418.

Koskela, A., Finnilä, M.A., Korkalainen, M., Spulber, S., Koponen, J., Håkansson, H., Tuukkanen, J., Viluksela, M., 2016. Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. Toxicology and Applied Pharmacology 301, 14-21.

Lake, B.G., 2009. Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumour formation. Xenobiotica 39, 582-596.

Lam, J., Koustas, E., Sutton, P., Johnson, P.I., Atchley, D.S., Sen, S., Robinson, K.A., Axelrad, D.A., Woodruff, T.J., 2014. The Navigation Guide-Evidence-Based Medicine Meets Environmental Health: Integration of Animal and Human Evidence for PFOA Effects on Fetal Growth. Environ Health Perspect 122, 1040-1051.

Lau, C., Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Stanton, M.E., Butenhoff, J.L., Stevenson, L.A., 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol Sci 74, 382-392.

Lau, C., Butenhoff, J.L., Rogers, J.M., 2004. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicol Appl Pharmacol 198, 231-241.

Lau, C., Thibodeaux, J.R., Hanson, R.G., Narotsky, M.G., Rogers, J.M., Lindstrom, A.B., Strynar, M.J., 2006. Effects of Perfluorooctanoic Acid Exposure during Pregnancy in the Mouse. Toxicol Sci 90, 510-518.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl Acids: A Review of Monitoring and Toxicological Findings. Tox Sci 99, 366-394.

Lau, C., 2012. Perfluoroalkyl acids: Recent research highlights. Reprod Toxicol 33, 405-409.

Lee, E.-S., Han, S., Oh, J.-E., 2016. Association between perfluorinated compound concentrations in cord serum and birth weight using multiple regression models. Reproductive Toxicology 59, 53-59.

Lenters, V., Portengen, L., Rignell-Hydbom, A., Jonsson, B.A., Lindh, C.H., Piersma, A.H., Toft, G., Bonde, J.P., Heederik, D., Rylander, L., Vermeulen, R., 2016. Prenatal Phthalate, Perfluoroalkyl Acid, and Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models Based on Elastic Net Regression. Environ Health Perspect 124, 365-372.

Leonard, R.C., Kreckmann, K.H., Sakr, C.J., Symons, J.M., 2008. Retrospective cohort mortality study of workers in a polymer production plant including a reference population of regional workers. Ann Epidemiol 18, 15-22.

Lewis, R.C., Johns, L.E., Meeker, J.D., 2015. Serum Biomarkers of Exposure to Perfluoroalkyl Substances in Relation to Serum Testosterone and Measures of Thyroid Function among

Adults and Adolescents from NHANES 2011-2012. Int J Environ Res Public Health 12, 6098-6114.

Li, Y., Fletcher, T., Mucs, D., Scott, K., Lindh, C.H., Tallving, P., Jakobsson, K., 2018. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occup Environ Med 75, 46-51.

Lin, C.Y., Lin, L.Y., Chiang, C.K., Wang, W.J., Su, Y.N., Hung, K.Y., Chen, P.C., 2010. Investigation of the Associations Between Low-Dose Serum Perfluorinated Chemicals and Liver Enzymes in US Adults. American Journal of Gastroenterology 105, 1354-1363.

Looker, C., Luster, M.I., Calafat, A.M., Johnson, V.J., Burleson, G.R., Burleson, F.G., Fletcher, T., 2014. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol Sci 138, 76-88.

Luebker, D.J., Case, M.T., York, R.G., Moore, J.A., Hansen, K.J., Butenhoff, J.L., 2005a. Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicology 215, 126-148.

Luebker, D.J., York, R.G., Hansen, K.J., Moore, J.A., Butenhoff, J.L., 2005b. Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharmacokinetic parameters. Toxicology 215, 149-169.

MacPherson, I.R., Bissett, D., Petty, R.D., Tait, B., Samuel, L.M., MacDonals, J., Smith, M., Birse-Archbold, J.A., Barnett, A.L., Wolf, C.R., Elcombe, C.R., Jeynes-Ellis, A., Evans, T.R.J., 2011. A first-in-human phase I clinical trial of CXR1002 in patients (pts) with advanced cancer. J Clin Oncol 29.

Macpherson, M., Bissett, D., Tait, B., Samuel, L.M., MacDonald, J., Barnett, A.L., Wolf, C.R., Elcombe, C.R., Jeynes-Ellis, A., Evans, T.R.J., 2010. 391 A phase I clinical trial of CXR1002 in patients (pts) with advanced cancer. EJC Supplements 8, 124-124.

Mogensen, U.B., Grandjean, P., Heilmann, C., Nielsen, F., Weihe, P., Budtz-Jorgensen, E., 2015. Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. Environ Health 14, 47.

Morken, N.-H., Travios, G.S., Wilson, R.E., Eggesbe, M., Longnecker, M.P., 2014. Maternal Glomerular Filtration Rate in Pregnancy and Fetal Size. PLos One 9, e101897.

Mundt, D.J., Mundt, K.A., Luippold, R.S., Schmidt, M.D., Farr, C.H., 2007. Clinical epidemiological study of employees exposed to surfactant blend containing perfluorononanoic acid. Occup Environ Med 64, 589-594.

Negri, E., Metruccio, F., Guercio, V., Tosti, L., Benfenati, E., Bonzi, R., La Vecchia, C., Moretto, A., 2017. Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological data. Critical reviews in toxicology 47, 482-508.

Nolan, L.A., Nolan, J.M., Shofer, F.S., Rodway, N.V., Emmett, E.A., 2009. The Relationship Between Birth Weight, Gestational Age and Perfluorooctanoic Acid (PFOA)-Contaminated Public Drinking Water. Reprod Toxicol 27, 231-238.

Nolan, L.A., Nolan, J.M., Shofer, F.S., Rodway, N.V., Emmett, E.A., 2010. Congenital Anomalies, Labor/Delivery Complications, Maternal Risk Factors and Their Relationship with Perfluorooctanoic Acid (PFOA)-Contaminated Public Drinking Water. Repro Toxicol 29, 147-155.

NTP. 2016. Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). In: N.T. Program (Ed), Research Triangle Park, NC.

OECD. 2007. OECD Guide for Testing of Chemicals No. 426: Developmental Neurotoxicity Study. https://doi.org/10.1787/20745788.

OECD. 2016. OECD Guide for Testing of Chemicals No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. http://dx.doi.org/10.1787/9789264264403-en, Accessed on July 17, 2017.

Okada, E., Sasaki, S., Kashino, I., Matsuura, H., Miyashita, C., Kobayashi, S., Itoh, K., Ikeno, T., Tamakoshi, A., Kishi, R., 2014. Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. Environ Int 65, 127-134.

Olsen, G.W., Burris, J.M., Mandel, J.H., Zobel, L.R., 1999. Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. J Occup Environ Med 41, 799-806.

Olsen, G.W., Burris, J.M., Burlew, M.M., Mandel, J.H., 2000. Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. Drug Chem Toxicol 23, 603-620.

Olsen, G.W., Burris, J.M., Burlew, M.M., Mandel, J.H., 2003. Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. J Occup Environ Med 45, 260-270.

Olsen, G.W., Zobel, L.R., 2007. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. International archives of occupational and environmental health 81, 231-246.

Olsen, G.W., Butenhoff, J.L., Zobel, L.R., 2009. Perfluoroalkyl chemicals and human fetal development: an epidemiologic review with clinical and toxicological perspectives. Reprod Toxicol 27, 212-230.

Olsen, G.W., Ehresman, D.J., Buehrer, B.D., Gibson, B.A., Butenhoff, J.L., Zobel, L.R., 2012. Longitudinal Assessment of Lipid and Hepatic Clinical Parameters in Workers Involved With the Demolition of Perfluoroalkyl Manufacturing Facilities. J Occup Environ Med 54, 974-983.

Onishchenko, N., Fischer, C., Ibrahim, W., Negri, S., Spulber, S., Cottica, D., Ceccatelli, S., 2011. Prenatal Exposure to PFOS or PFOA Alters Motor Function in Mice in a Sex-Realted Manner. Neurotox Res 19, 452-461.

Oppenheimer, J.H., Schwartz, A.L., Strait, K.A., 1995. An integrated view of thyroid hormone actions *in vivo*. In: B.D. Weintraub (Ed), Molecular Endocrinology: Basic Concepts and Clinical Correlations, Raven Press, Ltd., New York, pp. 249-265.

Parzefall, W., Erber, E., Sedivy, R., Schulte-Hermann, R., 1991. Testing for induction of DNA synthesis in human hepatocyte primary cultures by rat liver tumor promoters. Cancer Res 51, 1143-1147.

Peden-Adams, M.M., Keller, J.M., Eudaly, J.G., Berger, J., Gilkeson, G.S., Keil, D.E., 2008. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. Toxicol Sci 104, 144-154.

Perrone, C.E., Shao, L., Williams, G.M., 1998. Effect of rodent hepatocarcinogenic peroxisome proliferators on fatty acyl-CoA oxidase, DNA synthesis, and apoptosis in cultured human and rat hepatocytes. Toxicology and applied pharmacology 150, 277-286.

PFAS Expert Health Panel. 2018. Australia PFAS Expert Health Panel - Report to the Minister. http://www.health.gov.au/internet/main/publishing.nsf/Content/C9734ED9736BE9238EC9730CA2581BD00052C00003/$File/expert-panel-report.pdf.

Raleigh, K.K., Alexander, B.H., Olsen, G.W., Ramachandran, G., Morey, S.Z., Church, T.R., Logan, P.W., Scott, L.L., Allen, E.M., 2014. Mortality and cancer incidence in ammonium perfluorooctanoate production workers. Occup Environ Med 71, 500-506.

Robledo, C.A., Yeung, E., Mendola, P., Sundaram, R., Maisog, J., Sweeney, A.M., Barr, D.B., Louis, G.M., 2015. Preconception Maternal and Paternal Exposure to Persistent Organic Pollutants and Birth Size: The LIFE Study. Environ Health Perspect 123, 88-94.

Romano, M.E., Xu, Y., Calafat, A.M., Yolton, K., Chen, A., Webster, G.M., Eliot, M.N., Howard, C.R., Lanphear, B.P., Braun, J.M., 2016. Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. Environmental Research 149, 239-246.

Rosen, M.B., Schmid, J.E., Das, K.P., Wood, C.R., Zehr, R.D., Lau, C., 2009. Gene Expression Profiling in the Liver and Lung of Perfluorooctane Sulfonate-Exposed Mouse Fetuses: Comparison to Changes Induced by Exposure to Perfluorooctanoic Acid. Reproductive Toxicology 27, 278-288.

Rosen, M.B., Schmid, J.E., Corton, J.C., Zehr, R.D., Das, K.P., Lau, C., 2010. Gene Expression Profiling in Wild-Type and PPARα-Null Mice Exposed to Perfluorooctane Sulfonate Reveals PPARα-Independent Effects. PPAR Research 2010, 1-23.

Ross, J., Plummer, S.M., Rode, A., Scheer, N., Bower, C.C., Vogel, O., Henderson, C.J., Wolf, C.R., Elcombe, C.R., 2010. Human constitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR) Support the Hypertrophic but not the Hyperplastic Response to the Murine Nongentoxic Hepatocarcinogens Phenobarbital and Chlordane *In Vivo*. Toxicol Sci 116, 452466.

Roy, A., Pahan, K., 2009. Gemfibrozil, stretching arms beyond lipid lowering. Immunopharmacol Immunotoxicol 31, 339-351.

Ryffel, B., Mihatsch, M.J., 1986. Cyclosporine nephrotoxicity. Toxicol Pathol 14, 73-82.

Sakr, C.J., Kreckmann, K.H., Green, J.W., Gillies, P.J., Reynolds, J.L., Leonard, R.C., 2007a. Cross-Sectional Study of Lipids and Liver Enzymes Related to a Serum Biomarker of Exposure (ammonium perfluorooctanoate or APFO) as Part of a General Health Survey in a Cohort of Occupationally Exposed Workers. J Occup Environ Med 49, 1086-1096.

Sakr, C.J., Leonard, R.C., Kreckmann, K.H., Slade, M.D., Cullen, M.R., 2007b. Longitudinal study of serum lipids and liver enzymes in workers with occupational exposure to ammonium perfluorooctanoate. J Occup Environ Med 49, 872-879.

Savitz, D.A., Stein, C.R., Bartell, S.M., Elston, B., Gong, J., Shin, H.-M., Wellenius, G.A., 2012a. Perfluorooctanoic Acid Exposure and Pregnancy Outcome in a Highly Exposed Community. Epidemiology 23, 386-392.

Savitz, D.A., Stein, C.R., Elston, B., Wellenius, G.A., Bartell, S.M., Shin, H.-M., Vieira, V.M., Fletcher, T., 2012b. Relationship of Perfluorooctanoic Acid Exposure to Pregnancy Outcome Based on Birth Records in the Mid-Ohio Valley. Environ Health Perspect 120, 1201-1207.

Seacat, A.M., Thomford, P.J., Hansen, K.J., Olsen, G.W., Case, M.T., Butenhoff, J.L., 2002. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. Toxicol Sci 68, 249-264.

Seacat, A.M., Thomford, P.J., Hansen, K.J., Clemen, L.A., Eldridge, S.R., Elcombe, C.R., Butenhoff, J.L., 2003. Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. Toxicology 183, 117-131.

Smit, L.A., Lenters, V., Hoyer, B.B., Lindh, C.H., Pedersen, H.S., Liermontova, I., Jonsson, B.A., Piersma, A.H., Bonde, J.P., Toft, G., Vermeulen, R., Heederik, D., 2015. Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. Allergy 70, 653-660.

Sobus, J.R., DeWoskin, R.S., Tan, Y.M., Pleil, J.D., Phillips, M.B., George, B.J., Christensen, K., Schreinemachers, D.M., Williams, M.A., Hubal, E.A., Edwards, S.W., 2015. Uses of NHANES Biomarker Data for Chemical Risk Assessment: Trends, Challenges, and Opportunities. Environ Health Perspect 123, 919-927.

Staples, R.E., Burgess, B.A., Kerns, W.D., 1984. The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. Fundam Appl Toxicol 4, 429-440.

Starling, A.P., Engel, S.M., Richardson, D.B., Baird, D.D., Haug, L.S., Stuebe, A.M., Klungsoyr, K., Harmon, Q., Becher, G., Thomsen, C., Sabaredzovic, A., Eggesbo, M., Hoppin, J.A., Travlos, G.S., Wilson, R.E., Trogstad, L.I., Magnus, P., Longnecker, M.P., 2014. Perfluoroalkyl substances during pregnancy and validated preeclampsia among nulliparous women in the Norwegian Mother and Child Cohort Study. Am J Epidemiol 179, 824-833.

Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., Vaccarino, V., 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am J Epidemiol 170, 1268-1278.

Steenland, K., Tinker, S., Shankar, A., Ducatman, A., 2010. Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctanesulfonate (PFOS) with Uric Acid Among Adults with Elevated Community Exposure to PFOA. Environmental Health Perspectives 118, 229-233.

Steenland, K., Woskie, S., 2012. Cohort mortality study of workers exposed to perfluorooctanoic acid. Am J Epidemiol 176, 909-917.

Steenland, K., Savitz, D.A., Fletcher, T., 2014. Commentary: class action lawsuits: can they advance epidemiologic research? Epidemiology 25, 167-169.

Steenland, K., Zhao, L., Winquist, A., 2015. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). Occup Environ Med 72, 373-380.

Steenland, K., Barry, V., Savitz, D., 2018. Serum perfluorooctanoic acid (PFOA) and birthweight: an updated meta-analysis with bias analysis. Epidemiology.

Stein, C.R., Savitz, D.A., Dougan, M., 2009. Serum Levels of Perfluorooctanoic Acid and Perfluorooctane Sulfonate and Pregnancy Outcome. Am J Epidemiol 170, 837-846.

Stein, C.R., Ge, Y., Wolff, M.S., Ye, X., Calafat, A.M., Kraus, T., Moran, T.M., 2016a. Perfluoroalkyl substance serum concentrations and immune response to FluMist vaccination among healthy adults. Environmental Research 149, 171-178.

Stein, C.R., McGovern, K.J., Pajak, A.M., Maglione, P.J., Wolff, M.S., 2016b. Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. Pediatric research 79, 348-357.

Stuart, J.J., Bairey Merz, C.N., Berga, S.L., Miller, V.M., Ouyang, P., Shufelt, C.L., Steiner, M., Wenger, N.K., Rich-Edwards, J.W., 2013. Maternal recall of hypertensive disorders in pregnancy: a systematic review. J Womens Health (Larchmt) 22, 37-47.

Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Barbee, B.D., Richards, J.H., Butenhoff, J.L., Stevenson, L.A., Lau, C., 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. Toxicol Sci 74, 369-381.

Timmermann, C.A., Budtz-Jorgensen, E., Jensen, T.K., Osuna, C.E., Petersen, M.S., Steuerwald, U., Nielsen, F., Poulsen, L.K., Weihe, P., Grandjean, P., 2017. Association between perfluoroalkyl substance exposure and asthma and allergic disease in children as modified by MMR vaccination. J Immunotoxicol 14, 39-49.

Timmermann, C.A.G., Budtz-Jørgensen, E., Petersen, M.S., Weihe, P., Steuerwald, U., Nielsen, F., Jensen, T.K., Grandjean, P., 2016. Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl substances. Reproductive Toxicology in press.

Toren, K., Brisman, J., Jarvholm, B., 1993. Asthma and asthma-like symptoms in adults assessed by questionnaires. A literature review. Chest 104, 600-608.

USEPA. 1998. Guidelines for neurotoxicity risk assessment. Federal Register 63, 26926-26954.

USEPA. 2016a. Drinking Water Health Advisory for Perfluorooctane Sulfonate (PFOS). EPA Document Number: 822-R-16-004 https://www.epa.gov/sites/production/files/2016-2005/documents/pfos_health_advisory_final_2508.pdf.

USEPA. 2016b. Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA). EPA Document Number: 822-R-16-005, https://www.epa.gov/sites/production/files/2016-2005/documents/pfoa_health_advisory_final_2508.pdf.

Vanden Heuvel, J.P., Thompson, J.T., Frame, S.R., Gillies, P.J., 2006. Differential Activation of Nuclear Receptors by Perfluorinated Fatty Acid Analogs and Natural Fatty Acids: A Comparison of Human, Mouse, and Rat Peroxisome Proliferator-Activated Receptor-α, -β, and -γ, Liver X Receptor-β, and Retinoid X Receptor-α. Toxicological Sciences 92, 476-489.

Velez, M.P., Arbuckle, T.E., Fraser, W.D., 2015. Maternal exposure to perfluorinated chemicals and reduced fecundity: the MIREC study. Hum Reprod 30, 701-709.

Verner, M.A., Loccisano, A.E., Morken, N.H., Yoon, M., Wu, H., McDougall, R., Maisonet, M., Marcus, M., Kishi, R., Miyashita, C., Chen, M.H., Hsieh, W.S., Andersen, M.E., Clewell, H.J., Longnecker, M.P., 2015. Associations of Perfluoroalkyl Substances (PFAS) with Lower Birth Weight: An Evaluation of Potential Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model (PBPK). Environ Health Perspect 123, 1317-1324.

Vestergaard, S., Nielsen, F., Andersson, A.-M., Hjollund, N.H., Grandjean, P., Andersen, H.R., Jensen, T.K., 2012. Association between perfluorinated compounds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive. Human Reproduction 27, 873-880.

Vesterinen, H.M., Johnson, P.I., Atchley, D.S., Sutton, P., Lam, J., Zlatnik, M.G., Sen, S., Woodruff, T.J., 2015. Fetal growth and maternal glomerular filtration rate: a systematic review. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet 28, 2176-2181.

Wambaugh, J.F., Setzer, R.W., Pitruzzello, A.M., Liu, J., Reif, D.M., Kleinstreuer, N.C., Wang, N.C., Sipes, N., Martin, M., Das, K., Dewitt, J.C., Strynar, M., Judson, R., Houck, K.A., Lau, C., 2013. Dosimetric anchoring of in vivo and in vitro studies for perfluorooctanoate and perfluorooctanesulfonate. Toxicol Sci 136, 308-327.

Washino, N., Saijo, Y., Sasaki, S., Kato, S., Ban, S., Konishi, K., Ito, R., Nakata, A., Iwasaki, Y., Saito, K., Nakazawa, H., Kishi, R., 2009. Correlations Between Prenatal Exposure to Perfluorinated Chemicals and Reduced Fetal Growth. Environ Health Perspect 117, 660-667.

White, S.S., Calafat, A.M., Kuklenyik, Z., Villanueva, L., Zehr, R.D., Helfant, L., Strynar, M.J., Lindstrom, A.B., Thibodeaux, J.R., Wood, C., Fenton, S.E., 2007. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. Toxicol Sci 96, 133-144.

White, S.S., Kato, K., Jia, L.T., Basden, B.J., Calafat, A.M., Hines, E.P., Stanko, J.P., Wolf, C.J., Abbott, B.D., Fenton, S.E., 2009. Effects of Perfluorooctanoic Acid on Mouse Mammary Gland Development and Differentiation Resulting from Cross-Foster and Restricted Gestational Exposures. Reprod Toxicol 27, 289-298.

Whitworth, K.W., Haug, L.S., Baird, D.D., Becher, G., Hoppin, J.A., Skjaerven, R., Thomsen, C., Eggesbo, M., Travlos, G., Wilson, R., Cupul-Uicab, L.A., Brantsaeter, A.L., Longnecker, M.P., 2012. Perfluorinated Compounds in Relation to Birth Weight in the Norwegian Mother and Child Cohort Study. Am J Epidemiol 175, 1209-1216.

Whitworth, K.W., Haug, L.S., Sabaredzovic, A., Eggesbo, M., Longnecker, M.P., 2016. Plasma concentrations of perfluorooctane sulfonamide (PFOSA) and time to pregnancy among primiparous women. Epidemiology 27, 712-715.

Winquist, A., Steenland, K., 2014. Perfluorooctanoic Acid Exposure and Thyroid Disease in Community and Worker Cohorts. Epidemiology 25, 255-264.

Wolf, C.J., Fenton, S.E., Schmid, J.E., Calafat, A.M., Kuklenyik, Z., Bryant, X.A., Thibodeaux, J., Das, K.P., White, S.S., Lau, C.S., Abbott, B.D., 2007. Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. Toxicol Sci 95, 462-473.

Wu, K., Xu, X., Peng, L., Liu, J., Guo, Y., Huo, X., 2012. Association between maternal exposure to perfluorooctanoic acid (PFOA) from electronic waste recycling and neonatal health outcomes. Environment International 48, 1-8.

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

Zhu, Y., Qin, X.-D., Zeng, X.-W., Paul, G., Morawska, L., Su, M.-W., Tsai, C.-H., Wang, S.-Q., Lee, Y.L., Dong, G.-H., 2016. Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: By gender and asthma status. Science of The Total Environment 559, 166-173.

Executive summary

The 3M Company (3M) appreciates the opportunity to review and comment on the EFSA's draft scientific opinion on the "Risk to human health related to the presence of perfluoroalkyl substances in food." Given the myriad of scientific literature that has become available, we offer the following comments.

Taking the critical concepts for hazard characterization and risk assessment developed over the years in the evaluation of research studies, it is imperative to apply a weight of evidence approach for dose response, consistencies across studies and species, and known biological plausibility.

The EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) has previously performed risk assessments for PFOA and PFOS in 2008 and 2012. Based on the experimental toxicology data from laboratory animals actually exposed to high doses of PFOA and/or PFOS, the previous CONTAM panels concluded that "it is unlikely that adverse effects of PFOS or PFOA are occurring in the general population." The total daily intake (TDI) set by EFSA in 2008 and 2012 were the identical 1500 ng/kg b.w. and 150 ng/kg b.w. for PFOA and PFOS, respectively.

The provisional assessments released by the next EFSA CONTAM Panel in 2018 for PFOA and PFOS were much lower. The TDI for PFOA and PFOS were 0.8 ng/kg b.w. and 1.8 ng/kg b.w., respectively (or 6 ng/kg b.w. and 13 ng/kg b.w. if expressed as TWI). These values were not determined by the experimental animal data; rather, they were based on statistical associations gathered from cross-sectional epidemiological survey data. These epidemiological cross-sectional designs can only demonstrate statistical correlations but cannot be used as a proof of causation (i.e., it cannot determine if exposure to compound X causes effect Y).

The EFSA CONTAM Panel (2018) selected several cross-sectional epidemiological studies that reported positive associations between serum cholesterol and PFOA / PFOS; and the TWI values were derived from these results. These observational epidemiological findings, however, were in contrast to data obtained from (evidence-based) toxicological studies including: 1) studies with PFOA and PFOS using genetically engineered APOE*3-Lieden.CETP mouse model that mimics human lipoprotein metabolism; 2) a study where PFOS was administered to non-human primates with repeated cholesterol measurements made for about one year; and 3) a phase 1 clinical trial in humans where PFOA was administered as a chemotherapeutic in participants who had failed standard therapy. All of these studies showed decreased blood cholesterol when exposed to high doses of PFOA and PFOS at approximately 750000 - 100000 ng/mL or higher in the blood. In addition, according to EFSA, based on the hypothesis that PFOS and PFOA can affect CYP7A1 activity (the rate-limiting step in the metabolism of cholesterol in the hepatocyte to primary bile acids), enterohepatic circulation of bile salts and PFOS/PFOA concomitantly may result in a negative feedback regulation leading to a positive association between serum total cholesterol and PFOS/PFOA levels. In particular, it is the latter point that the current EFSA CONTAM Panel (2020) has acknowledged the uncertainty regarding its cholesterol assessment to be larger than what was assumed in 2018.

This change in opinion shifted the 2020 EFSA's CONTAM Panel's attention to calculate a TDI based on immunotoxicity. The critical effect study, however, remained, yet again, to be a cross-sectional epidemiological study (Abraham et al. 2020). This study was conducted more than 20 years ago and is much smaller than what was used for the cross-sectional cholesterol-related studies considered by EFSA CONTAM Panel in 2018. Abraham et al. evaluated 101 one-year old children in Germany between 1997 -1999 to assess tetanus and *Haemophilus influenza* type b (Hib) titers in relation to dioxins, PCBs, 'old' pesticides, and heavy metals. As a consequence of the CONTAM Panel provisional scientific opinion released in 2018, Abraham et al. then analyzed in 2019 the two remaining stored frozen (-80˚C) serum aliquots for diphtheria titers and ~~several~~ four PFAS compounds (PFOA, PFOS, PFHxS, and PFNA). The mean serum concentrations were 16.8 and 15.2 ng/mL for PFOA and PFOS for breast-fed babies and 3.8 and 6.8 ng/mL for the formula-fed babies, respectively. Abraham et al. reported a statistically significant association of reduced titers to 3 vaccines (*Haemophilus influenza* type b, tetanus, and diphtheria) and measured serum PFOA concentrations but there were no significant associations with serum PFOS, PFHxS, or PFNA concentrations. Nor was there any evidence of increased risk of infections related to any of these perfluoroalkyl measurements in these children. The CONTAM Panel obtained the access to the individual Abraham et al. data to do their own "mixture approach" analysis of the four perfluoroalkyls and they calculated a combined NOAEC of 31.9 ng/mL (summed for PFOA, PFOS, PFHxS, and PFNA). Using PBPK models, long-term daily maternal exposure was estimated to be 1.16 ng/kg b.w. (or 8 ng/kg b.w. per week). The CONTAM Panel (2020) did not analyze the other three perfluoroalkyls individually.

There are uncertainties in the analyses and estimation of this newly proposed TDI – many of which the CONTAM Panel (2020) acknowledged. Food data were primarily submitted by only 3 countries with 92% of the results reported below LOD/LOQ. Although upper bound expoauew estimates were available, the better scientific decision was to use lower bound estimates. Biomonitoring and food concentrations of PFAS continue to decline The conclusion of an adverse effect is based on reduced antibody response related to only PFOA exposure in the critical study from Abraham et al. with no evidence that there was an increased risk of infection, of any type, in these one-year old children. The CONTAM Panel conducted the PBPK models on grouped, not continuous data. There are several technical issues with EFSA's statistical approach which reflect faulty decision-making or implementation. These issues have resulted in the flawed No Observable Adverse Effect Concentration (NOAEC) of 31.9 ng/mL. The following points highlight these concerns:

1) The reported exposure data (*i.e.*, serum PFAS concentrations) should not have been arbitrarily assigned into groups (as quintiles and deciles). The exposure-response data are observational in nature and they cannot be considered as treatment groups.

2) The determination of the NOAEC based on NOAEL/LOAEL is unacceptable and problematic. This method is not robust and it does not account for the variability and uncertainty of the data resulting from the study design itself. A better approach would be use of benchmark dose methodology that EFSA has routinely used (including for its 2018 draft opinion on PFOS and PFOA) or even of a more sophisticated Bayesian analysis.

3) Albeit inappropriate in this particular case, a dose-response analysis is generally only valid for endpoint(s) that demonstrated clear evidence of a relationship between dose and response. Not only were the analyses described in the current draft opinion inappropriate, the p-values currently presented in Table K.1 appear to be incorrect. When the dose and response data were reanalyzed, the results did not support a concentration-dependent effect on Hib titer. This contradicts EFSA's conclusion that there was an "inverse association between serum levels of the sum of these 4 PFASs and antibody titers against *Haemophilus Influenza* type b (Hib)" (cf. page 7 of the draft scientific opinion).

4) The statistical approach based on the summation of the four PFAS compounds is not justified. When analyzing the data using an appropriate method by treating each compound separately, they would not have the same coefficients. EFSA's summation approach assumes that they do. Hence summation of the four PFAS compounds is an inaccurate approach and constrained the overall data interpretation. Furthermore, summing and applying equal potency for PFOA, PFOS, PFHxS, and PFNA is not supported by science.

To date, the available data on the PFOS and PFOA immunotoxicity studies have not demonstrated concordance and, the immune-related data for PFHxS and PFNA are even more limited. The critical laboratory animal study cited by EFSA (Peden-Adams et al., 2008) does not provide sufficient evidence for PFOS-related immune suppression; and the other epidemiological data cited do not support reduced resistance (via titer measurement) with tetanus for either PFOA or PFOS and they provide imprecise reduced antibody estimates for diphtheria. Collectively, the epidemiologic data do not suggest an increase propensity for infection. Given that EFSA has acknowledged that "a clear mode of action of immunotoxicity by PFOS and PFOA has not been established" (c.f. section 3.3.5.4), it is unclear how these inconsistent findings from the animal and human studies can be used by EFSA to develop a guidance value. Thus, the TDI proposed by the EFSA CONTAM Panel (2020) should only be considered, at best, **provisional.**

Provided below are our detailed comments which we trust these scientific emphases will be taken into consideration by EFSA with the final assessment.

2020 EFSA draft scientific opinion PFOA, PFOS, PFHxS, and PFNA TWI

EFSA CONTAM Panel proposed a tolerable weekly intake (TWI) of 8 ng/kg/bw for the sum of four perfluoroalkyl substances: PFOA, PFOS, PFHxS, and PFNA. This value was derived based on data obtained by EFSA from data reported by Abraham et al. (2020), which was a cross-sectional study of 101 healthy 1-year old children conducted more than 20 years ago. The study reported statistically significantly negative associations between serum levels of PFOA measured in the children and their vaccine antibody responses for Hib, tetanus, and diphtheria. Abraham et al. reported no statistically significant associations between reduced antibody responses and serum levels of PFOS, PFHxS, or PFNA.

Selection of the critical study (Abraham et al. 2020)

Between June 1997 and May 1999, a total of 101 children living in Berlin were examined, including obtaining their blood samples, in a study whose primary purpose was to examine deterimine persistent organic pollutant exposure at the end of their breastfeeding period. These exposures included biological measurements of dioxins, PCBs, 'old' pesticides, and heavy metals. To increase study exposures in this population, a subset of this population was recruited from a dioxin hotspot. According to Abraham et al., ages of the children had to be between 341 and 369 days old and had been breastfed maximally for 2 weeks (*i.e.*, referred to as formula-fed children) or breastfed equivalent to a duration of exclusive breastfeeding of 4 months or longer. Children had to be considered to be healthy and vaccinated according to the recommended vaccination schedules with at least two vaccinations against diphtheria and tetanus. Specific antibodies at the time were measured via ELISA test kits for tetanus toxoid and *Haemohilus influenza* type b. Standard clinical chemistry assessments were also conducted as well as thyroid hormone measurements. As a consequence of the EFSA's 2018 opinion that PFOS and PFOA may result in reduced vaccination antibody titers, the principal investigator (Abraham) in 2019 decided to conduct an additional assessment of the two remaining stored plasma aliquots (stored at -80^{o}C) to measure level of perfluoroalkyls: PFOA, PFOS, PFBS, PFHxS, PFHxA, PFNA, PFDA, PFDoDA, and ADONA. Abraham also decided to use an ELISA test kit to examine antibodies against diphtheria toxoid. Their study showed no associations with childhood infections (reported by mother). Although a standard c-reactive protein (CRP) determined samples below a level indicative of inflammatory processes, a subset of 22 children were above a more sensitive method to measure sCRP.

Mean serum concentrations were 16.8 and 15.2 ng/mL for PFOA and PFOS for the breast-fed babies and 3.8 and 6.8 ng/mL for the formula-fed babies, respectively. Correlations between adjusted antibody levels and PFOA were Hib ($r = -0.32$, $p = 0.001$), tetanus ($r = -0.25$, $p = 0.01$), and diphtheria ($r = -0.23$, $p = 0.02$). No significant associations were observed for PFOS for Hib ($r = -0.32$, $p = 0.66$), tetanus IgG2 ($r = -0.07$, $p = 0.52$), or diphtheria, $r = -0.02$, $p = 0.84$). No significant correlations were found either for PFHxS and PFNA (data were not shown). The four perfluoroalkyls themselves were correlated with the Spearman rank correlation coefficient of 0.67 between PFOA and PFOS. The effect size differences (means for PFOA quintiles Q1 vs Q5) were -86%, -54%, and -53%, respectively, for Hib, tetanus, and diphtheria antibodies. [Please Note: on page 122, line 4438 of the draft scientific opinion, EFSA describes these percentages as -78%, -53%, and -57%, respectively. EFSA should clarify this discrepancy in

reported percentages as found in Abraham et al. vs. the EFSA (2020) report when comparing the highest to lowest quintile of PFOA exposure.] Confidence intervals were not provided for these effect sizes. No observed adverse effect concentrations (NOAEC) were determined by fitting a 'knee' function for these three antibodies at 12.2,16.9, and 1.62 ng/mL PFOA, respectively. None of these mean effect size percentage differences were provided in the Abraham et al. paper for PFOS or the other two perfluoroalkyls.

In the current draft scientific opinion, EFSA relied on the detailed PFOA PBPK modelling results from the Abraham et al. (2020) study but did not provide any individual analyses for the other three compounds. While EFSA acknowledged and stated that "*associations for PFOS, PFHxS and PFNA were not significant*", these four compounds were summed together as a mixture by EFSA for the subsequent statistical analyses. This decision by EFSA to use a mixture alone is not justified when EFSA stated in their review of the supporting toxicological data that PFOS is more of the compound of toxicological interest because of its immunotoxicity. On page 110 of the draft scientific opinion, EFSA stated "*In summary, the literature database, including the publications that appeared after the publication of the EFSA CONTAM opinion (2018), if any, support the notion that PFOS exposure, possibly more than PFOA, causes immunosuppression, as evidenced by decreased antibody response to sensitization to an antigen, and that suppressed immune functionality may lead to reduced resistance to infection."* Also, on page 110, EFSA concluded the study by Peden-Adams et al. (2008) that evaluated the effect of PFOS (not PFOA) on immunotoxicity in mice "showing effects at the lower serum levels is the critical study for immune effects in animals."

Given EFSA had reached such a conclusion toxicologically about PFOS, it is unclear why EFSA did not provide detailed PBPK modelling results for PFOS based on the Abraham et al. (2020) study. EFSA had privileged communication with Abraham et al. for undoubtedly many months, perhaps a year or longer, before the public ever had a chance to read the Abraham et al. study. [Note: Abraham et al. (2020) did not appear in PubMed until March 30, 2020, two months after release of the draft EFSA (2020) report and only 3 weeks before public comments must be submitted to EFSA.]

Within the data reported in Abraham et al. (2020), most of the detailed analyses focused on PFOA as that was the only perfluoroalkyl that showed statistically significantly associations. There was no comparable Figure 1 for PFOS as there was for PFOA. Table 3 did provide correlations of adjusted levels with PFOS but the remaining data displayed in Table 3 were about PFOA. Abraham et al. described the effect size in the mean reduction in antibody response when comparing the highest to the lowest quintile of PFOA exposures but such information was not found in the text or supplemental material for PFOS, PFHxS or PFNA. While Table 4 provided side-by-side data for PFOA and PFOS with the biological parameters measured, only PFOA data were provided in Figure 3 a-c, Table S2, and Figure S2. This may be the prerogative of Abraham et al. to limit their data presentation or analyses to PFOA but not PFOS, however, it cannot be the prerogative of EFSA to do the same. Because EFSA requested the actual data on the four perfluoroalkyls from Abraham et al., it behooved EFSA to analyze and present the data for each individual compound separately, especially for PFOS, given the above immunotoxicology reasons (cf. page 110 of the draft scientific opinion). Neither Abraham et al.

nor EFSA provided these individual data for effect size mean reduction in antibody response from low to high quintiles and deciles for PFOS, PFHxS, or PFNA. EFSA should also provide the fitted knee functions for PFOS similar to Figure 3 (a – c) that Abraham et al. had reported for PFOA, as well as provide those for PFHxS and PFNA.

In order to conduct its own additional analyses, 3M noted that EFSA requested unpublished data from several researchers (*e.g.*, Abraham et al., Grandjean et al., and Peden-Adams et al.). These unpublished data should have been made available as appendices in the EFSA scientific draft opinion for the public to review and comment. If this is not possible due to the prerogative of the research investigators who originated the data, then EFSA should adopt the policy similar to IARC whereby only published data are considered in the review process.

Comments on EFSA's data analyses

A NOAEC as a point of departure was derived by EFSA via various statistical analyses based on the observational data reported in Abraham et al. (2020). 3M retained an independent statistics and modeling expert (Mr. Bruce Allen) to review EFSA's data analysis and Mr. Allen concluded that there are several technical issues with EFSA's statistical approach. The resulting faulty decisions and computations have led to a flawed NOAEC of 31.9 ng/mL (see Mr. Allen's report attached in Appendix A).

Provided below are four important findings from Mr. Allen's review on EFSA's data selection process:

> First, the reported exposure data (i.e., serum PFAS concentrations) should not have been arbitrarily assigned into groups (as quintiles and deciles). The exposure-response data are observational in nature and they cannot be considered as treatment groups.
>
> Second, the determination of the NOAEC based on NOAEL/LOAEL is unacceptable and problematic. This method is not robust and it does not account for the variability and uncertainty of the data resulting from the study design itself. A better approach will be use of benchmark dose methodology that EFSA has routinely used (including for its 2018 draft opinion on PFOS and PFOA) or even of a more sophisticated Bayesian analysis.
>
> Third, albeit inappropriate in this particular case, a dose-response analysis is generally only valid for endpoint(s) that demonstrated clear evidence of a relationship between dose and response. Not only were the analyses described in the current draft opinion inappropriate, the p-values currently presented in Table K.1 appear to be incorrect. When the dose and response data were reanalyzed, the results did not support a concentration-dependent effect on Hib titer. This contradicts EFSA's conclusion that there was an "inverse association between serum levels of the sum of these 4 PFASs and antibody titers against Haemophilus influenza type b (Hib)" (cf. page 7).
>
> Lastly, the statistical approach based on the summation of the four PFAS compounds is not justified. When analyzing the data using an appropriate method by treating each compound separately, they would not have the same coefficients. EFSA's summation

approach assumes that they do. Hence summation of the four PFAS compounds is an inaccurate approach. Use of the summation approach constrained the overall data interpretation.

For all of the reasons listed above, the derived NOAEC of 31.9 ng/mL is not supported scientifically.

Combining multiple perfluoroalkyls as a sum for risk assessment

In its draft scientific opinion, PFOA, PFOS, PFHxS and PFNA were summed together as a mixture by EFSA for its derivation of the TWI. Based on the general population biomonitoring data represented as measured serum concentrations, it is evident that humans are exposed to multiple perfluoroalkyls. Due to limited hazard data available, there have been some efforts to combine these multiple exposures as a sum for the purpose of risk assessment, however, this approach has not been fully developed nor validated for perfluoroalkyls.

Applying the critical concept of Toxic Equivalency Factors (TEFs) that was developed for dioxin-like compounds, Scialli et al. (2007) and Peters and Gonzalez (2011) independently evaluated the scientific feasibility of combining perfluoroalkyl exposures for risk assessment. Scialli et al. (2007) reviewed similar same-species studies performed with different perfluoroalkyls and they found large discordance in endpoints measured for PFOS, PFOA, PFBS, and PFDA. Peters and Gonzalez (2011) also concluded that perfluoroalkyl exposure should not be combined based on the following observations:

> *(1) lack of conclusive evidence demonstrating that a single receptor is required to mediate the toxicities of perfluoroalkyl chemicals; (2) the potential influence of species differences in the response to PPARα ligands that would significantly limit this approach; (3) inconsistent toxicities observed with different perfluoroalkyl chemicals; and (4) a limited toxicological database for a number of perfluoroalkyls chemicals (e.g., perfluorinated sulfonamide polymers and perfluorinated sulfonamide-based phosphate fluorosurfactants).*

Therefore, the available scientific data do not support EFSA's current approach to combining perfluoroalkyl exposures in its derivation of a TWI.

Applying equal potency for PFOA, PFOS, PFHxS, and PFNA

In its derivation for a combined TWI, EFSA had exerted and assigned equal (toxicity) potency for PFOA, PFOS, PFHxS, and PFNA. This was based on the following rationale:

- these four PFASs contribute most to the levels observed in human serum;
- these four PFASs share toxicokinetic properties and show similar accumulation and long half-lives in humans; and
- these compounds in general show the same effects when studied in animals.

The available data for these four perfluoroalkyls contradicted greatly with the assertions made by EFSA. Qualitatively, it is true that these four perfluoroalkyls do have longer serum elimination

half-lives in humans (see Table 1), however, there are distinct quantitative differences for the reported half-lives. In addition, given the key hazards identified by EFSA (c.f. section3.3.3 of the draft scientific opinion), Table 1 also illustrates several differences in these categorical effects with animal data. From a specific effect such as (frank) postnatal mortality in rodent pups or even adaptive liver weight increases in animals, there are large quantitative differences depending on the compounds and doses. Therefore, it is scientifically unjustifiable for EFSA to assume they all have the same effects (because they don't). The broad conjecture to assume equal potency is not supported by data, and the resulting uncertainties (as acknowledged by EFSA) bring into question the appropriateness of this methodology by EFSA.

Table 1:

Hazard characterization identified by EFSA (section 3.3.3)			**PFOA**	**PFOS**	**PFHxS**	**PFNA**
Human Data	Serum elimination half-lives		2 – 3 years	3 – 4 years	5 – 7 years	~ 3 years
Animal (rodent) Data	Liver Effect		Y	Y	Y	Y
	Developmental Effect	Postnatal mortality	Y	Y	N	Y
		Mammary gland effect	Y	--	--	--
	Neurotoxicity		Y (↑ activity)	Y (↓ activity)	Y (↓ activity)	--
	Immune effect	Thymus weight	Dose-dependent	Dose-dependent	--	↑ or ↓
		Spleen weight	Dose-dependent	Dose-dependent		Dose-dependent
		Thymus cell #	↔ or ↑ or ↓	↔ or ↑ or ↓		↓
		Spleen cell #	↔ or ↑ or ↓	↔ or ↑ or ↓		↓
		IgM	↓	↓		--
		IgG	↔ or ↑	↔ or ↑		--
	Genotoxicity		N	N	N	N
	Carcinogenicity		Y	Y	--	--

Y Yes, effects confirmed and reported
N No effect reported
-- Not available

Comments on EFSA's PBPK modelling

The contents provided in this section are based on the comments prepared by Dr. Anne Loccisano (at the request of 3M) to assist EFSA in its current effort with PBPK modelling. Dr. Loccisano is the principal investigator who developed a series of PBPK models for the human, primate, and rat for which EFSA adopted to conduct some their analyses. Funds to develop these PBPK models (Loccisano et al., 2011; Loccisano et al., 2012b; Loccisano et al., 2012a; Loccisano et al., 2013) were provided by an EPA Star grant, 3M, and DuPont.

Gestational and lactational models not used: On p. 155, it is stated that "The graphs [referring to Figures 12 and 13] do not include the effect of having a child and of subsequent breastfeeding,

which results in a (temporary) decline in the serum levels of the mothers." This is again stated on p. 163, where EFSA states that the models do not take into account possible physiological changes during pregnancy, which could alter the pharmacokinetics of PFASs during this period and could influence transfer to the fetus. There are separate pregnancy and lactation models for both PFOA and PFOS, both of which have been published (Loccisano et al., 2011). It is not clear why EFSA chose to modify the adult human model when these other models are available and could possibly provide a better modeling approach. As EFSA states that the inclusion of pregnancy could alter the pharmacokinetics and transfer to the child, rationale for not including the pregnancy life stage when there are available models should be provided.

Lack of a model schematic: Appendix M of the EFSA document describes the modifications made to a previously published PBPK model for PFOA and PFOS in adult humans (Loccisano et al., 2011). The modifications made to the code are straightforward (equations for placental transfer and breast milk transfer were added as well as an equation for growth). The added equations appear to be adequate for describing growth, the initial amount of PFAS at birth in the neonate, and amount of PFAS in milk over time. While equations and a description of the modifications are provided, a model schematic should be included. As EFSA modified existing models, it would be useful to have a figure of the structure of the modified model used. It is not clear if a single model was used for estimation of maternal daily intake and child intake or if the model was modified and used separately for the child and mother. A model schematic would help in clarifying this.

Clarification on maternal intake: The process by which EFSA used the PBPK models to estimate the maternal intake is not well-described and is difficult to follow. Each step in the process in estimating the maternal daily intake (for both PFOA/PFNA and PFOS/PFHxS) should be outlined. If the milk concentration was estimated first and then the maternal intake was estimated to give this milk concentration that should be clearly stated. In addition, the concentrations at which the mother was exposed to in utero and during breastfeeding should be clearly outlined.

Clarification on placental transfer coefficients**:** EFSA discusses fetal/maternal serum ratios (c.f. page 71), which are then used in the PBPK model for the placental transfer coefficients (this is the PT parameter in the model). EFSA reports that median values for PFHxS, PFOS, PFOA, and PFNA were 0.56, 0.36, 0.74, and 0.48, respectively, which were calculated from the ratios reported by various study authors. EFSA states that these were the values used for the calculation of the serum level in the infant at birth; however, EFSA should be specific about which values were actually used for the compounds of interest. As EFSA has assumed that PFOA and PFNA would behave similarly and that the PFOS and PFHxS would behave similarly and thus considered them collectively with model use, it should be stated if only the PFOA and PFOS values were used or if the values were averaged (i.e., PFNA and PFOA and PFOS and PFHxS).

Provide justification on the lactational transfer ratios used in the model: The maternal-infant transfer via breast milk was estimated using the ratio of the concentrations in milk versus plasma (this is the RATIO parameter in the model). EFSA reports that the ratio is in the range of 0.01 – 0.07 for PFHxS, PFNA, PFDA, PFUnDA, and FOSA, indicating that the transfer occurs at

similar levels as for PFOS (ratio of 0.01 – 0.02) but to a lesser extent than for PFOA (ratio of 0.03-0.12). Values of 0.03 and 0.015 are used for PFOA and PFOS, respectively in the model. EFSA should provide justification/rationale for use of these values, given that a range has been reported.

Specify PFAS clearance coefficients in the breast milk: Mondal et al. 2014 calculated that each month, breastfeeding was associated with 1 and 2% decreases in maternal serum concentrations of PFHxS and PFNA, respectively, compared to 3% for both PFOS and PFOA. Several other authors estimated the decline in PFAS concentration in breast milk due to breastfeeding. In addition, calculations can be based on intakes, distribution volumes, breastfeeding amount, and ratios between breast milk and serum concentrations. According to EFSA, all of these methods that various authors have used show that monthly decreases in breast milk concentrations ranged from 1.2-3.1% for PFOS and from 1.3-7.7% for PFOA. Percentages reported for PFHxS and PFNA varied between 1-6.7% and 1.1-2.8%, respectively. EFSA chose to use a value of 3.1% for PFOS and a value of 7.7% for PFOA in the model (this is the DECLINE parameter in the model). These values should be explicitly cited (i.e., the studies that reported these conclusions). If EFSA performed their own analyses to obtain these numbers, then that should be stated and described.

Technical clarification on kinetic behaviors for PFNA and PFHxS: EFSA used a modified PBPK model in order to estimate maternal daily intake. Data for PFNA and PFHxS were insufficient for modeling; thus, it was assumed that these compounds behave like PFOA and PFOS, respectively. Support should be provided for this this assumption; EFSA should describe how the kinetic behaviors are similar for these compounds.

Study by Peden-Adams et al. 2008 does not provide sufficient evidence for PFOS-related immune suppression

Among a myriad of outcomes that have been reported for PFOS in the laboratory animals, EFSA considered the study by Peden-Adams et al. (2008) to be the critical toxicological study for supporting its current scientific position. The study by Peden-Adams et al. reported immunosuppression based on decreased antibody IgM TDAR responses in splenocytes in mice (upon exposure to SRBC antigen) and accordingly to EFSA, this may lead to reduced resistance to infection.

The decreased IgM antibody response in splenocytes upon SRBC challenge alone is insufficient to support the notion that PFOS caused an immunosuppression effect. Antibody responses should be done corroboratively in other key immune organs such as thymus, lymph node, bone marrow, and serum. Peden-Adams et al. (2008) did not evaluate antibody response in thymus from the same animals. In a separate cohort of animals, they did report decreased serum IgM level, however, these animals were challenged once with trinitrophenyl conjugated to LP (TNP-LPS) instead of SRBC hence it was difficult to compare the results among these data; given two different antigens were used and the antigenic effect induced by TNP-LPS could be mediated by either B cells or T cells. In addition, in the NTP (2016) monograph which declared that "*PFOS is presumed to be an immune hazard to humans*", it concluded that PFOS exposure is associated

with suppression of NK cell activity in animals. However, Peden-Adams et al. (2008) actually reported increased NK cell activities.

Even though the conclusion that PFOS suppresses antibody responses in mice is supported by a number of other studies which also showed that exposure to PFOS at various life stages can affect the IgM levels (Dong et al., 2011; Keil et al., 2008; Peden-Adams et al., 2008), it is important to recognize that the antibody titers (to antigen vaccinations) are primarily of the IgG antibody isotype, not IgM. Peden-Adams et al. (2008) did not evaluate IgG response in mice; and even when IgG was evaluated in mice by others such as the studies by Dong et al. (2011) and Qazi et al. (2010), the secondary IgG response was not appropriately induced to elicit a *bona fide* memory response as only a single antigen challenge was used in these studies. A later study by Lee et al. (2018) evaluated serum IgG1 and IgE levels in mice that had been challenged by multiple ovalbumin treatments before PFOS administration and again one more time after PFOS administration, they did not see a suppression of serum IgG1 or IgE. Rather, increased serum IgG1 and IgE were reported.

It is worth noting that Peden-Adams et al. (2008) also reported non-dose-response changes in splenic CD4/CD8 subpopulations, however, this specific finding has not been replicated in the subsequent publications co-authored by Dr. Peden-Adams (Fair et al., 2011; Mollenhauer et al., 2011). As discussed by Fair et al. (2011):

> *Moreover, data from this study confirm that numbers of CD4+ cells were within normal ranges. This contrasts a previous report from this laboratory where absolute numbers of CD4+ cells were decreased in female B6C3F1 mice at 0.1 mg/kg [total administered dose] but not at 1.0 mg/kg TAD using a similar 28-day exposure regimen (Peden-Adams et al., 2008). This previous observation was from a single experiment, whereas in this study, the experiment was repeated twice for absolute numbers and three times for percent changes with all experiments yielding the same results. The effect previously reported was not dose-responsive and is likely to be a transient effect. Overall, these data indicate that T-helper cells, B-cells, and MHC-II+ cells were not selectively eliminated.*

Lastly, the animal evidence for an effect of PFOS on disease resistance/infectious disease outcome is weak. EFSA relies essentially on one study by Guruge et al. (2009) which reported decreased survival in PFOS-treated mice infected with a mouse-adapted strain of influenza virus (H1N1). This study did not provide any data to demonstrate "comprised" immune condition in the animals. It did not evaluate the amount of virus present in the lung after H1N1 virus injection, nor did it measure the number and the proportion of inflammatory cells (T cells and granulocytes) in the BALF or the lung. In addition, the study did not evaluate antigen-specific viral titers to conclude whether anti-viral activity was affected by PFOS treatment.

Epidemiological data do not support reduced resistance to infection

In section 3.3.4.4.3, EFSA cited a total of 9 papers with their opinion of mixed results regarding an association with infection and PFAS (cf. page 125 of the draft scientific opinion). It is unclear why EFSA (2020) did not cite Abraham et al. (the critical effect study used to calculate the TWI) who did not find any association with PFOA or PFOS related to parentally reported

infections with these 101 children. The lack of associations with PFOA or PFOS included total number of infections, number of infections with fever, 3-day fever experience, number of pneumonia diagnoses, number of diarrhea diagnoses, and varicella experience. Despite this omission of Abraham et al., EFSA concluded that there is some evidence to suggest that "*exposures to PFASs are associated with increased propensity of infections but more studies with objective measures of infections (not self-reports are needed).*" (cf. page 126 of the draft scientific opinion). In fact, as shown below in Table 2, an equally valid conclusion could have been reached by EFSA that there is insufficient evidence to lead to the suggestion of an increased propensity of associations with infections and exposure for PFOS or PFOA.

Table 2: Summary table of 9 epidemiology studies cited by EFSA that examined associations between serum PFOS and PFOA levels and infectious disease outcomes.

Reference Study	Study population	Study design	PFOS		PFOA	
			Blood measurement	Outcome	Blood measurement	Outcome
Abraham et al., (2020)	101 German 1-year old children	cross-sectional	1-year old child	Symptoms of infection -total number infections, infections with fever, 3-day fever, number antibiotic treatments, pneumonia, ear infection, diarrhea, variacella, napkin or oral candidiasis: all NS	1-year old child	Symptoms of infection -total number infections, infections with fever, 3-day fever, number antibiotic treatments, pneumonia, ear infection, diarrhea, variacella, napkin or oral candidiasis: all NS
Impinen et al., (2019)	2 Norwegian children cohorts (0 – 3 years N = 1270); (6-7 years, N = 972)	prospective	maternal	Symptoms of infection 0 -3 years: -common cold:↓ -bronchitis/pneumonia: ↑ -throat infection: NS -pseudocroup: NS -ear infection:↓ -diarrhea/gastric flu: NS -urinary tract infection (UTI):↓ 6-7 years: -bronchitis/pneumonia, ear infection, diarrhea, UTI: all NS	maternal	Symptoms of infection 0 -3 years: -common cold:↓ -bronchitis/pneumonia:↑ -throat infections: NS -pseudocroup:↑ -ear infection: NS -diarrhea/gastric flu: NS -urinary tract infection (UTI):↓ 6-7 years: - diarrhea ↑; - bronchitis/pneumonia, ear infection, and UTI:NS
Impinen et al., (2018)	641 Norwegian children (0-10 years)	prospective	cord blood	Symptoms of infection - common cold episodes from 0-2 years of age: NS - lower respiratory tract infection episodes 0-10 years of age:↑	cord blood	Symptoms of infection - common cold episodes from 0-2 years of age: NS - lower respiratory tract infection episodes 0-10 years of age: ↑

Dalsager et al., (2016)	359 Odense children (1-4 years)	prospective	maternal	Symptoms of infection - fever: ↑ - cough: NS - nasal discharge: NS - diarrhea: NS - vomiting: NS	maternal	Symptoms of infection - fever: ↑ - cough: NS - nasal discharge: NS - diarrhea: NS - vomiting: NS
Goudarzi et al., (2016)	1558 Japanese children (0-4 years)	Prospective	maternal	Symptoms of all infectious diseases* - all children: ↑ (3rd and 4th quartile) - girls: ↑ (4th quartile) - boys: ↑ (4th quartile)	maternal	Symptoms of all infectious diseases* - all children: NS - girls: NS - boys: NS
Looker et al., (2014)	411 U.S. adults with drinking water exposure	cross-sectional	adult	Symptoms of infection - any "flu" infection in last 12 months: NS - any cold in last 12 months: NS - cold or flu in last 12 months: NS	adult	Symptoms of infection - any "flu" infection in last 12 months: NS - any cold in last 12 months: NS - cold or flu in last 12 months: NS
Granum et al., (2013)	99 Norwegian children (0-3 years)	prospective	maternal	Symptoms of infection - common cold episodes: NS - common cold (y/n): NS - gastroenteritis episodes: NS - gastroenteritis (y/n): NS	maternal	Symptoms of infection - common cold episodes: ↑ - common cold (y/n): NS - gastroenteritis episodes: NS - gastroenteritis (y/n): NS
Okada et al., (2012)	343 Japanese infants (0-18 months)	prospective	maternal	Symptoms of infection Otitis media during the first 18 months of life - all infants: NS - males: NS - females: NS (< 5% reported chicken pox, bronchitis, RSV disease, rhinitis, pneumonia, skin infections, rotavirus, adenovirus and cytomegalovirus and were not included in the analyses)	maternal	Symptoms of infection Otitis media during the first 18 months of life - all infants: NS - males: NS - females: NS (< 5% reported chicken pox, bronchitis, RSV disease, rhinitis, pneumonia, skin infections, rotavirus, adenovirus and cytomegalovirus and were not included in the analyses)

Fei et al., (2010)	577 Danish children (average age = 8.2 years)	cross-sectional	maternal	Incidence of hospitalization for infectious diseases - all children: NS - age 0 - <1 year: NS - age 1 - <2 years: NS - age 2 - <4 years: NS - age ≥ 4 years: NS - girls: ↑ - boys: NS	maternal	Incidence of hospitalization for infectious diseases - all children: NS - age 0 - <1 year: NS - age 1 - <2 years: NS - age 2 - <4 years: NS - age ≥ 4 years: NS - girls: ↑ - boys: NS

* Infectious diseases included at least one case of self-reported otitis media, pneumonia, RS virus and varicella.

Note: ↑ = statistically significant increase; ↓ statistically significant decrease; NS = not statistically significant

Is there evidence for a diminished antibody response to vaccination for tetanus and diphtheria?

Excluding the very recently published results from Abraham et al. (2020) due to lack of providing confidence intervals, there have been 11 studies that have examined vaccine antibody response with exposure to PFOS and PFOA (Table 3). Percent change to tetanus antibody or diphtheria antibody per increase in PFOS or PFOA have been the outcomes most reported. As noted in Table 3, only one of these 11 studies reported results on Hib (Granum et al. 2013). In bivariate analyses of 50 children, they reported no statistically significant associations with reduced antibody levels to Hib, tetanus, or measles and exposures to PFOS, PFHxS, PFOA, or PFNA. Median concentrations for the total of 99 participants were 5.5, 0.3, 1.1, and 0.3 ng/mL, respectively. They did not provide a specific median for the 50 children. Granum et al. did report statistically significant exposure-related associations with rubella in bivariate and multivariate analyses.

Table 3:

Vaccine type	Number of studies	Reference(s)
Tetanus	6	Grandjean et al. (2012); Grandjean et al. (2017a); Grandjean et al. (2017b); Granum et al. (2013); Kielsen et al. (2016); Mogenson et al. (2015)
Diphtheria	5	Grandjean et al. (2012); Grandjean et al. (2017a); Grandjean et al. (2017b); Granum et al. (2013); Mogenson et al. (2015)
Rubella	3	Granum et al. (2013); Pilkerton et al. (2018); Stein et al. (2016b)
Measles	2	Granum et al. (2013); Stein et al. (2016b)
Influenza A (H1N1)	2	Looker et al. (2014); Stein et al. (2016a)
Influenza B	2	Looker et al. (2014)
Haemophilus influenza type b	1	Granum et al. (2013)
Influenza A (H1N2)	1	Looker et al. (2014)
Mumps	1	Stein et al. (2016b)
Enterovirus (EV71)	1	Zeng et al. (2019)
Coxsackievirus (CA16)	1	Zeng et al. (2019)

Based on the forest plots below, there is inconsistent evidence to suggest an association with reduced tetanus antibody response for either PFOS or PFOA (Figure 1A and Figure 1B). There is imprecise evidence suggestive of an association between decreased diphtheria antibody response and increased serum concentrations of PFOS or PFOA (Figure 2A and Figure 2B).

Figure 1A:

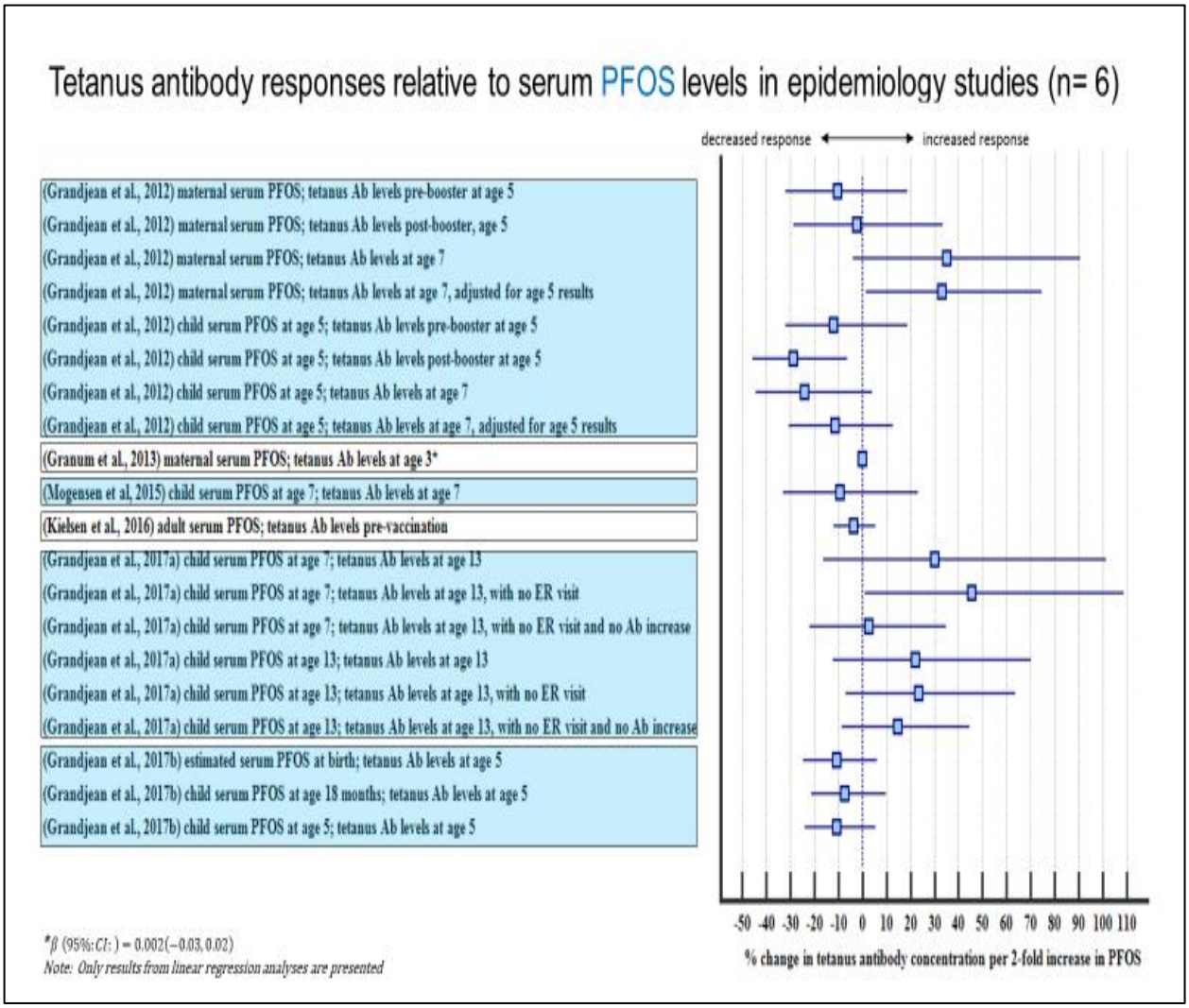


Figure 1B:

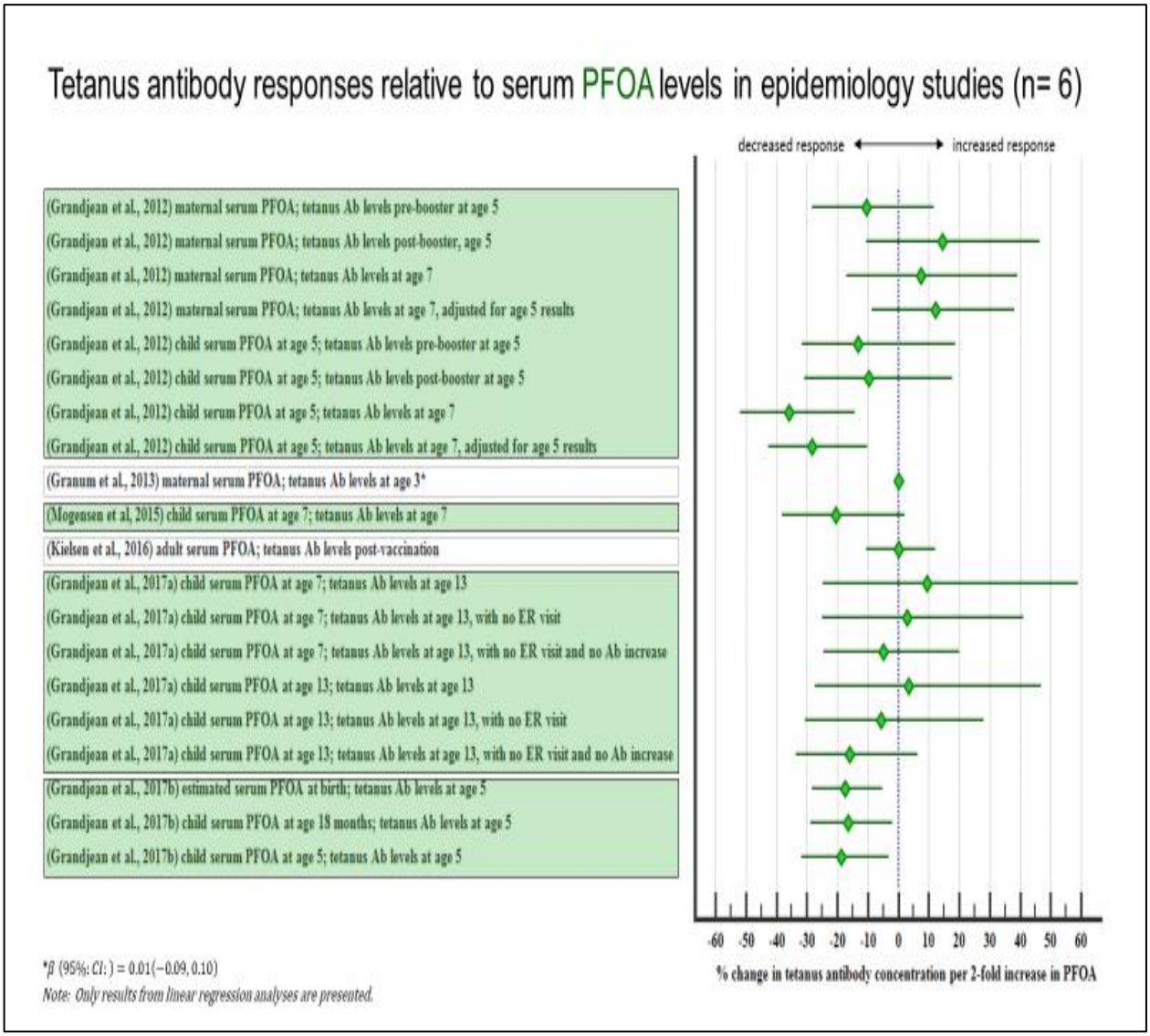

Tetanus antibody responses relative to serum PFOA levels in epidemiology studies (n= 6)
decreased response
increased response
(Grandjean et al., 2012) maternal serum PFOA; tetanus Ab levels pre-booster at age 5
(Grandjean et al., 2012) maternal serum PFOA; tetanus Ab levels post-booster, age 5
(Grandjean et al., 2012) maternal serum PFOA; tetanus Ab levels at age 7
(Grandjean et al., 2012) maternal serum PFOA; tetanus Ab levels at age 7, adjusted for age 5 results
(Grandjean et al., 2012) child serum PFOA at age 5; tetanus Ab levels pre-booster at age 5
(Grandjean et al., 2012) child serum PFOA at age 5; tetanus Ab levels post-booster at age 5
(Grandjean et al., 2012) child serum PFOA at age 5; tetanus Ab levels at age 7
(Grandjean et al., 2012) child serum PFOA at age 5; tetanus Ab levels at age 7, adjusted for age 5 results
(Granum et al., 2013) maternal serum PFOA; tetanus Ab levels at age 3*
(Mogensen et al, 2015) child serum PFOA at age 7; tetanus Ab levels at age 7
(Kielsen et al., 2016) adult serum PFOA; tetanus Ab levels post-vaccination
(Grandjean et al., 2017a) child serum PFOA at age 7; tetanus Ab levels at age 13
(Grandjean et al., 2017a) child serum PFOA at age 7; tetanus Ab levels at age 13, with no ER visit
(Grandjean et al., 2017a) child serum PFOA at age 7; tetanus Ab levels at age 13, with no ER visit and no Ab increase
(Grandjean et al., 2017a) child serum PFOA at age 13; tetanus Ab levels at age 13
(Grandjean et al., 2017a) child serum PFOA at age 13; tetanus Ab levels at age 13, with no ER visit
(Grandjean et al., 2017a) child serum PFOA at age 13; tetanus Ab levels at age 13, with no ER visit and no Ab increase
(Grandjean et al., 2017b) estimated serum PFOA at birth; tetanus Ab levels at age 5
(Grandjean et al., 2017b) child serum PFOA at age 18 months; tetanus Ab levels at age 5
(Grandjean et al., 2017b) child serum PFOA at age 5; tetanus Ab levels at age 5
-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60
% change in tetanus antibody concentration per 2-fold increase in PFOA
*β (95%: CI:) = 0.01(−0.09, 0.10)
Note: Only results from linear regression analyses are presented.

Figure 2A:

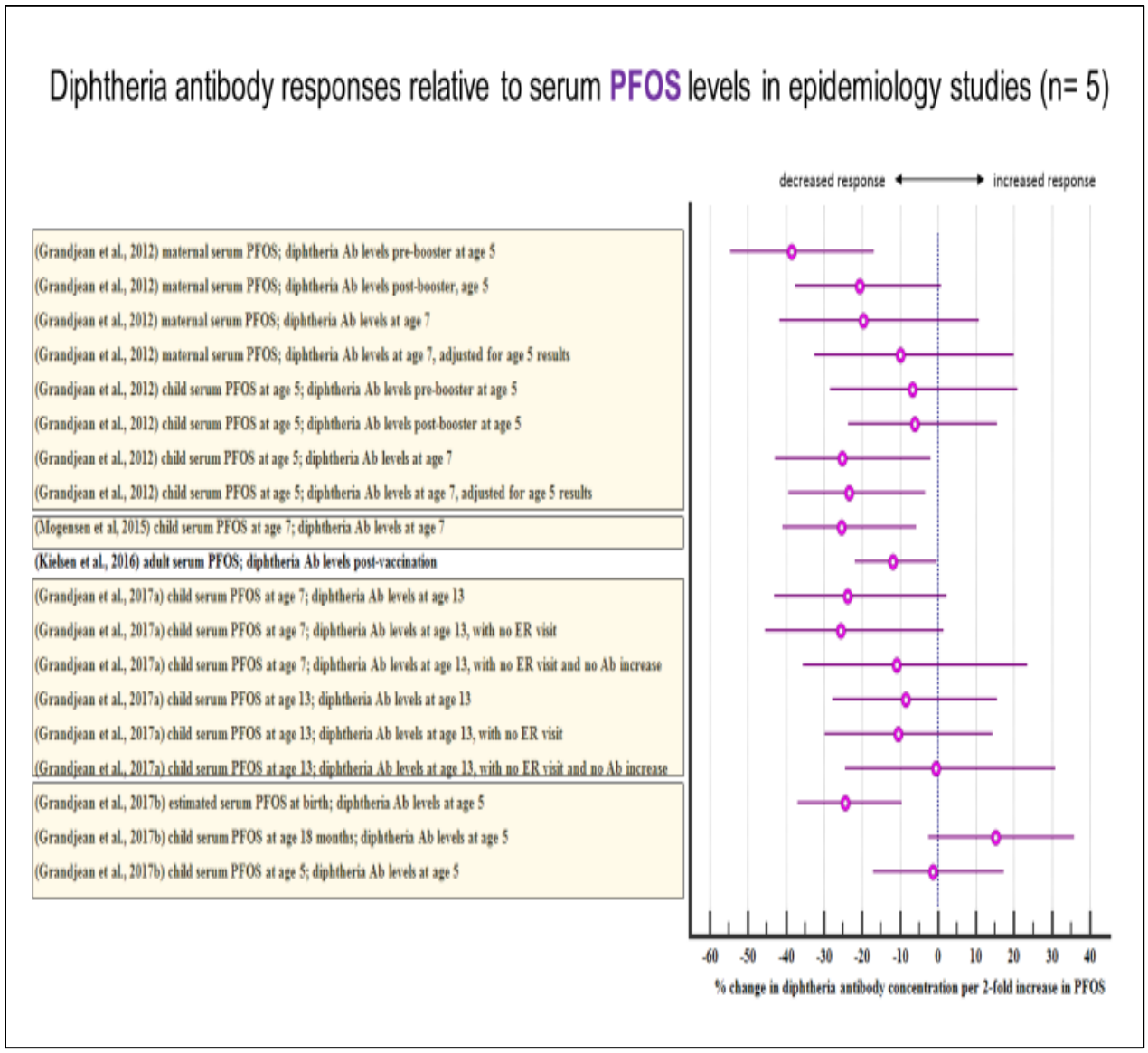
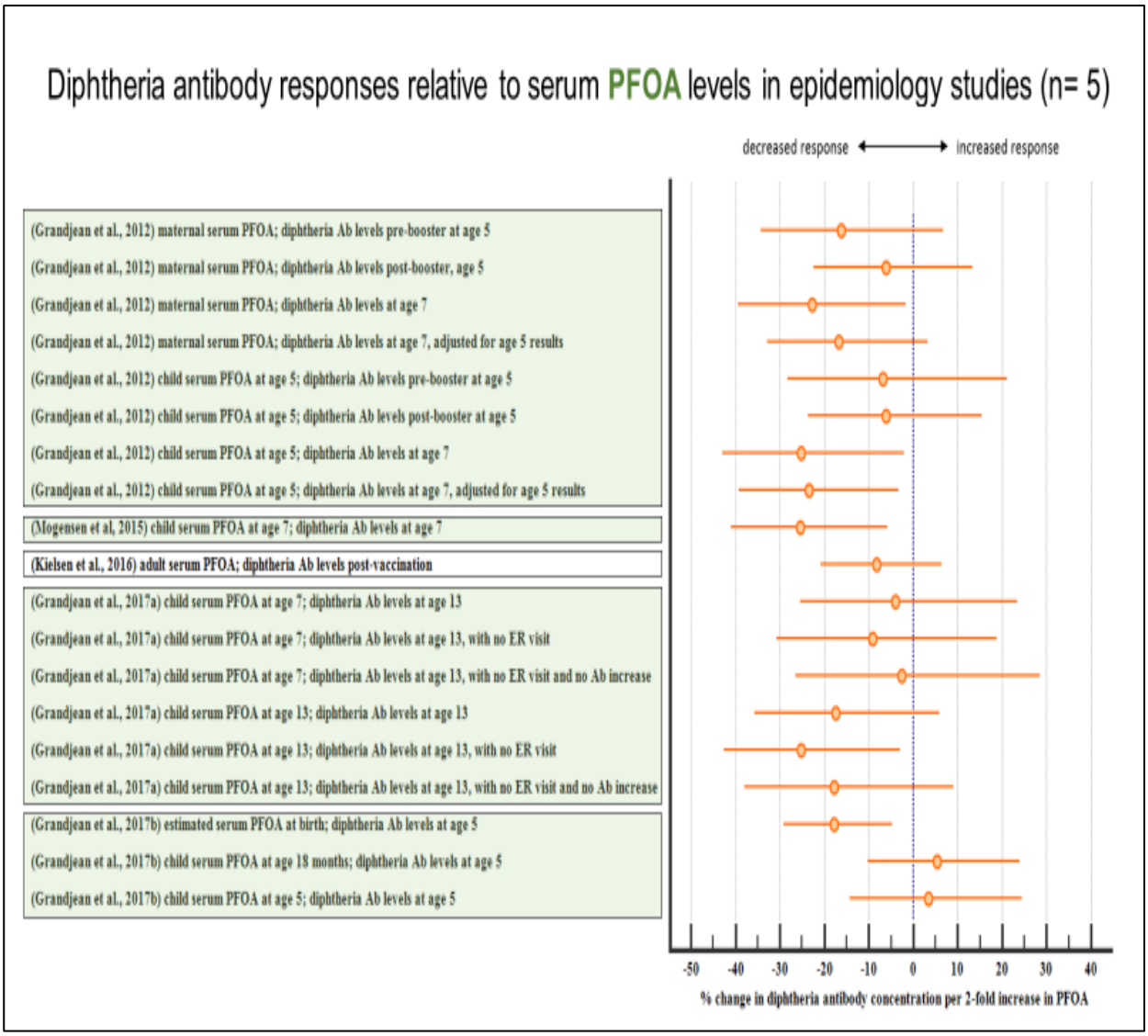

Diphtheria antibody responses relative to serum PFOS levels in epidemiology studies (n= 5)
decreased response
increased response
(Grandjean et al., 2012) maternal serum PFOS; diphtheria Ab levels pre-booster at age 5
(Grandjean et al., 2012) maternal serum PFOS; diphtheria Ab levels post-booster, age 5
(Grandjean et al., 2012) maternal serum PFOS; diphtheria Ab levels at age 7
(Grandjean et al., 2012) maternal serum PFOS; diphtheria Ab levels at age 7, adjusted for age 5 results
(Grandjean et al., 2012) child serum PFOS at age 5; diphtheria Ab levels pre-booster at age 5
(Grandjean et al., 2012) child serum PFOS at age 5; diphtheria Ab levels post-booster at age 5
(Grandjean et al., 2012) child serum PFOS at age 5; diphtheria Ab levels at age 7
(Grandjean et al., 2012) child serum PFOS at age 5; diphtheria Ab levels at age 7, adjusted for age 5 results
(Mogensen et al, 2015) child serum PFOS at age 7; diphtheria Ab levels at age 7
(Kielsen et al., 2016) adult serum PFOS; diphtheria Ab levels post-vaccination
(Grandjean et al., 2017a) child serum PFOS at age 7; diphtheria Ab levels at age 13
(Grandjean et al., 2017a) child serum PFOS at age 7; diphtheria Ab levels at age 13, with no ER visit
(Grandjean et al., 2017a) child serum PFOS at age 7; diphtheria Ab levels at age 13, with no ER visit and no Ab increase
(Grandjean et al., 2017a) child serum PFOS at age 13; diphtheria Ab levels at age 13
(Grandjean et al., 2017a) child serum PFOS at age 13; diphtheria Ab levels at age 13, with no ER visit
(Grandjean et al., 2017a) child serum PFOS at age 13; diphtheria Ab levels at age 13, with no ER visit and no Ab increase
(Grandjean et al., 2017b) estimated serum PFOS at birth; diphtheria Ab levels at age 5
(Grandjean et al., 2017b) child serum PFOS at age 18 months; diphtheria Ab levels at age 5
(Grandjean et al., 2017b) child serum PFOS at age 5; diphtheria Ab levels at age 5
-60 -50 -40 -30 -20 -10 0 10 20 30 40
% change in diphtheria antibody concentration per 2-fold increase in PFOS

Figure 2B:



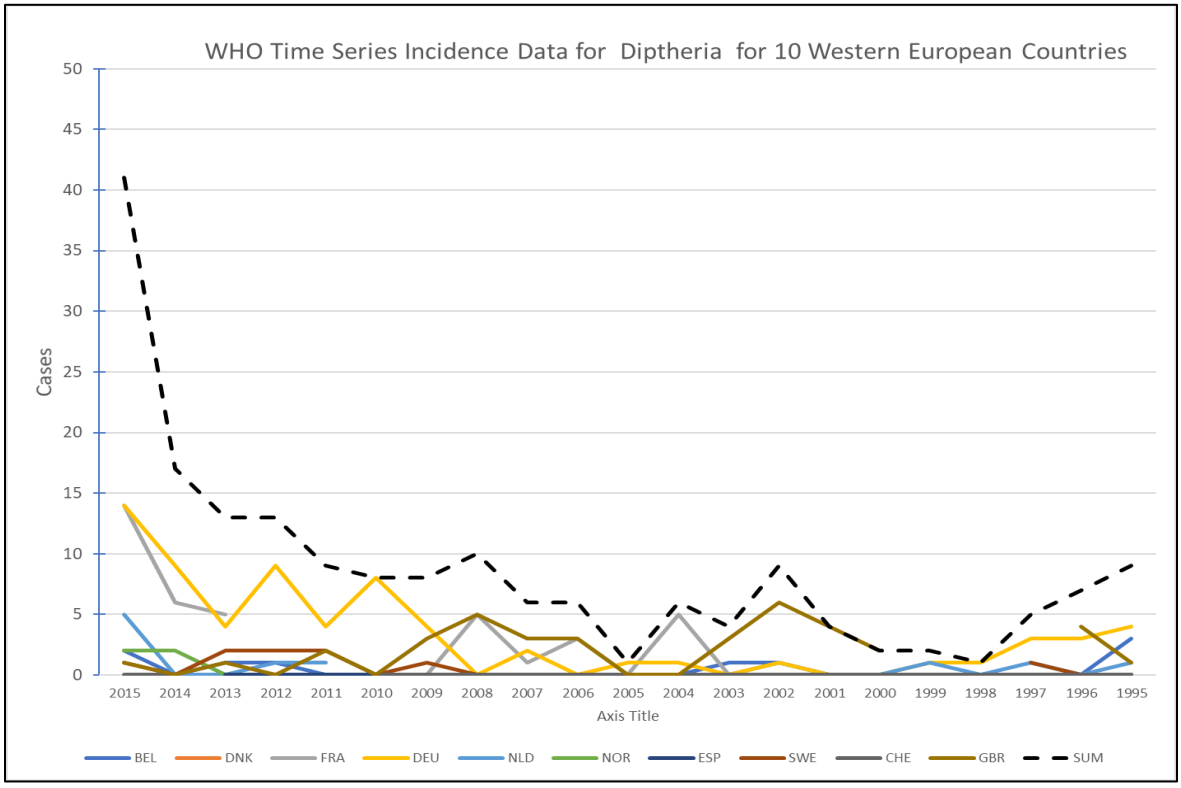
Commercially available vaccines differ depending on the nature of the vaccine antigen. Tetanus and diphtheria, for example, are toxoid vaccines whereas measles, mumps and rubella are live attenuated vaccines. Influenza vaccines are inactivated (killed), conjugate or live attenuated depending on the strain and method of administration (e.g., intranasal, injectable). Consequently, each vaccine type elicits an immune response through various molecular and cellular mechanisms of the immune system. Additionally, all vaccines contain various excipients including adjuvants to improve the antibody response, preservatives, stabilizers, and vehicles for delivering the vaccine which may differ substantially depending on the vaccine (Baxter, 2007).

Given the minimum evidence of increased infectious disease susceptibility (as shown in Table 3 for PFOS), it is questionable whether the imprecise decreases in antibody response, as seen in Figures 2A and 2B for diphtheria, are clinically relevant. One thing worth noting is that PFOS

and PFOA have high degrees of binding affinity with serum albumin proteins, therefore, there might be a potential interference between these compounds and the ELISA assay components used to determine serum antibody titers, which normally consist of protein-based buffers and diluents. We are currently unaware of any research that has examined of this question.

As shown in Figure 3, our ecological analysis of the WHO time series database between 1995 and 2015 demonstrated a counterintuitive observation to the hypothesis of reduced response leads to increased risk of disease. In this WHO reporting of diphtheria incident cases, there was an increased number of diphtheria cases reported in 10 Western European during the phase-out production of PFOS and PFOA that began in 2000 that resulted in the declining trend in PFOS and PFOA serum concentrations between 2000 and 2015 in Western Europe as well as elsewhere (Australia, Canada, United States). We speculate whether the increase in diphtheria cases in Germany and France, shown in Figure 3, might be attributable, at least in part, due to lower vaccination prevalence present in the increasing immigrant populations that occurred in these two countries (this trend continued to increase through 2018). Reported vaccination rates were fairly constant and the 'herd health' effect needs to be considered. Nevertheless, there is no suggestive evidence that a decline in serum PFOS and PFOA concentrations in the general population that occurred between 2000 - 2015 was also associated with a decline in diphtheria cases in these 10 countries.

Figure 3 (https://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencediphtheria.html)



A similar analysis of the WHO time series database was attempted with reported tetanus cases for the 10 countries. However, Germany did not report data regarding tetanus after 2000; therefore, this ecological analysis was considered uninterpretable.

Others have weighed in on the question of risk of infection with exposure to perfluoroalkyls:

National Toxicology Program (2016)

Although the 2016 NTP conclusion that "*exposure to PFOA or PFOS is presumed to be an immune hazard to humans*" it also concluded that there was low confidence it was associated with increased incidence of infectious disease.

Australia Expert Health Panel (2018):

"*The strongest evidence for a link between PFAS and clinically important immunological effects is for impaired vaccine response. However, the human dose-response/threshold for potential immune effects is very poorly characterized, and the overall human evidence is weak.*"

Food Standards Australia New Zealand, FSANZ (2016):

A literature review commissioned by FSANZ concluded that *"there are both positive and negative studies showing associations for increasing PFOS and PFOA concentrations to compromise antibody production in humans. However, to date there is no convincing evidence for increased incidence of infective disease associated with PFOS or PFOA effects on human immune function".*

Health Canada (2017a):

"Studies in environmentally-exposed populations have identified associations between PFOS levels and decreased antibodies against various illnesses, but the influence of PFOS exposure on clinical immunosuppression (i.e., incidence of illnesses) appears to be more tenuous." Health Canada further commented that "*a low level of consistency was observed across studies, with variations between genders, specific microbial immunoglobins, infections, mother vs. child exposure, and child years, amongst other characteristics. Moreover, the risk of residual confounding, bias, and chance cannot be discarded. These flaws impede concluding on a causative mechanism, and the nature of the association remains unclear*." Health Canada reached similar conclusions regarding PFOA (Health Canada, 2017b)

National Institute for Public Health and the Environment (RIVM, 2016):

RIVM concluded that "*associations have been found between exposure to PFOA and a decreased vaccination response*", but the "*evidence is unclear*".

Comments regarding about lipids

EFSA (2018) used increased serum cholesterol outcomes from cross-sectional epidemiologic studies to derive a provisional TDI for PFOA and PFOS. EFSA (2020) now considers the uncertainty regarding causality to be larger than it did in 2018 primarily due to a hypothesis of enterohepatic cycling of both PFASs and bile acids which the latter could affect cholesterol. In fact, this uncertainty is even likely larger than what EFSA (2020) acknowledges based on five studies (four of which were not cited by EFSA). Provided in Appendix B are comments on 2 epidemiologic studies related to serum cholesterol and 3 animal toxicological studies with exposure to PFAS. The latter were conducted on a genetically engineered mouse model that mimics human lipoprotein metabolism, on cynomolgus monkeys, and a small (n = 20) study on mice examining bile acids and PFAS.

Comments regarding about mammary glands

3M disagrees with EFSA's assessment that mammary gland development is a robust endpoint for PFOA-related toxicity in laboratory animals. There are a number of specific technical concerns that warrant careful consideration before using mammary gland data for risk characterization. Please see Appendix C for additional details on this topic as well as several other areas of concern that EFSA needs to address in their review of published studies. To date, there has not been a standardized or even internationally recognized method of evaluating mammary gland in laboratory rodents. The studies cited by EFSA had numerous instances of inappropriate data interpretation and most studies gave very few quantitative measures pertaining to mammary gland development. These data often built on a great deal of speculation without the corroborative reproductive and/ developmental data. They were not sufficient in supporting the phenotypic consequences such as reduction in mammary gland development with exposure to PFOA. The fact that the effects of PFOA on mammary gland development cannot be consistently described and quantified in studies (Table 4) brings into question the biological significance of this phenotype and its relevance to human health.

Table 4:

Study	Mouse Strain	Mammary Gland Development Outcomes (per Study Authors)
White et al. (2007)	CD-1	Stunted
White et al. (2009)	CD-1	Delayed
Yang et al. (2009)	C57BL6	Stimulatory (5 mg/kg); Inhibitory (10 mg/kg)
	Balb/c	Inhibitory
Zhao et al. (2010)	C57BL/6	Stimulated
Macon et al. (2011)	CD-1	Delayed
White et al. (2011)	CD-1	Delayed
Albrecht et al. (2013)	Sv/129 WT	No effect
	PPARα KO	No effect
	hPPARα	No effect
Tucker et al. (2015)	CD-1	Delayed

	C57BL/6	Delayed

References:

Albrecht, P. P., Torsell, N. E., Krishnan, P., Ehresman, D. J., Frame, S. R., Chang, S. C., Butenhoff, J. L., Kennedy, G. L., Gonzalez, F. J., and Peters, J. M. (2013). A species difference in the peroxisome proliferator-activated receptor alpha-dependent response to the developmental effects of perfluorooctanoic acid. *Toxicol Sci* **131**, 568-82.

Baxter, D. (2007). Active and passive immunity, vaccine types, excipients and licensing. *Occup Med (Lond)* **57**, 552-6.

Chang, S., Allen, B. C., Andres, K. L., Ehresman, D. J., Falvo, R., Provencher, A., Olsen, G. W., and Butenhoff, J. L. (2017). Evaluation of Serum Lipid, Thyroid, and Hepatic Clinical Chemistries in Association With Serum Perfluorooctanesulfonate (PFOS) in Cynomolgus Monkeys After Oral Dosing With Potassium PFOS. *Toxicol Sci* **156**, 387-401.

Chang, S. C., Thibodeaux, J. R., Eastvold, M. L., Ehresman, D. J., Bjork, J. A., Froehlich, J. W., Lau, C. S., Singh, R. J., Wallace, K. B., and Butenhoff, J. L. (2007). Negative bias from analog methods used in the analysis of free thyroxine in rat serum containing perfluorooctanesulfonate (PFOS). *Toxicology* **234**, 21-33.

Chang, S. C., Thibodeaux, J. R., Eastvold, M. L., Ehresman, D. J., Bjork, J. A., Froehlich, J. W., Lau, C., Singh, R. J., Wallace, K. B., and Butenhoff, J. L. (2008). Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). *Toxicology* **243**, 330-9.

Chang, S. C., Ehresman, D. J., Bjork, J. A., Wallace, K. B., Parker, G. A., Stump, D. G., and Butenhoff, J. L. (2009). Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: toxicokinetics, thyroid hormone status, and related gene expression. *Reprod Toxicol* **27**, 387-399.

Convertino, M., Church, T. R., Olsen, G. W., Liu, Y., Doyle, E., Elcombe, C. R., Barnett, A. L., Samuel, L. M., MacPherson, I. R., and Evans, T. R. J. (2018). Stochastic Pharmacokinetic-Pharmacodynamic Modeling for Assessing the Systemic Health Risk of Perfluorooctanoate (PFOA). *Toxicol Sci* **163**, 293-306.

Dalsager, L., Christensen, N., Husby, S., Kyhl, H., Nielsen, F., Host, A., Grandjean, P., and Jensen, T. K. (2016). Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years among 359 children in the Odense Child Cohort. *Environ Int* **96**, 58-64.

Dong, G. H., Liu, M. M., Wang, D., Zheng, L., Liang, Z. F., and Jin, Y. H. (2011). Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. *Arch Toxicol* **85**, 1235-44.

Fair, P. A., Driscoll, E., Mollenhauer, M. A., Bradshaw, S. G., Yun, S. H., Kannan, K., Bossart, G. D., Keil, D. E., and Peden-Adams, M. M. (2011). Effects of environmentally-relevant levels of perfluorooctane sulfonate on clinical parameters and immunological functions in B6C3F1 mice. *J Immunotoxicol* **8**, 17-29.

Fei, C., McLaughlin, J. K., Lipworth, L., and Olsen, J. (2010). Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. *Environ Res* **110**, 773-7.

Fletcher, T., Galloway, T. S., Melzer, D., Holcroft, P., Cipelli, R., Pilling, L. C., Mondal, D., Luster, M., and Harries, L. W. (2013). Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. *Environ Int* **57-58**, 2-10.

Goudarzi, H., Miyashita, C., Okada, E., Kashino, I., Kobayashi, S., Chen, C. J., Ito, S., Araki, A., Matsuura, H., Ito, Y. M., and Kishi, R. (2016). Effects of prenatal exposure to perfluoroalkyl acids on prevalence ofallergic diseases among 4-year-old children. *Environ Int* **94**, 124-132.

Grandjean, P., Andersen, E. W., Budtz-Jorgensen, E., Nielsen, F., Molbak, K., Weihe, P., and Heilmann, C. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA* **307**, 391-7.

Grandjean, P., Heilmann, C., Weihe, P., Nielsen, F., Mogensen, U. B., and Budtz-Jorgensen, E. (2017a). Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds. *Environ Health Perspect* **125**, 077018.

Grandjean, P., Heilmann, C., Weihe, P., Nielsen, F., Mogensen, U. B., Timmermann, A., and Budtz-Jorgensen, E. (2017b). Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. *J Immunotoxicol* **14**, 188-195.

Granum, B., Haug, L. S., Namork, E., Stolevik, S. B., Thomsen, C., Aaberge, I. S., van Loveren, H., Lovik, M., and Nygaard, U. C. (2013). Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotoxicol* **10**, 373-9.

Guruge, K. S., Hikono, H., Shimada, N., Murakami, K., Hasegawa, J., Yeung, L. W., Yamanaka, N., and Yamashita, N. (2009). Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice. *J Toxicol Sci* **34**, 687-91.

Impinen, A., Nygaard, U. C., Lodrup Carlsen, K. C., Mowinckel, P., Carlsen, K. H., Haug, L. S., and Granum, B. (2018). Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. *Environ Res* **160**, 518-523.

Impinen, A., Longnecker, M. P., Nygaard, U. C., London, S. J., Ferguson, K. K., Haug, L. S., and Granum, B. (2019). Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy and asthma related outcomes and infections in the Norwegian Mother and Child (MoBa) cohort. *Environ Int* **124**, 462-472.

Keil, D. E., Mehlmann, T., Butterworth, L., and Peden-Adams, M. M. (2008). Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. *Toxicol Sci* **103**, 77-85.

Kielsen, K., Shamim, Z., Ryder, L. P., Nielsen, F., Grandjean, P., Budtz-Jorgensen, E., and Heilmann, C. (2016). Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. *J Immunotoxicol* **13**, 270-3.

Koulouri, O., Moran, C., Halsall, D., Chatterjee, K., and Gurnell, M. (2013). Pitfalls in the measurement and interpretation of thyroid function tests. *Best Pract Res Clin Endocrinol Metab* **27**, 745-62.

Lau, C., Thibodeaux, J. R., Hanson, R. G., Rogers, J. M., Grey, B. E., Stanton, M. E., Butenhoff, J. L., and Stevenson, L. A. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. *Toxicol Sci* **74**, 382-92.

Lee, J.-K., Lee, S., Choi, Y.-A., Jin, M., Kim, Y.-Y., Kang, B.-C., Kim, M.-J., Dhakal, H., Lee, S.-R., Kim, S.-U., Khang, D., and Kim, S.-H. (2018). Perfluorooctane sulfonate exacerbates mast cell-mediated allergic inflammation by the release of histamine. *Molecular & Cellular Toxicology* **14**, 173-181.

Li, Y., Barregard, L., Xu, Y., Scott, K., Pineda, D., Lindh, C. H., Jakobsson, K., and Fletcher, T. (2020). Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. *Environ Health* **19**, 33.

Loccisano, A. E., Campbell, J. L., Jr., Andersen, M. E., and Clewell, H. J., 3rd (2011). Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. *Regul Toxicol Pharmacol* **59**, 157-75.

Loccisano, A. E., Campbell, J. L., Jr., Butenhoff, J. L., Andersen, M. E., and Clewell, H. J., 3rd (2012a). Evaluation of placental and lactational pharmacokinetics of PFOA and PFOS in the pregnant, lactating, fetal and neonatal rat using a physiologically based pharmacokinetic model. *Reprod Toxicol* **33**, 468-490.

Loccisano, A. E., Campbell, J. L., Jr., Butenhoff, J. L., Andersen, M. E., and Clewell, H. J., 3rd (2012b). Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model. *Reprod Toxicol* **33**, 452-467.

Loccisano, A. E., Longnecker, M. P., Campbell, J. L., Jr., Andersen, M. E., and Clewell, H. J., 3rd (2013). Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. *J Toxicol Environ Health A* **76**, 25-57.

Looker, C., Luster, M. I., Calafat, A. M., Johnson, V. J., Burleson, G. R., Burleson, F. G., and Fletcher, T. (2014). Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. *Toxicol Sci* **138**, 76-88.

Luebker, D. J., York, R. G., Hansen, K. J., Moore, J. A., and Butenhoff, J. L. (2005). Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters. *Toxicology* **215**, 149-69.

Macon, M. B., Villanueva, L. R., Tatum-Gibbs, K., Zehr, R. D., Strynar, M. J., Stanko, J. P., White, S. S., Helfant, L., and Fenton, S. E. (2011). Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry. *Toxicol Sci* **122**, 134-45.

Mendel, C. M., Frost, P. H., and Cavalieri, R. R. (1986). Effect of free fatty acids on the concentration of free thyroxine in human serum: the role of albumin. *J Clin Endocrinol Metab* **63**, 1394-9.

Mogensen, U. B., Grandjean, P., Heilmann, C., Nielsen, F., Weihe, P., and Budtz-Jorgensen, E. (2015). Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. *Environ Health* **14**, 47.

Mollenhauer, M. A., Bradshaw, S. G., Fair, P. A., McGuinn, W. D., and Peden-Adams, M. M. (2011). Effects of perfluorooctane sulfonate (PFOS) exposure on markers of inflammation in female B6C3F1 mice. *J Environ Sci Health A Tox Hazard Subst Environ Eng* **46**, 97-108.

NTP (2016). Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) (N. T. Program, Ed.^ Eds.), Research Triangle Park, NC.

Okada, E., Sasaki, S., Saijo, Y., Washino, N., Miyashita, C., Kobayashi, S., Konishi, K., Ito, Y. M., Ito, R., Nakata, A., Iwasaki, Y., Saito, K., Nakazawa, H., and Kishi, R. (2012). Prenatal

exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. *Environ Res* **112**, 118-25.

Oppenheimer, J. H., Schwartz, A. L., and Strait, K. A. (1995). An integrated view of thyroid hormone actions *in vivo* (B. D. Weintraub, Ed.^ Eds.), pp. 249-65. Raven Press, Ltd., New York.

Peden-Adams, M. M., Keller, J. M., Eudaly, J. G., Berger, J., Gilkeson, G. S., and Keil, D. E. (2008). Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol Sci* **104**, 144-54.

Peters, J., and Gonzalez, F. J. (2011). Why Toxic Equivalency Factors are not Suitable for Perfluoroalkyl Chemicals. *Chemical Research in Toxicology* **24**, 1601-1609.

Pilkerton, C. S., Hobbs, G. R., Lilly, C., and Knox, S. S. (2018). Rubella immunity and serum perfluoroalkyl substances: Sex and analytic strategy. *PLoS One* **13**, e0203330.

Pouwer, M. G., Pieterman, E. J., Chang, S. C., Olsen, G. W., Caspers, M. P. M., Verschuren, L., Jukema, J. W., and Princen, H. M. G. (2019). Dose Effects of Ammonium Perfluorooctanoate on Lipoprotein Metabolism in APOE*3-Leiden.CETP Mice. *Toxicol Sci* **168**, 519-534.

Qazi, M., Abedi, M. R., Nelson, B. D., DePierre, J. W., and Abedi-Valugerdi, M. (2010). Dietary exposure to perfluorooctanoate or perfluorooctane sulfonate induces hypertrophy in cetrilobular hepatocytes and alters the hepatic immune status in mice. *International Immunopharmacology* **10**, 1420-1427.

Refetoff, S., Robin, N. I., and Fang, V. S. (1970). Parameters of thyroid function in serum of 16 selected vertebrate species: a study of PBI, serum T4, free T4, and the pattern of T4 and T3 binding to serum proteins. *Endocrinology* **86**, 793-805.

Salihovic, S., Dickens, A. M., Schoultz, I., Fart, F., Sinisalu, L., Lindeman, T., Halfvarson, J., Oresic, M., and Hyotylainen, T. (2019). Simultaneous determination of perfluoroalkyl substances and bile acids in human serum using ultra-high-performance liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem*.

Scialli, A. R., Iannucci, A., and Turim, J. (2007). Combining perfluoroalkane acid exposure levels for risk assessment. *Regul Toxicol Pharmacol* **49**, 195-202.

Seacat, A. M., Thomford, P. J., Hansen, K. J., Olsen, G. W., Case, M. T., and Butenhoff, J. L. (2002). Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. *Toxicol Sci* **68**, 249-64.

Stein, C. R., Ge, Y., Wolff, M. S., Ye, X., Calafat, A. M., Kraus, T., and Moran, T. M. (2016a). Perfluoroalkyl substance serum concentrations and immune response to FluMist vaccination among healthy adults. *Environ Res* **149**, 171-178.

Stein, C. R., McGovern, K. J., Pajak, A. M., Maglione, P. J., and Wolff, M. S. (2016b). Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. *Pediatr Res* **79**, 348-57.

Thibodeaux, J. R., Hanson, R. G., Rogers, J. M., Grey, B. E., Barbee, B. D., Richards, J. H., Butenhoff, J. L., Stevenson, L. A., and Lau, C. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. *Toxicol Sci* **74**, 369-81.

Tucker, D. K., Macon, M. B., Strynar, M. J., Dagnino, S., Andersen, E., and Fenton, S. E. (2015). The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. *Reprod Toxicol* **54**, 26-36.

Vanden Heuvel, J. P. (2013). Comment on "associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans" by Fletcher et al., Environment International 57-58 (2013) 2-10. *Environ Int* **61**, 150-3.

White, S. S., Calafat, A. M., Kuklenyik, Z., Villanueva, L., Zehr, R. D., Helfant, L., Strynar, M. J., Lindstrom, A. B., Thibodeaux, J. R., Wood, C., and Fenton, S. E. (2007). Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. *Toxicol Sci* **96**, 133-44.

White, S. S., Kato, K., Jia, L. T., Basden, B. J., Calafat, A. M., Hines, E. P., Stanko, J. P., Wolf, C. J., Abbott, B. D., and Fenton, S. E. (2009). Effects of perfluorooctanoic acid on mouse mammary gland development and differentiation resulting from cross-foster and restricted gestational exposures. *Reprod Toxicol* **27**, 289-298.

White, S. S., Stanko, J. P., Kato, K., Calafat, A. M., Hines, E. P., and Fenton, S. E. (2011). Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 mice. *Environ Health Perspect* **119**, 1070-6.

Yang, C., Tan, Y. S., Harkema, J. R., and Haslam, S. Z. (2009). Differential effects of peripubertal exposure to perfluorooctanoic acid on mammary gland development in C57Bl/6 and Balb/c mouse strains. *Reprod Toxicol* **27**, 299-306.

Zeng, X. W., Bloom, M. S., Dharmage, S. C., Lodge, C. J., Chen, D., Li, S., Guo, Y., Roponen, M., Jalava, P., Hirvonen, M. R., Ma, H., Hao, Y. T., Chen, W., Yang, M., Chu, C., Li, Q. Q., Hu, L. W., Liu, K. K., Yang, B. Y., Liu, S., Fu, C., and Dong, G. H. (2019). Prenatal exposure to perfluoroalkyl substances is associated with lower hand, foot and mouth disease viruses antibody response in infancy: Findings from the Guangzhou Birth Cohort Study. *Sci Total Environ* **663**, 60-67.

Zhao, Y., Tan, Y. S., Haslam, S. Z., and Yang, C. (2010). Perfluorooctanoic acid effects on steroid hormone and growth factor levels mediate stimulation of peripubertal mammary gland development in C57BL/6 mice. *Toxicol Sci* **115**, 214-24.

APPENDIX A

Comments from Mr. Bruce Allen
Research Triangle Park, North Carolina

Comments regarding the EFSA draft opinion "Risk to human health related to the presence of perfluoroalkyl substances in food"
Use of Abraham et al. (2020) data

The following comments suggest that EFSA's use of the very recent Abraham et al. (2020) data is not appropriate. There are several issues involved in that determination and the following text attempts to present those issues in a sequence delineating the erroneous decisions that have been made to arrive at a point of departure (POD) of 31.9 ng/ml.

Based on my understanding, the value of 31.9 ng/ml was determined by selecting a "NOAEC" from the "Hib" response from Abraham et al., as summarized in Table K.1. The errors made in that regard are as follows.

Issue 1: The grouped data should not have been used as the basis for any analysis.

The underlying data from this study do not come from a study with dose (or "treatment") groups defined by PFAS exposure (PFAS-sum). As evidenced by Figures K1 – K3, the observations are scattered over varying PFAS-sum values. The data are observational rather than experimental[1]; they are not naturally grouped into convenient, homogeneous groups. The groupings shown in Table K.1 are arbitrary.

In fact, the grouping of the observations is completely unnecessary. Analyses can, and should be, based on the individual values. Analyses based on arbitrary groupings may mask the true underlying relationships (if any) between PFAS-sum and the response variables. An indication of this is shown in the EFSA draft opinion (Table K.1) wherein the determination of whether or not responses differ with differing PFAS-sum values depends on the coarseness of the grouping. More will be said about this in the discussion of Issue 3.

Issue 2: The determination of a POD is based on an outdated and discredited approach.

The NOAEC of 31.9 ng/ml is determined by the debunked "NOAEL/LOAEL" approach. As far back as Crump (1984), the problems with that approach were identified and a much more robust replacement (the BMD approach) was described. In the meantime, many more publications have described the issues associated with the NOAEL/LOAEL approach (see for example Kimmel and Gaylor, 1988; Gaylor and Slikker, 1990). In fact, EFSA itself has routinely used, and in some ways has spearheaded, the BMD approach, through its use of PROAST and BMDS software for BMD estimation.

This reversion to the discredited NOAEL/LOAEL approach appears to be at odds with standard EFSA practice. The EFSA draft opinion justifies this choice because the resulting bounds on BMD estimates were very wide (data or results not shown; see Section 3.4.3.1), which they ascribe to the fact that the models must "extrapolate to zero" because the data do not have observations with 0 PFAS-sum values. That rationale is not pertinent. The parameters of a dose-response model are estimated together; there is no separate and independent estimation of the parameter corresponding to background response.

[1] That is, there was no planned experiment where the subjects were manipulated to have designed independent variable (PFAS sum) values. Rather, each PFAS sum value as presented by a selected child was observed and reported as-is.

Hence, the absence of observations that correspond to the exact exposure level associated with one of those parameters (i.e., the background parameter corresponding to 0 exposure) is no methodological barrier.

Furthermore, the width of a confidence interval (the BMDL to the BMDU) is not a reflection of the appropriateness of the methodology. Rather it is a reflection of the ability of the data to estimate a particular quantity, the BMD. The wide confidence interval may indicate that the data are not sufficient to be very certain about the value of the BMD; it does not argue against the application of the dose-response modeling approach. Moreover, the BMD approach is inherently conservative (health-protective) with respect to data deficiencies. Unlike the NOAEL/LOAEL approach, data sets with fewer observations (e.g., with fewer observations at or near zero) will tend to produce smaller BMDL values (i.e., generate smaller PODs) than would be estimated from data sets that have the same underlying relationships but more observations (Crump, 1984).

Note, finally, that the use of the NOAEL/LOAEL approach is totally unnecessary. Given the individual response data (the appropriate basis for analysis) or even with the faulty grouped summarization thereof (see the discussion of Issue 1), a dose-response analysis can (and should) be done to identify a BMD that would serve to replace the NOAEC. There are undoubtedly more sophisticated approaches to dose-response modeling that could be used. For example, Bayesian analyses of the dose-response relationship could account for supplemental information about background levels of response by defining prior distributions for the background parameter, among others. Nevertheless, the perspective of dose-response modeling, as opposed to statistical testing, is one that should be applied whenever possible. As described, it is possible in this instance of PFAS exposure and the immune response.

Issue 3: The statistical analyses appear to be erroneous.

Even if one were to pursue analyses of the grouped data (i.e., if one persisted in making the error of arbitrarily grouping the results), it would only be appropriate to do so for endpoints that demonstrated a dose-response relationship. I.e., as an operating principle, dose-response analyses are restricted to endpoints that exhibit some change as a function of exposure. Valid statistical tests are the basis for whether or not such change is evident.

In the case of the three endpoints under consideration (reported by Abraham et al. and labeled Hib, Tet, and Dip here – see Table K.1 and figures K1 – K3 in the EFSA draft opinion), we suggest that the p-values reported for the overall test of a concentration-dependent effect on response are wrong. That conclusion also appears to apply to the analyses reported in the supplemental material to the Abraham et al. manuscript (Table S2), which reports results for similar tests, but for which PFOA alone is considered as the explanatory variable.

The exact methodology used for deriving the p-values in Table K.1 is not clearly specified; the table title (and the text of Abraham et al., 2020) merely states that ANOVA results are shown. In the following alternative analysis, we have used BMDS software (USEPA, BMDS version 3.1.1) to evaluate the grouped data for the presence of exposure-related differences among the groups. That software uses likelihood-ratio tests applied to the data, and makes the following assumptions

a. The responses within the groups are normally distributed around group-specific means. Given that the response metric is the logarithm of antibody levels, this assumption appears to be appropriate (and would be standard for an ANOVA analysis).
b. The log of the ratio of the likelihoods under various "nested models" is approximately chi-squared with degrees of freedom equal to the difference in the number of parameters specifying the models. Again, this is a standard statistical assumption and the likelihood-ratio testing paradigm is well-established in the statistical literature.
c. The appropriate test is the one that compares the model assuming equal means and equal standard deviations across all groups (called model R in BMDS) against the "fully saturated" model (model A2 in BMDS) that assumes that each group has its own independent mean and its own independent standard deviation. Model R has 2 parameters (the common mean and the common standard deviation) whereas model A2 has 2*G parameters (a mean and a standard deviation for each of the G groups). This particular test is encoded in BMDS as "Test 1" in the "Tests of Interest" table presented for each response analyzed using BMDS. As noted by that software, "if this test fails to reject the null hypothesis ($p > 0.05$), there may not be a dose-response." Absent any additional information, we interpret the failure to reject the null hypothesis to be evidence that the endpoint in question should not be used for the purposes of deriving a POD.

The results are summarized with respect to the test of a dose-response relationship in the following table:

Table 1

Endpoint	Data Grouping	p-value for test of effect	
		PFAS-sum	PFOA
Hib	5-groups	0.267	0.024
	10-groups	0.279	0.071
Tet	5-groups	0.202	0.107
	10-groups	0.285	0.012
Dip	5-groups	0.081	0.001
	10-groups	0.005	0.008

Note that for Hib (the response used as the basis for the 31.9 ng/ml POD for PFAS-sum in the EFSA draft opinion) neither data grouping suggests a dose-related effect on that endpoint. The same is true for the Tet endpoint. For Dip, the issue is less clear, but note that the p-value for this test depends on the choice of grouping. The dependence, for Dip and for the other two endpoints, of the hypothesis test results on the group definition is another indicator of the problematic practice of grouping what are fundamentally ungrouped data. As noted in the discussion of Issue 1, there is no need to do such grouping: tests for determining if there is a dose-response relationship exist for ungrouped data. Consistent with the results from Abraham et al., PFOS appears to have no effect on the Dip response, and PFAS-sum appears to have no impact on TeT. PFAS-sum appears to induce Dip responses that differ

across the groupings, as in the Abraham et al. data set.[2] The results for PFOA are dependent on the grouping and so again suggest the disadvantage of arbitrarily grouping the results.

Issue 4: Using PFAS-sum appears to be inappropriate

In all of the analyses reported in the EFSA draft opinion, the explanatory variable is PFAS-sum, the summation of the concentrations of four specific PFAS. EFSA must have obtained the raw data from Abraham et al. in order to compute that sum for each individual; Abraham et al. (2020) merely reports results relative to PFOA and PFOS, separately, not combined.

This highlights another issue with the analyses reported in the EFSA draft opinion. Note that the results in Table 1 suggest, in general, that PFOA has some effect on the responses examined. But Abraham et al. (2020) concluded that that was not the case for PFOS. Thus, using PFAS-sum as the explanatory variable mixes (on an equal footing, see below) a contributor to the effect(s) with a non-contributor. It is hardly surprising then that the correct results for testing for an effect of PFAS-sum are not significant or shifted in that direction.

Let us consider the definition of PFAS-sum and its use in a regression type analysis further. Here we reference a "regression type" analysis, because of the arguments above that have shown that grouping (as needed for ANOVA or the discredited NOAEL/LOAEL procedure) is not appropriate to begin with.

First, there is an extensive literature on the problems that colinear explanatory variables pose for regressions (see https://en.wikipedia.org/wiki/Multicollinearity for a brief overview and references to more technical material). Note that this is a salient problem for the use of PFAS-sum because of the high degree of correlation noted by Abraham et al. (2020), Table S1 (supplemental material). The correlation coefficient for PFOA and PFOS in their data was 0.67. The other contributors to PFAS-sum (PFHxS and PFNA) were also relatively highly correlated with PFOA (correlation coefficients of 0.51 and 0.72, respectively). These relatively high degrees of correlation are consistent with colinearity being an issue.

But, the issue with using PFAS-sum goes beyond the generic colinearity concern. Note, as an example, that a linear regression using PFAS-sum would have the form

$$\text{Response} = \alpha + \beta * \text{PFAS-sum}$$

$$= \alpha + \beta * \text{PFOA} + \beta * \text{PFOS} + \beta * \text{PFHxS} + \beta * \text{PFNA}.$$

In other words, the response would be predicted to change equally for the same change in PFOA, PFOS, or the other two components. The results of Abraham et al. do not support this, at least in relation to PFOA and PFOS.

On the other hand, a regression considering each component separately would look like this:

$$\text{Response} = \alpha + \beta_1 * \text{PFOA} + \beta_2 * \text{PFOS} + \beta_3 * \text{PFHxS} + \beta_4 * \text{PFNA}.$$

Note the different β terms for each component. This approach would at least allow the effects of each component to modify the response (if at all) at a component-specific rate. There would still be issues

[2] It is not clear from these EFSA summaries if the PFAS components summed for the Abraham et al. analyses are the same as those summed for the Grandjean et al. analyses.

with colinearity for such modeling, and one would not want to extrapolate from such an analysis to predict the separate effects of each component. That is to say, one would not expect that the predicted effect of changing PFOA alone would be β_1 times the change in PFOA. All we are claiming here is that the use of PFAS-sum is even worse than using a multivariate regression that is not constrained to have the same coefficients across all components.

These more statistically based observations are consistent with the conclusions reached by Scialli et al. (2007), who looked at empirical dose-response relationships across PFASs, and Peters and Gonzalez (2011), who looked at the differences in receptor mediation across PFASs. Both of those investigations concluded that even a toxic equivalency factor (TEF) approach would not be appropriate for combining the effects of different PFASs. The PFAS-sum variable proposed by EFSA in its draft opinion is much more "restrictive" than a TEF approach, in that it inherently assumes the TEF =1 for all of the components contributing to PFAS-sum.

Conclusion

For the several reasons discussed above, the analyses in the EFSA draft opinion that led to the identification of 31.9 ng/ml as a POD are flawed. EFSA should not have used grouped data. But, given that they did, they should not have based the POD on the unacceptable NOAEL/LOAEL approach. And, even for that approach, they appear to have made errors in the statistical evaluation. Finally, the use of a summation of PFAS components is not supported, either in the specific data set that they examined (Abraham et al., 2020) or in the literature (Scialli et al., 2007; Peters and Gonzalez, 2011). We suggest that the path forward would be to use the ungrouped data, do dose-response modeling of those data, when and only when they demonstrate a dose-related effect, and to do such analyses separately for each PFAS component.

References

Crump, K. S. (1984). A new method for determining allowable daily intakes. *Toxicological Sciences*, *4*(5), 854-871.

Gaylor, D. W., & Slikker Jr, W. T. (1990). Risk assessment for neurotoxic effects. *Neurotoxicology*, *11*(2), 211-218.

Kimmel, C. A., & Gaylor, D. W. (1988). Issues in Qualitative and Quantitative Risk Analysis for Developmental Toxicology 1. *Risk analysis*, *8*(1), 15-20.

Peters, J. M., and Gonzalez, F. J. (2011). Why toxic equivalency factors are not suitable for perfluoroalkyl chemicals. *Chemical research in toxicology*, *24*(10), 1601-1609.

Scialli, A. R., Iannucci, A., and Turim, J. (2007). Combining perfluoroalkane acid exposure levels for risk assessment. *Regulatory Toxicology and Pharmacology*, *49*(3), 195-202.

U.S. EPA. Benchmark dose software (BMDS). Version 3.1.1. Washington, DC: U.S. Environmental Protection Agency, Center for Public Health and Environmental Assessment; 2019. https://www.epa.gov/bmds.

APPENDIX B

Comments on lipids

Convertino et al. (2018):

EFSA provides a critique of the Convertino et al. study that obfuscates the critically important points of this study by writing "[while] the CONTAM Panel noted that this report (Convertino et al.) cannot shed additional light on effects in humans at the much lower PFOA intake levels occurring from normal diet or contaminated drinking water. Of course, it cannot. Convertino et al. was never designed to do this; nor did it suggest otherwise. The Convertino et al study does, however, unequivocally show that at very high dosages of PFOA administered for 6 weeks in these human subjects resulted in decreased serum total cholesterol and LDL while HDL remain unchanged.

The Convertino et al. report reviews the clinical chemistry results from a phase 1 dose escalation trial conducted in Scotland that assessed the chemotherapeutic potential of the ammonium salt of PFOA. EFSA criticizes the Convertino et al. study for the lack of a control group that hinders both the internal and the external validity of this study. This EFSA criticism lacks merit. Each subject provided their own baseline evaluation one week before the first dose was administered. As stated in Convertino et al., "for each subject, pre-treatment evaluations included a full medical history tumor evaluation, chest X-ray and 12-lead ECG, full blood count and coagulation screen, biochemical provide including urea, electrolytes, alanine transaminase (AL), aspartate transaminase (AST), alkaline phosphatase, bilirubin, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, albumin, calcium, urea, uric acid, thyroid stimulating hormone (TSH), free thyroid hormone (fT4), blood glucose, urinalysis, and a physical examination."

EFSA questions the non-dietary administration of PFOA in this study where gelatin capsules were administered. This is a reasonable scientific question as it was a bolus administration. However, dose was not the primary metric of exposure; internal concentrations were. This is essentially no different than EFSA accepting the daily gavage administration of PFOS in immunotoxicity studies of mice and rats. Internal serum concentrations were considered in the exposure assessment of these animals.

Convertino et al. presented strong evidence of a lipid lowering effect in the phase 1 trial subjects at the very high dosages of administered PFOA . EFSA describes this as a 'slight' reduction in cholesterol. This is not slight at the very high dosages of PFOA administered in this phase 1 trial whether analyzed and shown as linear (generalized estimating equations - GEE) or non-linear (probability density function) analyses. Based on the GEE analyses, between 420 and 566 μM PFOA resulted in a ~ 0.5 to 0.7 mmol/L reduction in cholesterol (~20 mg/dl – 25 mg/dl). Between ~ 708 to 870 μM PFOA there was a 0.8 to 1.0 mmol/L reduction in cholesterol (~30 – 40 mg/dL). EFSA did not cite the lack of changes in HDL or in liver enzymes in this six-week study.

EFSA (2020) wrote serum PFOA concentrations were about 10,000 – 150,000 ng/mL after the first dose which is a fair approximation. EFSA then stated the concentrations ranged between 150,000 – 500,000 ng/mL after six weeks. This is not correct. Based on the PK/PD modeling (shown in Figure 1 of Convertino et al.), as well as the data

themselves, a 50 mg dose of PFOA taken weekly by the "average" 75 kg subject (see Table S1) resulted in a dose of approximately 0.67 mg/kg/week and predicted and actual PFOA concentrations of approximately 60,000 ng/mL, not 150,000 ng/mL Regardless, these are much higher concentrations of PFOA in the general or environmentally exposed populations and exceeds those found in the upper range found in occupational studies (Olsen et al. 2000).

EFSA also criticizes the Convertino et al. study for its small sample size of 49 subjects. This longitudinal study lasted 7 weeks (includes the first week that had the pre-examination in order to obtain the baseline measurements). Yet EFSA did not describe as "small" the critical effects study they chose to calculate their TDI, i.e., the Abraham et al. cross-sectional study of 101 infants.

Pouwer et al. (2019):

It is unfortunate that EFSA decided not to include in their review the Pouwer et al. (2019) toxicological study who used a genetically engineered APOE*3-Lieden.CETP mouse model that mimics human lipoprotein metabolism. This study confirmed the lipid lowering high dose PFOA findings from the phase 1 clinical trial in humans (Convertino et al.). This mouse model is designed to assess cholesterol ester transfer protein (CETP) expression and a delayed apoB clearance. CETP is responsible in both humans and in this mouse model for the transfer of cholesterol ester from HDL to the apoB-containing lipoproteins in exchange for triglyceride. In three different experiments lasting 4 to 6 weeks, Pouwer et al. fed these mice a Western-type diet that had four doses of PFOA (control, 10 ng/g/d PFOA, 300 ng/g/d, and 30,000 ng/g/d PFOA which resulted in plasma PFOA concentrations of approximately < 1.0 ng/mL, 50 ng/mL, 1500 ng/mL, and 90,000 – 144,000 ng/mL, respectively. In humans these concentrations reflect environmental, occupational, and toxicological exposures with the latter concentration similar to Convertino et al. Statistically significant results were observed only at the highest dose and involved decreased total cholesterol, non-HDL cholesterol, triglycerides, and elevated HDL-cholesterol. Also reported at this high dose group were increased liver weight and elevated ALT. The plasma lipid change at the high dose was explained through a decrease in very low-density lipoprotein(VLDL) production and increased VLDL clearance by the liver via increased lipoprotein lipase activity. The increase in HDL was mediated by a decrease CETP and changes in protein expression involving HDL metabolism. This APOE*3-Leiden .CETP mouse model has considerably higher concentrations of CETP than what is found in humans.

It is the combination of the findings from Convertino et al. and Pouwer et al., under their study conditions reported, that strongly suggest that at toxicological dosages of PFOA that yield very high serum concentrations of PFOA, a decrease in total cholesterol and LDL cholesterol is likely a causal effect. Understanding of the findings from these studies allows for those who opine there is a causal effect between low concentration of PFOA and higher cholesterol levels in humans to direct their resources on the mode of action for this hypothesis, let alone a mechanism of action. Continued publication of

cross-sectional epidemiologic studies at low concentrations of PFOA are unlikely to "shed additional light."

Salihović et al. (2019):

As EFSA notes, one current leading non-causal hypothesis to explain the association between general population or environmental levels of PFOA (or PFOS) and increased cholesterol may be due to their enterohepatic circulation. Significant associations between plasma levels of PFOA and PFOS concentrations of 1.42 and 4.2 ng/mL, respectively, and bile acid levels have been reported by Salihović et al. (2019) in a "small" study of 20 subjects. Perfluoralkyls are known to downregulate CYP7A1 which is the rate-limiting step in the metabolism of cholesterol in the hepatocyte to primary bile acids. The primary bile acids are conjugated in the hepatocctyes and then both perfluoralkyls and these conjugated bile acids enter the enterohepatic circulation where the bile acids are unconjugated into bile salts by the gut microbiome. In the ileum both perfluoroalkyls and bile acids are reabsorbed into the portal venous circulated and actively transported back into hepatocytes by sodium-taurocholate co-transporting polypeptide (NTCP). Hence, the reported cross-sectional epidemiological associations between higher cholesterol levels observed in humans with lower levels of PFOA may be a consequence of this potential mode of action.

Chang et al. (2017):

Although EFSA (2020) lists Chang et al. 2017 in the references, EFSA does not discuss this insightful toxicological study. Nor was it mentioned or referenced in the earlier EFSA (2018) report. EFSA should acknowledge that Chang et al. undertook a six-month oral dose study with PFOS administered to male and female cynomolgus monkeys, with scheduled clinical assessments through 1 year, in order to evaluate markers for coagulation, lipids, hepatic, renal, electrolytes, and thyroid-related hormones. There was a time-matched control group as well as 4 weeks of baseline values for the dosed groups. The low dose group (n = 6/sex) received 1 single K^+PFOS dose (9 mg/kg) with the highest mean serum concentration measured at 68000 ng/mL. The high-dose group (n = 4-6/sex) received 3 separate doses (11 – 17.2 mg/kg) during the six-month treatment phase with the highest mean serum concentration measured at 165000 ng/mL. Liver needle biopsies performed two months after completion of the study showed the highest mean liver PFOS concentrations at 112000 ng/g. At end of study all the animals were considered healthy, gained weight, and released back to the colony. Throughout the entire study, there were no K^+PFOS treatment related changes in serum liver enzymes, serum BUN or creatinine. There was a decrease in serum total thyroxine without a concomitant change in in the clinically-relevant TSH and free T4. Authors considered the decreased total thyroxine observed was likely due to competitive displacement by PFOS with of thyroxine and its subsequent increased metabolism and elimination. The most notable observation in this study was upon treatments, the mean serum total cholesterol was decreased by approximately 4 – 12% at 1 and 3 weeks post-dose when compared with mean time-matched control or baseline values. The reduction in cholesterol was

used to determine a lower-bound fifth percentile benchmark concentration ($BMCL1_{SD}$) of 74000 and 76000 ng/mL in male and female monkeys, respectively.

Li et al. (2020):

EFSA should also be aware of a very recent study that was just published that suggested a causal association between cholesterol and PFOS and PFHxS by Li et al. (2020).

Li et al. (2020) studied associations between the perfluoroalkyls PFOS and PFHxS (and to a lesser degree PFOA) and serum lipids in Ronneby, Sweden, where one of two waterworks had been contaminated from aqueous film forming foams (AFFF). The original exposure occurred between the mid-1980s and cessation of exposure that occurred in 2013 through GAC filter installation. Three populations were reported: control (N =130) in a neighboring community that had not been exposed; 2) a recently exposed population (N = 1160) who lived in Ronneby defined as anytime between 2005 to 2013; and 3) a non-recent/uncertain exposure group of N = 655 who lived in the contaminated after works distribution area in Ronneby before 2005 but not after as well as participants who lived in the non-contaminated waterworks area in Ronneby anytime between 1985 to 2013. All participants were between 20 – 60 years of age. Median serum concentrations (ng/mL) were for the control, non-recent/uncertain, and recently exposure groups were, respectively (ng/mL): PFOS 4.8, 45, 240; PFHxS 0.98 40, 210; and PFOA 1.6, 3.5, 13.

Comparing the control to total (combined) exposure group revealed a significantly increase in total and LDL cholesterol but not HDL, triglycerides or the total/HDL ratio. Analyzed each separately, the strongest positive lipid associations were reported for the recently exposed. Even among the controls there were modest significant associations with PFOS and PFHxS. Similar to Steenland et al. 2009, decile analyses suggested the strongest associations (slopes) were observed at the lowest concentrations (up to about the 40^{th} percentile). The recently exposed group had the highest odds ratio for high cholesterol. Li et al. conclude their findings provided additional evidence of a causal association between PFAS and serum lipids, including PFHxS, as is not necessarily confounded by the possibility of reabsorption of bile acids with the measurement of serum PFAS as some of their analyses were by categories of exposure not measurement. However, they acknowledged important limitations including (again) a cross-sectional study design, disparate socioeconomic (SES) differences between the exposed and control populations (the latter having higher SES), the lack of information on cholesterol lowering medications, and unknown dietary habits of the population. Also, the preference by the authors to cite literature that supported their position of a causal association was present. For example, they wrote humans may be less active than the rodent to the lipid lowering effects of PPARα but neither the Pouwer et al. nor Convertino et al. studies mentioned suggesting hypolipidemic associations are likely to occur at very high (i.e., toxicological) perfluoroalkyl concentrations. Li et al. also cited a paper that had promoted the idea of a "hypercholesterolemic environment" with PFOA through its effect on the expression of genes involved in human cholesterol transport and

metabolism (Fletcher et al., 2013). However, they did not to cite the evidence presented against this hypothesis by Vanden Heuval (2013).

APPENDIX C

Other Comments

Biomonitoring

Based on the summary data provided on lines 2504 – 2511 of the draft scientific opinion, 3M requests that EFSA provides the actual concentration trends and percentage of declines in graphical formats instead of the text data presented in Appendices B1 – B4. On lines 6284 – 6289, 3M again requests that actual data be presented rather the general terminologies used to describe general biomonitoring data. Clearly, PFOS and PFOA have declined in the European general population since 2000.

PFBS and thyroid hormones in rats (cf. NTP 2019a in the draft scientific opinion):

The NTP 28-day with PFBS (identified as NTP 2019a in the EFSA draft scientific opinion) reported decreased serum total T4, total T3, and free T4 in rats at the end of 28 days dosing, however, these three endpoints alone did not provide adequate (clinical) evidence to suggest that thyroid was being affected.

First, NTP study did not sufficiently recognize the sensitivity of the assays used to measure serum thyroid hormones to the presence of compounds, such as PFBS, that can interfere and compete with thyroxine for protein bindings. In such situations, this interference can negatively bias the free T4 results when conventional analog methods are used. This is in fact the case with PFBS and other PFAS such as PFBA and PFOS (Chang et al. 2007 Toxicology 234 21-33; Weiss et al. 2009 Toxicol Sci 109 206-216; Butenhoff et al. 2012 Reprod Toxicol 33 513-530). Therefore, the workaround is to measure free T4 by *equilibrium dialysis*-based methods. This was not done by NTP, as acknowledged in its report. Furthermore, total T4 and total T3 represent primarily biologically inactive T4 and T3. Thus, the total T4 and T3 and the analog free T4 do not provide sufficient or definite answers as to thyroid effects. Because of the resulting questionable confidence in the analog assays, thyroid histology in laboratory animals should be used as the gold standard to determine whether there was a thyroid effect. The thyroid histology was normal as reported in the NTP study, as well as in other subchronic studies (28-day and 90-day) in rats with PFBS (3M 2001; Lieder et al. 2009 Toxicology 255 45-52).

Second, it is imperative to recognize the serum TSH levels is the primary diagnostic indicator for thyroid hormone status. Given that there were normal TSH levels (primary diagnostic indicator for thyroid hormone status) and normal thyroid histology in these same rats (where decreased serum total T4, total T3, and free T4 were reported as measured by analog method only), this suggested that overall thyroid hormone status in these rats was normal.

PFBS and thyroid hormones in mice (cf. Feng et al. 2017 in the draft scientific opinion):

Similar to the comments provided above for the NTP 28-day study, the mouse developmental study (identified as Feng et al. 2017 in the draft scientific opinion) reported decreased total T4, decreased total T3, and normal TSH in serum at birth for female pups. Again, total T4 and total T3 alone did not provide adequate (clinical) evidence to suggest that thyroid was being affected, especially when TSH, the primary

diagnostic indicator for thyroid hormone status was normal. Feng et al. did not provide the sufficient information to allow a full interpretation of thyroid status.

In addition, there are several technical concerns regarding about this study:

- The observations from Feng et al. (2017) study need to be validated.

  There was a total of eight individual serum hormones measured and reported by Feng et al. (2017) based on the blood samples collected from the newborn mice; and each of the hormones was measured using the commercial ELISA kits obtained from USCN Life Science Inc., as described in the paper. According to the manufacturer's information (see https://www.cloud-clone.us), each ELISA kit requires 50 uL of serum sample volume. Given that a newborn mouse pup is quite small in size (approximately 1 gram), it is not clear how Feng et al. was able to measure all the hormones with such a limited blood volume. To better understand this, 3M consulted with Charles River Laboratories who concluded that, if they were to repeat the Feng et al. study, at least 75 dams per dose group would have been needed to achieve the blood sample volume required for the specified hormone measurements. Feng et al. only had 30 dams per dose group.

- The discrepancies between mouse and rat developmental data need to be addressed.

  The developmental endpoints from the short-term gestation exposure study in mice by Feng et al. (2017) were vastly different than the outcomes from the full 2-generation study in rats by Lieder et al. (2009). These differences need to be properly assessed before a scientific conclusion can be made. Key observations included effects reported by Feng et al. lacked dose-responses; the effects from 200 mg/kg-d were usually similar in magnitude to 500 mg/kg-d. It is worth noting that the study design and PFBS dosing regimen by Lieder et al. (2-generation in rats) was more rigorous than Feng et al. (gestational only in mice) in terms of treatment duration, doses, as well as direct treatments to developing

			Lieder et al. 2009	Feng et al. 2017
Species			Sprague Dawley rats	ICR mice
Test guideline			OECD 416 / OPPTS 870.3800 (2-gen)	None
GLP			Yes	No
Daily K⁺PFBS treatments (direct gavage)	Daily doses		30, 100, 300, 1000	50, 200, 500
	P-generation	Pre-mating, males	Yes, 70 days	No
		Pre-mating, females	Yes, 70 days	No
		Gestation, dams	Yes	Yes
		Lactation, dams	Yes	No
	F1-generation pups (before mating)	Weaning and on	Yes, ≥ 70 days	No

fetuses and pups during sensitive life stages, see Table below for comparison.

In addition, It was not clear why Feng et al. did not include male offspring in their evaluation. The female mouse offspring in the Feng et al. study were not directly dosed with K^+PFBS, however, the reported myriad of adverse developmental outcomes occurred in these female mouse pups (e.g., reduced body weight and changes in reproductive organ morphology). In contrast, female rat offspring (from Lieder et al. 2009) were not only exposed to PFBS during gestation and lactation, they were also directly dosed with PFBS (at higher dose levels than the Feng et al. study) after weaning and into their adulthood. There were no developmental effects noted in the female rat pups in Lieder et al. study.

- Regarding the alterations in ovary and uterus-related data, as reported by Feng et al., there were several technical details not provided by the study authors which precluded a meaningful interpretation of the data. They include:

  - Evaluation was reported for female pups at PND 60 only, not on PND 30 and not for dams (who were directly dosed with PFBS).
  - "Impaired" development reported by Feng et al. was based on decreased surface area (on microscopic slides) and limited morphological measurements. Surface area can be also attributed from different sectioning location (of the tissue). Feng et al. did not address how this was controlled among different animals. In addition, Feng et al. only provided relative organ-to-body weight data. There were no absolute organ weight data for the readers to interpret. Organ-to-brain weight data were not presented either.
  - Feng et al. did not take body weight into consideration when interpreting estrous cycle data which is unfortunate because they are related (Bermejo-Alvarez et al. 2012).
  - In Feng et al. (2017), albeit there were changes in female reproductive organ morphology, functional aspects of reproduction appeared not to be affected according to study authors (i.e., maternal body weight, maternal body weight-gain, and various pregnancy outcomes).

<u>PFASs and mode of action related to alterations in thyroid hormone levels in rats:</u>

Speaking of thyroid hormone status, it is imperative to understand that in the laboratory animal studies, thyroid pathology is considered as the gold standard (when feasible). If is not feasible to obtain thyroid pathology, then the primary clinical indicator for diagnosing thyroid function is TSH, which is essential in maintaining the regulatory functions in the hypothalamus-pituitary-thyroid (HPT) axis. In the event there is a need for supplementary verification, it would be appropriate to measure free thyroxine (FT4), the metabolicaly-active hormone, in conjunction with TSH. It is inappropriate to use serum TT4 as a diagnostic index because it is a measurement of bound (inactive) hormone (Mendel et al., 1986; Oppenheimer et al., 1995; Refetoff et al., 1970).

Studies in laboratory animals have reported that PFOS and other PFAS treatment may impact serum thyroid hormones. Using studies with PFOS as an example, serum total thyroxine (TT4) was decreased without a concomitant compensatory increase in TSH

(Lau et al., 2003; Luebker et al., 2005; Seacat et al., 2002; Thibodeaux et al., 2003). This condition is usually referred to as hypothyroxinemia, which is a condition where inactive protein-bound thyroxines are being displaced from binding proteins (i.e, due to competition with PFOS for binding proteins) without altering overall thyroid hormone homeostasis. Hypothyroxinemia is a condition where thyroid homeostasis is maintained and it is commonly observed with people taking aspirin, heparin, or free fatty acids (Koulouri et al., 2013).

In rodents, hypothyroxinemia is prone to occur due to their susceptibility to hepatic nuclear receptor activation. Upon activation of PPARalpha and CAR/PXR (like many PFAS do), the increased hepatic metabolic enzyme activities are often accompanied by increased conjugation and elimination of displaced thyroxines which ultimately lead to a net loss of thyroxine. However, the loss of thyroxine alone is not a disease diagnosis because the body has a rather reservoir of inactive (protein-bound) thyroxine and only very little of free (unbound) thyroxine is needed to maintain normal thyroid homeostatic functions. Hypothyroxinemia is not to be confused with hypothyroidism, the latter is a disease condition characterized by high serum TSH level with concomitant low (active) free thyroxine as well as thyroid hypertrophy.

In the case of PFAS, as discussed earlier, one needs to recognize the sensitivity of the assays used to measure serum thyroid hormones to the presence of compounds that can interfere and compete with thyroxine for protein bindings. In such situations, this interference can negatively bias the free T4 results when conventional analog methods are used. This is in fact the case with PFBS and other PFAS such as PFBA and PFOS (Chang et al. 2007 Toxicology 234 21-33; Weiss et al. 2009 Toxicol Sci 109 206-216; Butenhoff et al. 2012 Reprod Toxicol 33 513-530). Therefore, it is a must to measure free T4 by *equilibrium dialysis*-based methods when there are high levels of PFAS present in the serum.

Due to the misinformation and incorrect interpretation on thyroid-related parameters, subsequent studies with PFOS have highlighted the need to properly assess thyroid hormone function (Chang et al., 2017; Chang et al., 2009; Chang et al., 2008; Chang et al., 2007). Studies with PFOS in rats and monkeys did not show any effect on serum TSH and/or thyroid pathology when available (Chang et al., 2017; Chang et al., 2009; Chang et al., 2008; Chang et al., 2007; Lau et al., 2003; Luebker et al., 2005; Seacat et al., 2002; Thibodeaux et al., 2003). The observation of hypothyroxinemia indded corresponded to the increased hepatic gene transcripts reflecting increased metabolic turnover of thyroxine. The deiodinases, when evaluated, were not affected.

<u>PFHxS and liver and reproductive / developmental effects in mice (cf. Chang et al. 2018 in the draft scientific opinion):</u>

3M respectfully disagrees with EFSA's interpretation on the results of liver findings from Chang et al. (2018). As the primary contributing authors of this study, our conclusion on liver effects remained the same in that "*the microscopic findings in the liver were considered to be consistent with an adaptive response*" (cf. Chang et al. 2018). This conclusion was based the weight of evidence and corroborative data. The observation of hepatocellular lipid vesicles was of uncertain genesis and significance, it was likely to be

associated with the lipid metabolic changes induced by nuclear receptor-mediated effects. The necrotic hepatocytes were some minimal randomly distributed foci of necrosis in the liver (including one male mouse from the control group), but this type of necrosis is occasionally seen as an incidental background finding in laboratory rodents. Even though there were increased incidence of single cell necrosis noted in the highest dose group males, however, RT-PCR transcript markers associated with cellular stress were not increased in these mice.

3M also respectfully disagrees with EFSA's interpretation on EFSA's conclusion in that "the most sensitive reproductive endpoint for PFHxS exposure was reduced litter size" (cf. Section 3.3.3.3.4 of the draft scientific opinion). Even though there was a slight but statistically significant decrease in the mean live litter size in the 1 and 3 mg/kg-d groups; however, the toxicological significance of this finding is unclear based the lack of a clear dose-response; pup to implant ratios were similar among control and the treated groups; and the lack of other negative effects on development or reproduction. Similarly, at similar serum PFHxS concentrations, live litter size was not affected in Wistar rats (Ramhoj et al. 2018 Toxicol Sci 163 579-591).

Kidney effects and PFSAs (cf. section 3.3.3.232 summary of the draft scientific opinion):

3M respectfully disagrees with EFSA's conclusion that "alterations in the kidney" was documented repeatedly from toxicity studies with PFBS, PFHxS, and PFOS. Kidney has not been identified as a target organ for repeated dose studies with PFHxS or PFOS. Even under the most enduring dosing administration of 2-year chronic bioassay in rats with PFOS, there was no PFOS treatment-related effects in kidneys (Butenhoff et al. 2012 Toxicology 293 1-15). With PFBS, the kidney alteration effects were only noted under extremely high dosing conditions where the microscopic kidney effects (mild tubular hyperplasia and papillary edema) were not associated with functional impairment or damage. This may have been due to a response to high concentrations of PFBS passing through the kidney and into the urine; because PFBS is excreted rapidly in the urine with a serum elimination half-life of several hours in rats, almost 90% of each daily administered dose would be present in the daily urine output of these rats.

PFOA and mammary gland effects:

For PFOA, 3M respectfully disagrees with EFSA's conclusion that "the most sensitive developmental effect observed is delayed and impaired development of the mammary gland upon prenatal and early postnatal exposure". In addition to the high-level summary stated above in the main text, the technical comments that 3M submitted to ECHA in 2014 regarding about the study by Macon et al. (2011) are provided below to illustrate the fact that there are numerous areas of scientific uncertainties on this particular topic. Macon et al. (2011) was considered (and then withdrawn) by ECHA for its ANNEX XV restriction proposal on PFOA in 2014

(1). Study design & outcome

Macon et al. examined the effects of exposure to various ammonium PFOA (APFO) concentrations during gestation on mammary gland development in progeny born to CD-1 mice. A subset of females was dosed with APFO during almost the entire gestation when they received either 0 (vehicle control, DI water), 0.3, 1, or 3 mg/kg/day APFO from gestational day (GD) 1 – 17. In a separate study, other gestating females received APFO from GD10-17, at levels of 0 (vehicle control, DI water), 0.01, 0.1, or 1 mg/kg/day APFO. Treatment with APFO, a known agonist for xenosensor nuclear receptors such PPARα and CAR/PXR (Elcombe et al. 2010) resulted in increased liver weight in all offspring from dams exposed to APFO from GD 1 – 17, and also in offspring from dams exposed to 1 mg/kg/day APFO from GD 10 – 17. Even though hepatic hypertrophy appeared to be dose-dependent, the authors concluded there was "*significantly stunted mammary epithelial growth*" concomitant with fewer terminal end buds (TEBs) for all offspring and that a no observable adverse effect level for delayed mammary gland development could not be established.

(2). Inadequate animal acclimation procedure

The pregnant mice used by Macon et al. were only acclimated to the new environment for *one* day between the time of arrival and the administration of APFO at the study facility. Given the fact that these mice were newly impregnated and had gone through various physical and environmental stresses in less than a week (*e.g.*, co-habituation with a male, mating, becoming pregnant, transportation-induced physiological changes, adaptation to a new vivarium with inherent differences in environmental conditions), it is hard to imagine that these mice did not experience undue stress between the day of arrival and the start of the study. For reasons such as these, many institutions require a minimum acclimation period for mice of 3 - 5 days prior to the initiation of any experimentation on animals (ILAR 1996).

A further reason for concern with maternal stress arises when the authors stated that 15% of females were not pregnant "*as expected*". The rationale for this so-called expectation was not explained or justified. Given that such rates of loss were stated to be unrelated to PFOA exposure, this outcome further suggested that dams were stressed. Macon et al. did not provide any indication of what treatment groups these losses occurred in or to what extent. They described that n=13 pregnant dams were assigned to each treatment group, yet went on to say that 15% of dams were not pregnant; thus, group sizes of n=13 could not have been realized in the final study.

On a related note, dams in the full-gestation study were transported around day 0 of gestation, whereas dams in the latter study (late gestation exposure) were transported around day 8 of gestation. Thus, while all females experienced the same aforementioned short (1 day) acclimation period, this stressful experience was superimposed on different stages of fetal development, which may confound any extension of results.

(3). Maternal health

Guidance from the European Union, Section 3.7.2.4.1. states:

> *"Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms."*

Therefore, it is important to be able to differentiate whether the developmental effects associated with APFO occurred in the presence or absence of marked maternal toxicity. The fact that Macon et al. did not provide any body weight data for the pregnant dams is unusual and disconcerting. Body weight data is an easy and objective clinical endpoint to measure and it is often the primary clinical index used to ascertain the well-being of an animal, especially those that are pregnant. In addition to lack of maternal body weight data, no data were provided for maternal liver weight or maternal PFOA concentrations.

(4). Litter handling / Sample selection bias

In their study, Macon et al. took newborn pups on postnatal day (PND) 1 and randomly distributed them with pups from other dams in the same treatment group. This allocation resulted in unequal numbers of pups per litter, with 7 – 9 pups per litter (4 – 7 females per litter).

A well-planned developmental study would have attempted to cull and reach equal number of pups (per litter) with natural dams when possible. The authors stated they mixed pups and litters to be consistent with the approach used in their previous study (White et al. 2007). It was not clear why pups were randomly distributed to different dams because the instinctive and protective nature of a lactating dam (i.e., sensory recognition) can compromise the quality of the care for, and even the survival of, the foster pups. This oversight may be the reason why there were unequal numbers of pups per litter.

This oversight in experimental design may also be the reason why there were insufficient control female pups survived until PND 63 for sampling. Based on the experimental description, given that n = 13 dams (a seemingly sufficient number) was assigned to each treatment group, approximately n = 11 dams would have been expected to produce litters (with the expected 15% parturition loss). Given that the litter sizes were normalized to 4 - 7 females/litter, there should have been approximately 44 - 77 female pups available for necropsy across the 7 different postnatal ages (PND 7, 14, 21, 28, 42, 63 and 84) with a minimum of 6 female pups or more per postnatal time point for evaluation. It was not clear nor discussed by the authors as to why there were insufficient control female pups on PND 63. This not only raises the question as to the cause(s) and occurrence of postnatal death in the control group, it also reflected a poor study design and a lack of knowledge in animal handling.

(5). Mammary gland biology end points

a. Subjective scoring system for mammary gland development

The methods used by Macon et al. for assessing mammary gland development in offspring were performed *subjectively* on whole mounts using a categorical scale of 1 – 4 (1 = poor development and 4 = best development). In using this approach, the authors attempted to describe many different variables within the mammary glands as a single value rather than scoring or quantifying each variable. It is critical to recognize that the mammary glands undergo several developmental processes at once (i.e., ductal growth, branching, alveolar budding) and each of these landmark events must be quantified individually. Also, each of these processes is sensitive to different developmental and reproductive cues, and any comparisons of mammary gland development need to take the accompanying biology into account, such as age, metabolic bodyweight, stage of estrous cycle, and onset of ovarian function. It is worth noting that Macon et al. did not provide any information regarding stage of the estrous cycle, sex hormone concentrations, or histology of the reproductive organs. These baseline facts should have been adequately established to allow for a proper overall assessment.

What is most disconcerting is Macon et al. combined a subjective assessment of each variable within the mammary glands (i.e., ductal growth, branching, and alveolar budding) and integrated them into a single score that was not generated mathematically. The relative contribution or weighting of each variable in the final subjective score was never defined. The statement "*It should be noted that statistical differences found in a single quantitative endpoint did not necessarily determine aberrant development; rather, all quantitative and qualitative measurements were collectively utilized to determine overall developmental mammary gland scores*" reflected the fact that an undefined method was employed to generate their final scores for mammary gland development.

There are several significant limitations to this subjective scoring approach. A subjective scoring system precludes repetition by other laboratories, even those skilled in the art of mammary gland biology, given that the precise nature of the categorical scale is never documented. Moreover, even within the same laboratory there appears to be inconsistent definition and use of this scoring system. In their study, Macon et al. used a scale where 1 = "*poor development*" and 4 = "*best development*" that they described as being similar to methods described by other laboratories (Hilakivi-Clarke et al. 1997a, Hilakivi-Clarke et al. 1997b, Welsch et al. 1988). It is interesting to note that 2 of the 3 referenced papers (Hilakivi-Clarke et al. 1997b, Welsch et al. 1988) used rats (not mice) as the test subjects. In addition, the development of the mammary glands in rats is considerably different from that in mice, thereby raising questions as to how the scale was developed or implemented. The remaining referenced study also used CD-1 mice (Hilakivi-Clarke et al. 1997a), but that paper did not provide sufficient information that would enable replication of their scoring method.

Macon et al. described that "*Scores were based on qualitative and quantitative histological characteristics of each developmental time point, including, but not limited to, lateral and longitudinal epithelial growth, change in epithelial growth, appearance of budding from the ductal tree, branching density, and number of differentiating duct ends (Hilakivi-Clarke et al. 1997a). Where applicable, at a given time point, mammary glands from both studies were compared on the microscope to ensure consistency in the scoring scale between studies*". It is unclear what other variables contributed to the subjective score given the results were not limited to those variables detailed above.

By contrast, in a similar study from the same research group, White et al. (2011) used a scale where 4 = "*excellent development/structure*" and 1 = "*poor development/structure*". The number of primary ducts and large secondary ducts, lateral side branching, appearance of budding from the ductal tree, and longitudinal outgrowth were assessed. Thus, in two studies from the same laboratory (Macon et al. and White et al.) published in the same year, there was variation between the scoring criteria and strategies used. Likewise, in both cases, it was not clear whether "*best development/structure*" and "*excellent development/structure*" scores are synonymous, and whether a score of 4.0 represents that of an average control gland for a given age, which one might expect. In another instance, while Macon et al. reported the control mammary glands at PND21 with average scores of 3.3 (see Table 1, Macon et al. 2011) and 3.4 (see Supplemental Table 3, Macon et al. 2011), a recent study from the same laboratory, control glands from CD-1 mice at PND21 received a mean developmental score of 2.9 (Tucker et al, 2014). Even though the exact measures used to compute this score was not documented, it did appear that a score of 4.0 was realizable for control glands, as occurred at PND 84 (Macon et al. 2011).

Similarly, there appeared to be considerable variation among the population of CD-1 mice in this laboratory at PND21. The only data that were found to be statistically different at the 0.01 mg/kg dose at PND 21 was the value for this subjective developmental score (see Table 4, Macon et al. 2011). Even at 0.1 mg/kg, statistical differences were only detected for this subjective score and another quantitative measure of terminal end bud number. The ductal tree in the mammary glands of control females at PND 21 had only outgrown a few millimeters (see Figure 1A, Macon et al. 2011). By contrast, in comparable control CD-1 females at PND 21 in a recent paper from the same laboratory, the mammary ducts at PND21 have already reached the supramammary lymph node (see Figure 2A, Tucker et al. 2014), although again, no quantification of mammary growth was performed in that study. This difference is on the order of several millimeters, which relative to the size of the ductal tree at PND21, is substantial. *This dramatic difference in mammary gland development within the control population of CD-1 mice from this facility raises concern about how the mammary gland is being used as a toxicological end point*.

Another consideration that warrants further evaluation concerns the incorrect statistical inferences that have been made in analyzing the subjective mammary gland scores. By using a subjective scale, Macon et al. utilized a categorical method to generate their data. In performing their statistics by analysis of variance they assumed (incorrectly) that their mammary development scoring system increased linearly and/or with consistent increments. This assumption and statistical test is fundamentally invalid, further calling into question any conclusion about the low dose effects reported by Macon et al.

b. Lactation performance of dams as a critical variable

Another important consideration for this study and any study of gestational exposure concerns the consequences for the dam as she goes on to rear offspring. Specifically, the process of lactation is sensitive to a number of factors that can impact a dam's ability to provide milk to her offspring, thereby suppressing their development and that of their organs, including the mammary glands. Two processes that are most susceptible to such exposures are: 1) functional development of the dam's mammary glands during pregnancy in readiness for lactation; and 2) dam's ability to metabolically adapt to the massive nutrient demands of milk synthesis and secretion.

Studies by the same research group had suggested that exposure of pregnant mice to PFOA impaired the ability of the maternal mammary glands to undergo full growth and functional differentiation (White et al. 2007; White et al. 2009). In these studies, dams exposed to 5 mg/kg PFOA during gestation weaned pups at PND20 that were 33% lighter in bodyweight than controls (White et al. 2007), while White et al (2009) also found reductions in weaned bodyweight following *in utero* plus lactational exposure to 3 mg/kg PFOA. It is unclear why Macon et al. did not find this same effect on progeny bodyweight at the 3 mg/kg dose.
Regardless, one must consider the potential for one or more aspects of pre-weaning development to be disrupted as a result of impacts on the lactational capacity of the exposed dams. A point that is relevant to the findings by Macon et al. is that growth of the mammary glands in female mice offspring before the onset of allometric growth at puberty is isometric – that is, mammary gland development is proportional to body size when it is expressed as a function of their "metabolic bodyweight" (typically considered to equal $BW^{0.66\text{-}0.75}$). Hence, any measure of mammary gland development should be expressed relative to metabolic bodyweight, not merely total bodyweight. This type of correction cannot be performed for subjective scores.

A parallel consideration that must be taken into account is the energy expenditure by dams when litter size varies as it did in this study. Each unit volume of milk secreted contains considerable energy derived from the dam's reserves and from her nutrient intake. This point is relevant when considering the work by Macon et al. given that litter size varied. For example, in full-gestation exposure study the authors state they balanced litters to 10 pups despite not being able to realize a 50/50 male/female target ratio. In late-gestation exposure study the authors declared they had litter sizes ranging from 7-9 pups. A difference in litter size such as this can dramatically affect maternal performance – a dam feeding 7 pups expends considerably less energy for milk production than a dam feeding 10 pups in a litter. In turn, these differences in metabolic state of the dam can have major ramifications for milk yield and quality that can then go on to affect many aspects of pup growth and development.

(6). Data presentation bias

The authors provide a few quantitative measures in terms of mammary gland measurement for a subset of the study samples (from the late gestation study; see Table 1, Macon et al. 2011) although it is not clear (or explained) why no similar data were shown for the mice from full-

gestation study (subjective scores only, supplemental data Table 3). Thus, one must consider that the data set, as presented, is incomplete. It should be noted that Macon et al. disregarded significant outliers without explanation.

Regarding the mammary gland assessments for the full gestation study, Macon et al. stated that there were histological characteristics similar to previous findings; unfortunately they did not show any histological data at all.

The representative mammary glands presented in Figure 1 from Macon et al. (2011) did not align well with the author's claims. Macon et al. stated that mammary glands from the 0.3 and 1.0 mg/kg treatment groups were less developed, however the variation was substantial and much of this could be explained by variables such as individual differences, stage of estrous cycles, or lack of, for that matter. In particular, it should be noted that being an outbred strain, CD-1 mice have more inherent variation within their phenotypes. Macon et al. also emphasized that in the mature mouse mammary gland "….*in the adult mouse at PND 84, there are no TEB*s". However, there did not appear to be any visual differences in the distribution of TEBs presented as the examples in histological sections of the mammary glands for PND 84 between control (Figure 1D) and female pups from 0.3 mg/kg (Figure 1E) and 1 mg/kg (Figure 1F) dose groups. This raises the question whether the qualitative scores used by Macon et al. have a strong foundation based on histological analyses. The Restriction Report should re-examine the histology data presented by Macon et al.

Regarding the late gestation study, the authors reported reduced elongation at PND 14 by 14.4 and 37% in the 0.1 and 1 mg/kg doses (see Table 1, Macon et al. 2011), whereas in Figure 4 the most pronounced reduction is at PND 21. This figure would have benefited from counting number of ductal branches. The authors show reduced TEB number at PND 21 in Table 1; it is unclear however where the other quantitative data for the rest of the experiment are.

Conclusion

The study by Macon et al. (2011) was flawed in several important aspects of study design and had numerous instances of inappropriate data interpretation. The authors failed to consider all aspects of biology and rather than scope out the best objective endpoints for the assessment, the study gave very few quantitative measures. The authors had attributed various phenotypic consequences (i.e., reduction in mammary gland development) to the direct effects of PFOA. Alternative interpretations suggest that PFOA may be affecting mammary gland function in the lactating dams. Without any supporting evidence for maternal well-being, the data presented by Macon et al. are built on a great deal of speculation with a lack of definitive reproductive data combined with a lack of quantitative mammary gland analysis.

Subsequent to this report, a follow-up manuscript was recently published by the same group (Macon et al. 2014, doi: 10.1093/toxsci/kfu253) in which they examined gene expression within the mammary glands of mice exposed to PFOA. However, this publication was later *retracted* due to erroneous data presentation. In conclusion, 3M believes there are important technical reasons to question the study data by Macon et al. (2011). The fact that the effects of PFOA on mammary gland development cannot be consistently described and quantified in all mouse models brings into question the biological significance of this phenotype as described, and its relevance to human health is unclear.

Comments on Pachkowsky et al. (2019):

3M respectfully disagrees with EFSA's review of the Pachowski et al. paper. According to Pachowski et al (2019), they attempted benchmark dose modeling of the data for the dcreased PFC response endpoint from the Dong et al. (2009) study using EPA benchmark dose software (version 2.6.0.1). Using all six data points from the Dong et al study,

Pachowski reported that none of the available benchmark dose models gave an acceptable fit. Eliminating the largest dose group because it gave a disproportionately large decrease PFC response, Pachowski stated there were some models gave acceptable fits with 5 doses, none met the assumption of constant variance. Consequently, Pachowski et al. wrote that since a BMDL could not be derived, they identified a NOAEL serum concentration of 674 ng/mL for decreased PFC response from the Dong et al. 2009. study as the POD.

Using this NOAEL, Pachkowski calculated a Target Human Serum Level:

Target Human Serum Level = NOAEL / Uncertainty Factor
= 624 ng/mL x 30
= 22.5 ng/mL

Pachowski then calculated Clearance (CL) as follows assuming a human-life for PFOS = 5.4 years (based on an arithmetic mean from Olsen et al. 2007) and a PFOS-based volume of distribution (Vd) of 0.23 L/kg from the USEPA:

$CL = Vd \times (\ln 2 / t_{1/2})$
$= 0.23 \text{ L/g} \times (0.693 / 1972 \text{ days})$
$= 8.1 \times 10^{-5} \text{ L/kg/day}$

To calculate the reference dose (RfD), Pachowski et al. used the following equation:

RfD = Total Human Serum Level x CL
$= 22.5 \text{ ng/mL (which } = 2.25 \times 10^{4} \text{ ng/L)} \times 8.1 \times 10^{-5} \text{ L/kg/day}$
= 1.8 ng/kg/day

Pachkowsi et al. are all employees in the State of New Jersey Division of Environmental Protection which relied on the NJ Drinking Water Quality Institute for which two of the three authors are members. Within this state agency, this RfD was used to calculate a draft drinking water advisory value for PFOS.

The DWQI states that "The first step in dose-response analysis is identification of a Point of Departure (POD), which is the dose within or close to the dose range used in the study from which extrapolation begins." DWQI also recognized that "if a Benchmark Dose can be developed, it is **preferred** for use as the POD." Additionally, DWQI recognized that "Benchmark dose modeling is identified by the USEPA as **the preferred** approach for dose-response modeling when the available data are sufficient to support it."
Relying on Pachowski et al., DWQI reported that it was unsuccessful in its attempts to compute a BMD or BMDL based on the PFOS-included plaque forming cell response (PFCR) reported by Dong et al. (2009). As a result, it subsequently used the serum NOAEL of 674 ng/mL from the study as the POD for its MCL derivation.

3M's review of DWQI's BMD modeling discovered a major technical error in DWQI's BMD modeling (see details below). If corrected, an acceptable serum PFOS BMDL can be derived; specifically, a $BMDL_{1SD}$ of 3,400 ng/mL.

As NJDEP has recognized, a BMD and/or BMDL is the recommended and "preferred" approach for deriving a POD value. Accordingly, NJDEP should adopt the serum

$BMDL_{1SD}$ and revise its POD value for PFOS. Because the serum $BMDL_{1SD}$ (3,400 ng/mL) is five times higher than the serum NOAEL (674 ng/mL), the PFOS MCL should be raised by a factor of five to 0.065 µg/L (0.013 µg/L x 5 = 0.065 µg/L).

1) 3M believes DWQI erroneously used standard error and not the required standard deviation in its BMD modeling.

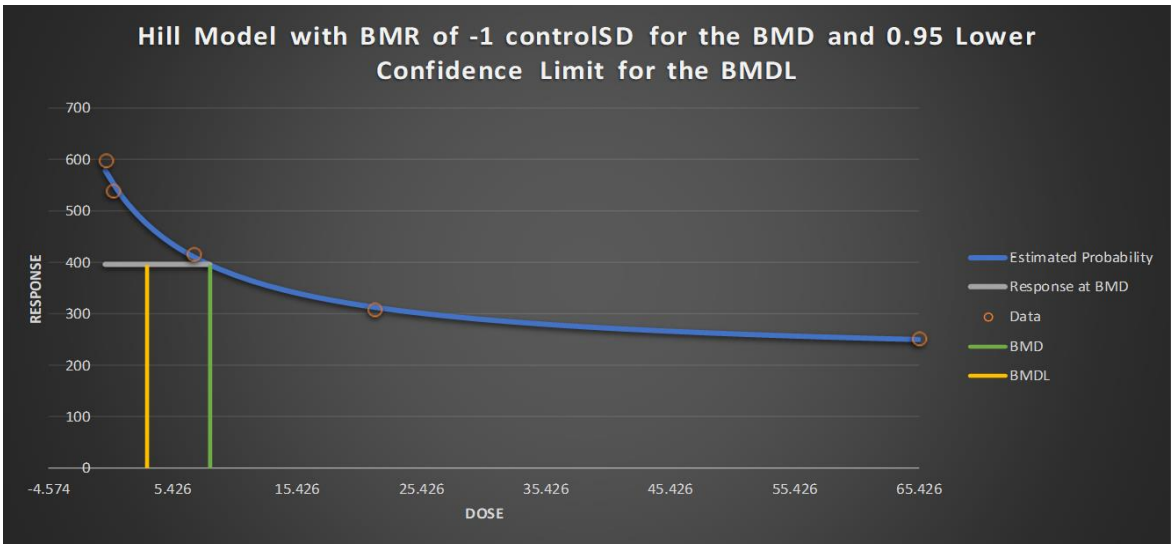
   Doses, number of animals, mean responses, and standard deviation are required to model summarized continuous response data using USEPA's Benchmark Dose Software (BMDS). According to DWQI's BMD modeling results for Dong et. al. (2009) PFCR data (*cf*. pages 236, 891 – 972, Appendix A - Health-Based Maximum Contaminant Level Support Document Perfluorooctane Sulfonate (PFOS)), the values in the standard deviation column are instead the standard error of mean values (SEM) provided by the study authors. This was a major modeling mistake by the DWQI. DWQI should have converted standard error to standard deviation by multiplying the standard error values by $\sqrt{N}$ ($\sqrt{10} \approx 3.16$). Therefore, its conclusion that the BMD modeling of the Dong et al. (2009) data did not give an acceptable fit to the data was based on faulty information.

2) $BMDL_{1SD}$ 3,400 ng/mL should be the POD for Dong et al. (2009) PFCR data

   The "correct" standard deviation can be derived by taking SEM x $\sqrt{10}$. With this corrected value, the dataset from Dong et. al. (2009) was modeled using USEPA Benchmark Dose Software (BMDS) version 3.1., a lowest $BMDL_{1SD}$ (3,400 ng/mL serum PFOS) and lowest AIC and was deemed to be the "best" fit for the dataset. Specifically, the serum PFOS concentration vs. PFCR response dataset (minus the high dose group) was modeled using Exponential, Hill, Linear, Polynomial, and Power models, both with and without parameter restrictions. All models were run using 3 user-defined options sets which assumed 1.) responses are normally distributed and variance is constant across dose groups; 2.) responses are log-normally distributed and variance is constant across dose groups; and 3.) responses are normally distributed and variance is non-constant (i.e. varies as a power function of the mean response. For all model runs, the benchmark response (BMR) was set to one control standard deviation and a BMDL equal to the 95% lower confidence limit on the BMD was calculated. Model viability was assessed on the basis of goodness-of-fit P-value, AIC, and visual inspection of graphs in accordance with BMDS technical guidance. The restricted Hill model assuming normally-distributed responses and non-constant variance had the lowest BMDL (3,400 ng/L serum PFOS) and lowest AIC and was deemed to be the "best" fit for the dataset (see Table 3).

Table 3: Benchmark Dose analysis (V3.1) for a 1 control standard deviation change in plaque forming cell response from PFOS administration in mice (Dong et al. 2009) – excluding highest dose group

Model	Serum PFOS (µg/mL)			Test 4 P-Value	AIC	BMDS Recommendation	
	BMD	BMDL	BMDU			Viable?	Notes
Exponential 4 (NCV)	10.03	5.10	24.02	0.74	626.74	Viable - Alternate	
Exponential 5 (NCV)	9.98	5.09	24.02	0.74	626.74	Viable - Alternate	
Hill (NCV)	***8.43***	***3.40***	***25.59***	***0.78***	***626.65***	***Viable - Recommended***	***Lowest AIC***



3) DWQI's rationale for concluding that the Dong et al. (2009) PFCR data is not amenable to benchmark dose modeling was incorrect.

DWQI performed benchmark dose modeling after excluding the high dose group which yielded 4 models with acceptable fits to the dataset:

- Restricted Hill Model, constant variance
- Restricted Hill Model, non-constant variance
- Unrestricted Hill Model, constant variance
- Restricted Hill Model, non-constant variance

The models that assumed constant variance were rejected because the constant variance test failed (Test 2 P-value was < 0.05), and we agree that the BMDLs calculated for these models should be used with caution. However, the version of BMDS that DWQI used (ver. 2.6.0.1) was unable to calculate BMDLs for non-constant variance Hill models. This software-based limitation has since been resolved in the more recent release of BMDS version 3.1. In fact, when we repeated DWQI's analysis (dropping the top dose and incorrectly entering standard error into the standard deviation column) using the most up-to-date version of the

software, there were 3 viable models with calculated BMDLs obtained under the assumption of non-constant variance: Restricted Exponential 4, Restricted Exponential 5, and Restricted Hill.

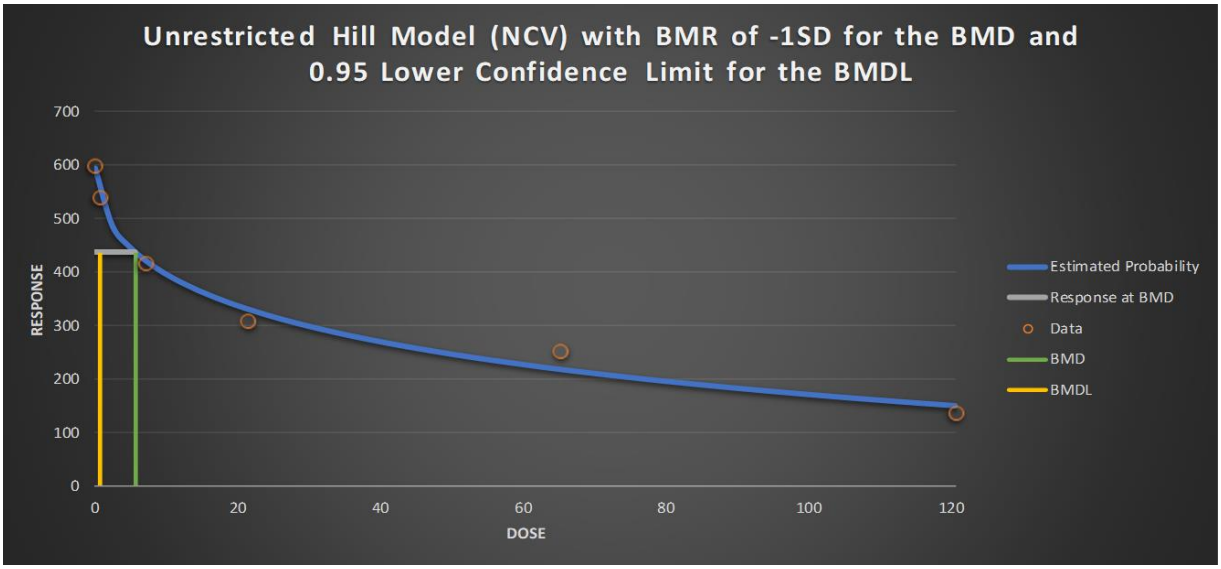
4) It should be noted that even if the highest dose group is included in the BMD modeling with the more recent release of BMDS version 3.1, there are no viable models that can be attained with the full dataset.
The complete dataset would yield 3 potential models for BMDL consideration (Table 4):

- Unrestricted Hill Model, non-constant variance
- Unrestricted Polynomial, Degree 4 Model, non-constant variance
- Unrestricted Polynomial Degree 3 Model, non-constant variance

Table 4: Benchmark Dose analysis for a 1 control standard deviation change in plaque forming cell response from PFOS in mice (Dong et al. 2009) – all dataset

Model	Restriction	Serum PFOS (μg/mL)			Test 4 P-Value	AIC	BMDS Recommendation	
		BMD	BMDL	BMDU			Viable?	Notes
Hill (NCV)	Unrestricted	5.6892	0.8301	22.0466	0.3025	736.7911	Viable - Recommended	Lowest BMDL WARNING: BMD/BMDL ratio > 5
Polynomial Degree 4 (NCV)	Unrestricted	11.9140	3.7914	13.3917	0.1881	738.8790	Viable - Alternate	Note: multiphasic curves
Polynomial Degree 3 (NCV)	Unrestricted	11.2946	7.8669	18.5970	0.4703	736.6554	Viable - Alternate	Note: multiphasic curves

However, in the unrestricted Hill Model, the ratio between BMD:BMDL > 5 reflects large uncertainty associated with the "true" shape of the dose-response curve in the low-dose region and caution should be used when selecting BMDLs from such models (Haber et. al., 2018).



The other 2 viable models (Poly 4 and Poly 3) have multiphasic curves with multiple inflection points which indicated non-monotonicity.

Taken together, these results suggest that all 3 unrestricted models should be excluded from consideration with BMDL selection which would mean no viable models were attained with the full dataset.

Meng et al. (2018)

3M respectfully finds that EFSA misinterpreted the Meng et al.(2018) study and its relationship to the Steenland et al (2018) meta-analysis of birthweight and PFOA.

EFSA writes of the Meng et al. 2018 study "For example, the large (n = 3,535) study by Meng et al. (2018) observed consistent inverse associations between PFOS and PFOA and birth weight. In that study, samples were drawn in early pregnancy (first trimester), which should minimize the risk of confounding by physiological changes in pregnancy (Steenland e tal. 2018)." In prior lines (4044 – 4053), EFSA, however, correctly acknowledged about the Meng et al. study (as clearly discussed in Meng et al.), "First trimester serum samples from 3,535 women were analyzed for PFOS and PFOA, and from 2120 women for other PFASs. *This included 1400 women who had previously been described in another publication (Fei et al. 2007)* (emphasis added)." What EFSA failed to also acknowledge is that the Fei et al. (2007) study of 1400 women was, in fact, included in the Steenland et al. meta-analysis of birth weight and PFOA. And Fei et al. was part of the stratified first trimester analysis as seen in Figure 3 of the Steenland et al. paper. Thus, to argue, as EFSA writes on lines 5672-5675 that, 'the association with reduced birth weight might at least partly be explained by changes in the physiology during pregnancy, although a recent study seemed to strengthen the causality of the effect (Meng et al.,, 2018)" is quite misleading because 40% of the 3,535 subjects in Meng et

al. were already included in Steenland et al. who had reported “Restricting to studies where blood was sampled from mothers early in the pregnancy or shortly before conception (5,393 births), we found little association of PFOA with birthweight (−3.3 g [−9.6, 3.0]). In studies where blood was sampled late in the pregnancy (7563 pregnancies), lower birthweight was associated with higher PFOA (−17.8 [−25.0, −10.6]).” In essence, only 60% of the Meng et al. 2018 paper were actually “new” data that had not been considered by Steenland et al. 2018. EFSA should, therefore, correct their misinterpretations of these two studies.

EFSA should also acknowledge in their summary, besides Johnson et al. (2014) and Negri et al. (2017) in lines 4109-4117, the conclusions in the meta-analyses conducted by Verner et al. (2015), that also included Fei et al. (2007) in their meta-analyses of PFOS and PFOA, as well as their simulated PBPK models, that confirmed a lack of reduced birthweight associations when PFOS or PFOA are measured in the maternal first trimester.

3M General Offices

3M Center
St. Paul, MN 55144-1000
651 733 1110

September 3, 2021

Washington State Department of Health
Office of Drinking Water

To Whom It May Concern:

The 3M Company (3M) appreciates the opportunity to again review and provide comments on the Washington State Board of Health (Board) revisions to the Group A public water supplies rule (chapter 246-290 WAC). The revisions propose to set State Action Levels (SALs) for Perfluorooctanoic Acid (PFOA), Perfluorooctaine Sulfonic Acid (PFOS), Perfluorhexane Sulfonic Acid (PFHxS), Pefluorbutane Sulfonic Acid (PFBS), and Perfluorononanoic Acid (PFNA) (collectively "Proposed Regulated PFAS"). The Washington Department of Health (DOH) authority for this rulemaking stems in large part from RCW 70.142.010, which authorizes the Board to establish standards for chemical contaminants in drinking water, and RCW 43.20.050(2)(a), which authorizes the Board to adopt rules for Group A public water systems. Taken together these two rules require the Board to consider the best available scientific information in establishing the rules necessary to assure safe and reliable public drinking water and to protect public health.

3M supports regulation based on sound science. The patent inaccuracies and flaws in certain key studies and assumptions that DOH relies upon in setting the proposed SALs is not sound science. 3M submitted extensive written comments on essentially the same proposal during the informal comment period. *See* 3M Comments dated January 31, 2020 (included as Attachment A). DOH does not appear to have taken *any* of 3M's comments into account, including those that identify fundamental scientific errors. 3M is concerned that DOH is not evaluating the comments received to ensure the accuracy and integrity of its proposal, but rather going through the procedural motions while moving forward with its desired outcomes. 3M urges DOH to closely evaluate these issues and either revise its proposal to align with sound science or, at minimum, explain why it does not believe such action is necessary given the available science.

As a science-based company, 3M continues to have significant concerns with the proposed SALs. These SALs do not reflect the best available science regarding these substances. They are overly conservative and technically flawed. DOH bases much of its proposal on other agency actions, but those agencies were also unduly conservative in their assumptions and selective in the portions of the studies relied on. In addition, these proposed SALs are premature as the process and criteria for adopting SALs were not yet finalized prior to proposing the SALs.

I. DOH Should Finalize the Selection Criteria Before Finalizing the SALs

DOH establishes both the process and criteria to set SALs for unregulated contaminants at the same time it proposes to establish the SALs for PFBS, PFHxS, PFNA, PFOA, and PFOS. Taking regulatory action on the proposed SALs prior to finalizing the criteria for decision making is inconsistent with Administrative Procedure Act requirements to ensure meaningful public participation in agency decisions. DOH must ensure that it selects the PFAS based on rigorously developed criteria that include a thorough evaluation of and response to public comments (See Ctr. For Biological Diversity v. Dept. of Fish & Wildlife, 474 P.3d 1107, 1126 (Wash Ct. App., 2020) "the purpose of such rulemaking procedures is to ensure that members of the public can participate meaningfully in the development of agency policies which affect them"). DOH must finalize the criteria first, rather than propose SALs based on criteria that are still subject to review and modification. In making the SAL proposal prior to finalizing the SAL criteria for decision making, DOH bases its decision on factors that have not been firmly established.

II. SALs Must Be Established Based on Sound Science

To establish a SAL based on the DOH criteria proposed in this rulemaking, the contaminant must have adverse impacts on human health. At a minimum, the criteria require that DOH determine that the Proposed Regulated PFAS be "known or likely to occur . . . at levels of public health concern" and have a "possible adverse effect on health of persons exposed based on peer-reviewed scientific literature or government publications . . ." The scientific literature and other information upon which DOH relies does not support such conclusions.

Overall, the proposed SALs are not derived using the best available science. There are many deficiencies and unduly conservative and scientifically flawed assumptions associated with these proposed SALs. DOH must use accurate data in determining the SAL for the Proposed Regulated PFAS, as it must for any substance. An agency is required to "examine the relevant data and articulate a satisfactory explanation for its action including a rational connection between the facts found and the choice made." *Motor Vehicle Mfrs. Ass'n v. State Farm Mut. Auto. Ins. Co.,* 463 U.S. 29, 43, 103 S.Ct. 2856, 77 L.Ed.2d 443 (1983). An agency "must not act cursorily in considering the facts and circumstances surrounding its actions." *Puget Sound Harvesters Ass'n*, 157 Wash. App. 935, 951, 239 P.3d 1140 (2010). DOH must consider the deficiencies of the information it relies on, as further described herein.

a. <u>The body of scientific evidence does not show adverse effects in humans from PFAS</u>

In *Recommended State Action Levels for Per-and Polyfluoroalkyl Substances (PFAS) in Drinking Water: Approach, Methods, and Supporting Information*, dated August 1, 2021 (Supporting Information), DOH states that some PFAS are highly bioaccumulative in people, but admits that researchers are still learning about how exposure to certain PFAS may affect human health.[1] The vast body of scientific evidence does not show that the Proposed Regulated PFAS cause adverse health effects in humans. While there remains some uncertainty in the science, the

[1] Supporting Information at 11.

evidence available today does not support the conclusions drawn in the Supporting Information. 3M has provided extensive comments to DOH and other agencies, including the Agency for Toxic Substances and Disease Registry (ATSDR) and the Minnesota Department of Health, each of which DOH relies on heavily to establish the SALs. These comments document the lack of support or consensus around claimed impacts on fetuses and infants, cancer, antibody response, and other issues.

For example, ATSDR has repeatedly concluded that while some studies suggest an association between PFAS exposure and health outcomes, "*cause and effect relationships have not been established for these outcomes*."[2] Moreover, the two studies selected by ATSDR to rely on in establishing Minimum Risk Levels (MRLs) – Onishchenko et al. (2011) and Kodkela et al. (2016) - lacked fundamental scientific rigor (e.g., using a single dose study without any dose-response, small sample size with only six pregnant dams; no details on the reproductive nor the developmental hallmarks, litter bias, non-standard testing methods, no internal serum PFOA dosimetry data).[3] Given these flaws, the proposed ATSDR MRLs were not derived using best available science and do not provide adequate support for the DOH proposal.

The Australian Expert Health Panel also concluded that "there is mostly *limited or no evidence for any link with human disease* from these observed differences. Importantly, there is no current evidence that supports a large impact on a person's health as a result of high levels of PFAS exposure."[4] The report further stated that "after considering all of the evidence, the Panel's advice . . . is that the evidence does not support any specific health or disease screening or other health interventions for highly exposed groups in Australia, except for research purposes."[5] Like ATSDR, the Australian Expert Health Panel analyzed hundreds of studies when reaching this conclusion.[6]

b. <u>DOH continues to rely on flawed analysis conducted by other agencies</u>

DOH also continues to rely on analysis by the Minnesota Department of Health (MDH), despite the fact that 3M previously submitted comments demonstrating that the MDH analysis relies on a flawed study, as there was a technical omission by Dong et al. (2011) that critically impacts the point of departure (POD). DOH should not accept the no observed adverse effect level (NOAEL) as the POD since the Dong et al. (2011) study related to an incomplete dataset presented in the manuscripts.[7] Had MDH used the complete dataset and performed the recommended benchmark dose modeling, the resulting BMDL1SD would become 3.0 mg/L, which is 21% higher than the existing NOAEL. Likewise, the resulting RfD and drinking water guidance value for PFOS would be 21% higher (which will yield 3.9 ng/kg-d as RfD and 18 ug/L

---

[2] ATSDR *Toxicological Profile for Perfluoroalkyls*, May 2021 at 6, 26, 751, (emphasis added). Available at https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf

[3] See Attachment A.

[4] Expert Health Panel for PFAS: Summary at 2 (emphasis added). Available at https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-pfas-expert-panel.htm

[5] Id.

[6] Expert Health Panel for Per- and Poly-Fluoroalkyl Substances (PFAS), March 2018 at 382-403. Available at https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-pfas-expert-panel.htm

[7] Id.

as the water guidance value). Furthermore, DOH should acknowledge that because of the numerous technical deficiencies in the Dong et al. study, it does not provide any robust or compelling scientific evidence to support the claim that PFOS is associated with immune suppression in mice. DOH should review the information provided by Dong, the study author, that completes the dataset for the study at issue.[8]

DOH also relies on information on the "C8 Health Project" but this is misleading and outdated. In 2020, scientists and collaborators who had formed the "C8 Science Panel" reviewed the current literature with respect to each of the health conditions potentially linked to PFOA.[9] These scientists concluded that epidemiological evidence remains limited and question the broader implications drawn from their prior work, noting that their work assessed a single population and that additional studies would be expected to vary. Their findings include:

- Increased blood cholesterol – the authors reviewed additional studies regarding the effects of PFOS and PFOA on serum cholesterol levels. While these more recent studies did generally support an association between exposure and increased levels of cholesterol, the magnitude of the cholesterol effect is inconsistent across different exposure levels in the epidemiologic studies and is not supported in the toxicological studies. The article notes there is not consistent evidence that exposure translates to an increase in cardiovascular disease risk. Furthermore, two workshop panels have recently recommended additional pharmacokinetic and mechanistic research be conducted to understand the epidemiological association of low concentrations of PFAS and higher serum lipids, which is contrary to the toxicological research reported in some studies at much higher concentrations.[10]

- Ulcerative colitis – the authors reviewed four additional published studies and concluded that while the evidence still supports a possible link, more studies are needed to reach definitive conclusions.

- Thyroid function – the authors concluded the evidence of an association of PFOA with thyroid disease has, in fact gotten weaker. The review focused on studies of a 2019 Swedish community regarding exposure to PFOS and PFOA.

- Testicular cancer – based on their review, the authors concluded that as a general matter, the evidence does not support PFOA being considered carcinogenic for any

---

[8] See Attachment A.

[9] See Kyle Steenland, Tony Fletcher, Cheryl R. Stein, Scott M. Bartell, Lyndsey Darrow, Maria-Jose Lopez-Espinosa, P. Barry Ryan, David A. Savitz, "Review: Evolution of evidence on PFOA and health following the assessments of the C8 Science Panel," Environment International, Volume 145, 2020 (available at https://doi.org/10.1016/j.envint.2020.106125).

[10] See Styliani Fragki, et al. "Systemic PFOS and PFOA exposure and disturbed lipid homeostasis in humans: what do we know and what not?" Critical Reviews in Toxicology, April 15, 2021 (available at https://www.tandfonline.com/doi/full/10.1080/10408444.2021.1888073); and Melvin E. Andersen, et al. "Why is elevation of serum cholesterol associated with exposure to perfluoroalkyl substances (PFAS) in humans? A workshop report on potential mechanisms," Toxicology Volume 459, July 2021, 152845 (available at https://www.sciencedirect.com/science/article/pii/S0300483X21001682).

given site. Specific to testicular cancer, the authors noted that the evidence for an association is suggestive but noted it is a rare type of cancer, limiting possible conclusions.

- Kidney cancer – likewise, the authors concluded the evidence for an association between exposure to PFOA and kidney cancer remains suggestive. They cautioned, however, that this determination is inconsistent with a 2014 study of high-exposure workers.

- Pre-eclampsia and elevated blood pressure during pregnancy – the authors determined the C8 Science Panel conclusions were relatively insensitive to potential errors in exposure and toxicokinetic models. Two new studies reviewed proved inconclusive as to an association between PFOA and pre-eclampsia.

In its Supporting Information, DOH relies on a number of publications by other states and federal agencies, many of which are flawed, in draft form, or otherwise problematic in this context. For instance, the document relies on state and federal materials, including those published by New Jersey, New Hampshire, and EPA, upon which 3M has already provided extensive technical comments. In short, the State should avoid duplicating erroneous and incomplete work done by other agencies. It does not appear DOH has evaluated the technical deficiencies in these studies that were highlighted during the informal comment period.

c. <u>DOH should rely on the most reliable and current scientific information</u>

In developing SALs, DOH should review and incorporate the latest scientific research and rely primarily on peer-reviewed information that has been published in its final form, taking into account comments from the public and experts. In addition to the information above from the Steenland report, 3M notes:

- PFOS and PFOA do not cause an increase in serum lipid in laboratory animals. Several observational epidemiological studies have reported an association between PFOA exposure and increased cholesterol levels but the magnitude of effect is entirely inconsistent across exposure levels. These findings are inconsistent with experimental studies which have observed decreased cholesterol levels with markedly higher PFOA concentrations. These experimental studies now include a Phase 1 clinical trial in humans (Convertino et al. 2018) and a transgenic mouse model that mimics human lipoprotein metabolism (Pouwer et al. 2019). There is no evidence of increased risk of cardiovascular mortality in the highest exposed occupational cohorts (Steenland and Woskie 2012; Raleigh et al. 2014) based on worker analyses in these studies which minimized the healthy worker effect.

- There is no known association between PFOA or PFOS and human liver disease including enlarged liver, fatty liver, cirrhosis, or liver cancer. Small percentage changes in alanine aminotransferase (ALT) have been reported, albeit inconsistently in epidemiology studies across vastly different perfluoroalkyl

concentrations but are within normal physiological ranges. This small magnitude of change, if it is even present, does not indicate liver damage by any standard clinical practice of medicine.[11]

- The absence of clinical immunosuppression along with inconsistent findings both within and across studies do not support a link between PFAS levels and decreased antibody responses to vaccines in humans. There is highly inconsistent evidence to suggest an association of PFAS with an increased risk of infection in children.[12]

- There is insufficient evidence in the literature to conclude that an association between thyroid disease and exposure to PFAS exists in humans.[13]

- The levels of PFOS or PFOA causing a potential reproductive or developmental toxicity in rodents are several orders of magnitude higher than the levels experienced by the general human population, demonstrating an ample margin of safety. In laboratory animals, fetal effects generally occurred at maternally toxic dose levels and no fetal changes were present at nontoxic material doses.[14] Similarly, EPA has been unable to establish a causal relationship between PFOS or PFOA and reproductive toxicity in humans. The evidence from two meta-analyses now indicate a non-causal association with lower birthweight for PFOA (Steenland et al. 2018) and PFOS (Dzierlenga et al. 2020) as it is likely due to confounding related to the maternal timing of the blood measurement and the physiological changes in pregnancy between first and second/third trimesters as related to the glomerular filtration rate.

III. DOH improperly relies on outdated information to establish the proposed SALs

Not only does DOH continue to rely on outdated information to establish the proposed SALs, it now relies on this same information to drastically reduce the SAL for PFBS. In determining the SALs for PFHxS and PFBS, DOH relied on serum T4 measurement to determine thyroid impacts. However, this measurement alone does not fully represent overall thyroid function. Thyroid histology and/or serum TSH (the primary diagnostic indicator for serum thyroid hormone status) should be included in any determination of thyroid status in laboratory studies when feasible. The available rodent studies do not lead to a conclusion that the collective data supports a hazard for a thyroid effect with either PFHxS or PFBS. In addition, with respect to PFBS, the developmental outcomes reported from the non-GLP[15] short-term gestation exposure in mice (Feng et al. 2017) were vastly different than those reported from the full GLP two-generation study in rats by Lieder et al. (2009). The discrepancies from

[11] See Attachment A, which includes 3M Comments on ATSDR Draft Toxicological Profile for Perfluoroalkyls (August 20, 2018), (hereinafter "3M ATSDR Comments").
[12] See 3M ATSDR Comments and Attachment B.
[13] See 3M ATSDR Comments; Li et al. 2021;5 Andersson et al. 2019. Prepublication draft available at https://www.sciencedirect.com/science/article/abs/pii/S0013935120315449.
[14] Id.
[15] Good Laboratory Practices

the short-term study need to be carefully evaluated prior to any meaningful risk assessment for humans.

IV. PFAS are not known to occur in public water systems at levels of public health concern

The proposed DOH criteria for a SAL include that the "contaminant is known to occur in public water systems at levels of public health concern . . ." DOH has not provided information that PFAS occur in public water systems at levels of public health concern. The known areas of PFAS occurrence in Washington are limited and contrary to a finding of occurrence in public water systems at any meaningful frequency. In Washington, 132 public water systems, including all Class A systems, were monitored for six PFAS, and only one water system had a well that exceeded EPA's lifetime health advisory level for PFOA and PFOS.[16] Furthermore, the data relied on was not particularly current. The Washington data was originally collected from 2013 to 2015, and the federal data is, at minimum, 5 years old. Not only has the occurrence of many PFAS likely declined since the cited data was collected, it should continue to decline, and the Board should have accounted for these declines in its evaluation of the occurrence of the PFAS at issue here. Although Washington partially updated its data with voluntarily collected information between 2016-2020, the State recognizes that it does not "yet know the full extent of PFAS contamination in our drinking water supplies, and the science around PFAS is evolving quickly."[17]

V. The Cost-Benefit Analysis Fails to Consider Costs Associated with Establishing the SALs

Costs to the public water systems are evaluated in the Significant Legislative Rule Analysis, but DOH does not include any costs associated with establishing the SALs. In fact, DOH states explicitly that "there are no known or anticipated direct compliance costs associated with the board establishing the SALs in the Rule."[18] This statement ignores any costs associated with long-term impacts of the SALs, which can serve as the foundation for future MCLs or remediation cleanup standards. DOH recognizes that such long-term applications of the SALs are expected to occur and must evaluate the anticipated costs of these actions. While DOH fails to include any costs associated with future actions based on the SALs, it considers the long-term benefits. For example, DOH evaluates as a benefit the fact that these SALs will be used by the Department of Ecology to set clean up standards.[19] The corresponding evaluation of the costs are significant and must be evaluated in the cost-benefit analysis.

3M appreciates the opportunity to provide these comments on the Proposed SALs. 3M urges DOH to evaluate and consider 3M's comments, as they address significant technical and procedural concerns that undermine the validity of DOH's proposed SALs.

---

[16] Significant Legislative Rule Analysis, dated August 3, 2021 at 3-4.
[17] Id.
[18] Id. at 14.
[19] Id. at 15.

Attachment A: 3M Comments to Washington Department of Health, January 31, 2020.
Attachment B: 3M Comments on European Food Safety Authority Draft Scientific Opinion on the “Risk to human health related to the presence of perfluoroalkyl substances in food” (April 20, 2020)

Rules Comment

Aug 13 2021 8:40AM

We the PEOPLE of Spokane county have repeatedly voted AGAINST ANY FLORIDATION being put into our drinking water! We DO NOT WANT THIS!! DO NOT PUT ANY FLORIDATION INTO OUR WATER SUPPLY!!

Sep 2 2021 6:53PM

Hello and thank you for your efforts to bring to light these toxic and dangerous forever chemicals. I'm grateful for the proposed increase in testing and the heightened awareness it will trigger across Washington State. I approve of this first step in establishing thresholds and monitoring regimes and standards for PFAS in Group A public drinking water systems encoded as State Action Levels for this first subset of forever-chemicals. I hope that over time: - the levels in various drinking water supplies will be monitored and openly published - other toxic PFAS will be added to this list - that the State will help local water districts with monitoring, remediation, and funding - that the State will seriously look at regulating and limiting the production and usage of such chemicals in the first instance. - that the State will set MCLs As a member of the Washington Water Advocates I hope that you will include our group in future relevant communications. For further information please contact us at info@washingtonwateradvocates.org Thank you, Adam Wells

Go Back

Access Washington® Official State Government Website

Washington State Department of Health
Center for Public Affairs
111 Israel Road SE
PO Box 47890
Olympia, WA 98504-7876

Last Update: 9/4/2021

Send inquires about DOH and its programs to the Health Consumer Assistance Office
Comments or questions regarding this web site? Send mail to the Subsite Developer.

BY ELECTRONIC MAIL

September 3, 2021

Ms. Jocelyn Jones
Office of Drinking Water
Department of Health
111 Israel Road SE
Tumwater, WA 98501

Re: Proposed Revision to the Group A Public Water Supplies Rule (Chapter 246-290 WAC) to set State Action Levels for Five Per- and Polyfluoroalkyl substances

Ms. Jones:

The Chemical Products and Technology Division of the American Chemistry Council (ACC/CPTD) submits the following comments on the proposed state action levels (SALs) for five per- and polyfluoroalkyl substances (PFAS) – perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorohexane sulfonate (PFHxS), and perfluorobutane sulfonate (PFBS). ACC/CPTD represents a number of companies with an interest in the development of regulatory guidelines and standards for these substances, such as the proposed SALs.

In addition to the substance-specific comments discussed below, ACC/CPTD offers several general comments on the proposal. These include concerns about the proposed definitions, monitoring and follow-up requirements, sources for assessing the need for establishing an SAL, and health effects language. We also are concerned about the Department's approach to estimating exposures to substances for which the selected health endpoint is a developmental effect.

WAC 246-290-010 – Definitions, Abbreviations, and Acronyms

The Department proposes overly broad definitions for "adverse effect" and "PFAS," that create uncertainty about the scope of the proposed regulation and may lead to confusion about its implementation. ACC proposes the following definitions to provide more clarity

> "Adverse effect" means a biological change, functional impairment, or pathological lesion that causes harm to the normal functioning of an organism.

This definition is consistent with that used by the US Environmental Protection Agency (USEPA) and recognizes that some biological changes observed in animal testing studies may be adaptive in nature and may not be indicative of an adverse effect. Many factors, including environmental, dietary, and physiological stressors, can cause changes in biology that do not impact the functioning of the organism.

> "PFAS" - means non-polymeric perfluoroalkyl and polyfluoroalkyl substances that are a group of man-made chemicals that contain at least 2 fully fluorinated carbon atoms, excluding gases and volatile liquids.

This definition incorporates the definitions suggested by USEPA,[1] the Organization of Economic Co-operation and Development (OECD),[2] and the Interstate Technology Regulatory Council (IRTC)[3] with an added focus on those substances that can reasonably be expected to be found in water.

WAC 246-290-300 – Monitoring Requirements

Paragraph 10 of 246-290-300 (Contaminants with a SAL under WAC 246-290-315, Table 9) inappropriately conflates the general term PFAS with those five substances for which SALs have been proposed and the 29 substances for which the Department has proposed state detection reporting limits (SDRLs) in Table 7 of WAC 246-390-075. Contrary to the proposed title of the paragraph, DOH is establishing SALs for only five of the substances that are referenced in Table 3 and subparagraph (b) of the proposed language. In addition, Table 3 erroneously indicates that SALs exist for all PFAS. ACC urges the Department to clarify the language of Paragraph 10 to accurately reflect the proposal. This may be best achieved by deleting the general term "PFAS" and creating separate paragraphs outlining the requirements for those PFAS for which an SAL exists and those requirements for PFAS which an SRDL has been established.

WAC 246-290-315 – State Action Levels (SALs) and State Maximum Contaminant Levels (MCLs)

Although the proposal recognizes the importance of peer review for scientific literature that can be used as a basis for identifying possible adverse effect on the health of persons, it does not appear to extend the same requirement for government publications. This is

---

1 86 *Federal Register* 33926 (June 28, 2021).

2 OECD. Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl substances: Recommendations and Practical Guidance. OECD Environment, Health and Safety Publications Series on Risk Management No. 61. (2021)

3 https://pfas-1.itrcweb.org/. The state's Department of Ecology is an ITRC member.

particularly important for state government science assessments that may not be subject to rigorous scientific review that is generally required for USEPA and Agency for Toxic Substances and Disease Registry (ATSDR) publications. ACC encourages the Department to delete the reference to state assessments or to clarify that government assessments also must undergo rigorous external peer review. We also recommend that the reference to USEPA guidelines for exposure assessments be deleted since it is unlikely to provide insight into adverse effects for individual PFAS.

ACC also recommends that paragraph (4)(b) be revised to require that at least one additional sample be collected and submitted to a certified lab within a specified number of days (*e.g.*, 14 days) for any sample that indicates that an SAL has been exceeded. Given the exceedingly low levels proposed for the identified PFAS, ACC urges the Department to require additional monitoring to confirm an SAL exceedance prior to requiring public notice under WAC 246-290-71006. Such verification of the original observation will help to eliminate reporting of inaccurate results and unnecessary public concern. Although the Department's separate rulemaking on laboratory certification and data reporting may help to reduce the incidence of false positives, the possibility for PFAS contamination of a sample during collection, storage, and transport of samples remains significant. Requiring confirmation sample testing will help to ensure the integrity of the program and maintain public confidence.

WAC 246-290-320 – Follow-Up Action

While the summary of the proposed regulation indicates that a decision to order a water system to take action to remedy an SAL exceedance will be made on a "case-by-case" basis, Section 246-290-480 would require purveyors to maintain "records of actions to correct violations of primary drinking water standards and exceedances of SALs." Such language suggests that remedial action to address an SAL exceedance is required and should be revised to indicate that correction is not a requirement of general application. DOH also should clarify what additional actions it may require of a purveyor who reports an SAL exceedance, and the basis for a determination that action is necessary. This information could significantly impact the cost implications of the proposed regulation. It should be considered before moving ahead with the proposal.

ACC also believes that the monitoring requirements following detection of a contaminant with an SAL be revised to better reflect laboratory capabilities. The trigger of 20 percent of the SAL means a value below the minimum reporting level (MRL) proposed by USEPA for the fifth Unregulated Contaminant Monitoring Rule (UCMR 5) for PFOS, PFOA, and PFNA.[4]

[4] USEPA defines the "MRL as the minimum quantification level that, with 95% confidence, can be achieved by capable analysts at 75% or more of the laboratories using a specified analytical method." (86 *Federal Register* 13846, March 11, 2021).

Requiring measurements below the MRL specified by USEPA jeopardizes the defensibility, consistency, and quality of the information reported to the Department. The Department should not establish monitoring thresholds below the MRL established by USEPA for UCMR 5 reporting.[5]

WAC 246-290-72012 – Regulated Contaminants

The health effects language specified for PFOA, PFOS, PFNA, and PFHxS includes language indicating that when water concentrations of the substance are much higher than the SAL "shorter periods of exposure are of concern." The Department has provided no evidence to support this claim for these four PFAS and it should be deleted in each case.

Evaluating Exposures for Assessing Developmental Effects

The SALs proposed for three of the five PFAS (PFOA, PFNA, and PFBS) are based on reports of effects in animals exposed during gestation. Although the studies chosen for these three substances are discussed later in this comment, ACC/CPTD wishes to provide a general comment on the Department's approach to estimating exposures. In each case, the Department uses the water intake model developed by the Minnesota Department of Health which includes both pre- and post-natal exposures – even though the offspring in the studies were exposed *in utero*. For the purposes of evaluating many developmental effects, estimates of exposures should be limited to prenatal exposure which can be based on serum levels of the mother. Including post-natal exposures significantly increases the estimate of internal dose (**Figure 1**).

[5] Similarly, the state detection reporting limits (SDRLs) proposed in Table 7 of WAC 246-390-075 should be equal to the MRLs established for UCMR 5.

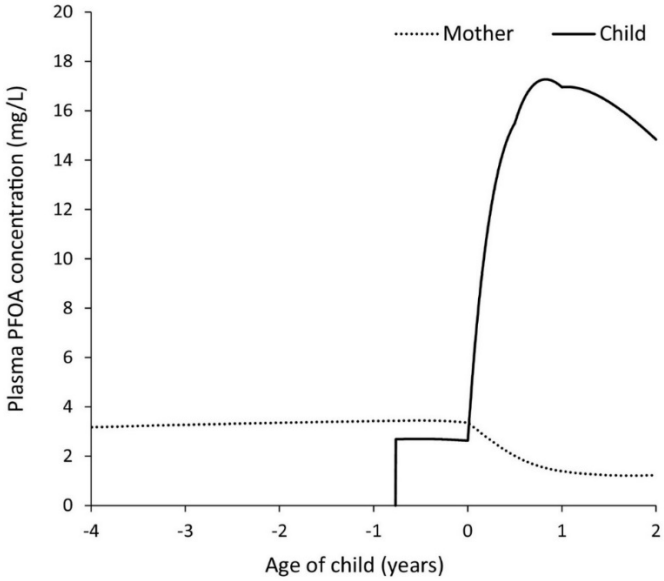


Figure 1. Simulated plasma PFOA concentrations in human mother/child.[6]

Perfluorooctanoic Acid (PFOA)

The proposed SAL for PFOA is based on reports of altered activity and skeletal effects in the adult offspring of mice exposed to PFOA through gestation.[7] The study includes a single-dose group which greatly limits its value for evaluating low doses because of the absence of a dose-response relationship. The first publication from this study, Onishchenko *et al.* (2011) reported mild gender-specific differences in exploratory behavior patterns were reported after 5 weeks of age. PFOA-exposed males were more active, while PFOA-exposed females were less active, than their respective controls.[8]

In the second publication, Koskela *et al.* (2016) reported mild alterations in bone morphometry and mineral density of femurs and tibias in mice while noting that the biomechanical properties of the bones were not affected.[9] Based on the absence of an impact on mechanical function, the biological significance of bone geometry and mineral density

---

6 Kieskamp KK *et al.* Incorporation of fetal and child PFOA dosimetry in the derivation of health-based toxicity values. *Environ Intl* 111:260-267 (2018).

7 The Health Department's assessment is based on the 2021 Toxicological Profile for Perfluoroalkyls developed by the Agency for Toxic Substances and Disease Registry.

8 Onishchenko N *et al.* Prenatal exposure to PFOS or PFOA alters motor function in mice in a sex-related manner. *Neurotox Res* 19(3): 452-61 (2011).

9 Koskela A *et al.* Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. *Toxicol Appl Pharmacol* 301: 14-21 (2016).

alterations is uncertain and may not be a suitable basis for the SAL calculation.[10] Notably, no increases in the occurrence of malformations/variations were observed in similar studies conducted in rats.[11,12] Koskela *et al.* also appear to have conducted their statistical analysis on a per-fetus basis, rather than per-litter as advised by USEPA's guidelines for assessing developmental toxicity, which has been widely critiqued as a study deficiency in the past.[13]

Lau *et al.* (2006) also reported skeletal effects in the offspring of mice exposed to PFOA, but the effects did not increase in a dose-related manner. Consequently, the effects noted by Lau *et al.* would generally not be considered biologically significant.[14] In noting the striking difference between their results and the minor effects reported in the two-generation study in rats by Butenhoff *et al.* (2004), the authors suggest that they are most likely related to pharmacokinetic differences between the two species. Considering the significant differences in pharmacokinetics between these two closely related rodent species, careful consideration should be given when extrapolating these studies to humans.

To address, in part, the issues surrounding species extrapolation, effects in primates may be considered. In its earlier assessment of PFOA,[15] the Agency for Toxic Substances and Diseases Registry (ATSDR) chose the increase in liver weights in *Cynomolgus* monkeys reported by Butenhoff *et al.* (2002) as the basis for its proposed MRLs.[16] Although the 2002 study included a small number of animals and reported early deaths at several dose levels, the effects seen in the non-human primates are consistent with those reported in rats by Butenhoff *et al.*

---

10 Carney WE Kimmel CA. Interpretation of skeletal variations for human risk assessment: delayed ossification and wavy ribs. *Birth Defects Res B Dev Reprod Toxicol* 80(6):473-96 (2007). https://www.doi.org/10.1002/bdrb.20133

11 Staples RE *et al.* The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. *Fundam Appl Toxicol* 4(3 Pt 1): 429–440 (1984).

12 Butenhoff JL *et al.* The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. *Toxicol* 196(1–2):95–116 (2004).

13 USEPA. Guidelines for Developmental Toxicity Risk Assessment. Risk Assessment Forum. EPA/600/FR-91/001(December 1991). (EPA Guidelines 1991). https://www.epa.gov/risk/guidelines-developmental-toxicity-risk-assessment

14 USEPA Guidelines 1991, at 13. The 1991 guidelines note that a dose-related increase in variations in skeletal ossification is interpreted as an adverse developmental effect, but caution that assessing the biological significance of the variation must consider what is known about the developmental stage.

15 ATSDR. Draft Toxicological Profile for Perfluoroalkyls. US Department of Health and Human Services. Agency for Toxic Substances and Disease Registry. Atlanta, GA (2015).

16 Butenhoff JL *et al.* Toxicity of ammonium perfluorooctanoate in male *Cynomolgus* monkeys after oral dosing for 6 months. *Toxicol Sci* 69(1):244-257 (2002).

(2012).[17] Moreover, the evidence of histological hepatic effects observed in the rats coupled with increased liver weight and hypertrophy observed in the 2012 rat study provide an indication that the effects are adverse – rather than adaptive.[18] As such, the results from Butenhoff *et al.* 2012 may provide a more appropriate basis for the SAL.

Perfluorooctane Sulfonic Acid (PFOS)

The immune system effects in mice reported by Dong *et al.* (2011), [19] that are the basis of the SAL, conflict with the findings reported by other researchers. In addition, the decision to focus on immune effects as the basis for its proposed SAL runs directly counter to the specific concerns expressed about these data by both USEPA [20] and Health Canada. [21]

Sensitivity to immunological effects appears to be dependent on several factors.[22] The influence of species on effects is difficult to ascertain, as the only rat study specifically designed to measure immune effects reported a no observed adverse effect level (NOAEL) several orders of magnitude higher than the lowest observed adverse effect levels (LOAELs) from the studies in mice. [23] Even within a single species, differences in sensitivity have been reported among strains—effects on sheep red blood cell (SRBC)-specific IgM levels were observed at lower levels in B6C3F1 mice[24] than in C57BL/6 mice,[13,25] even after a shorter duration of exposure (28

---

17 Butenhoff JL *et al.* Toxicological evaluation of ammonium perfluorobutyrate in rats: Twenty-eight-day and ninety-day oral gavage studies. *Reprod Toxicol* 33(4):513-530 (2012).

18 Hall AP *et al.* Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes—conclusions from the 3rd International ESTP Expert Workshop. *Toxicol Pathol* 40(7): 971–994 (2012).

19 Dong *et al.* Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. *Arch Toxicol* 85(10): 1235–1244 (2011).

20 USEPA. Drinking water health advisory for perfluorooctane sulfonate (PFOS). EPA 822-R-16-004 (May 2016). https://www.epa.gov/sites/production/files/2016-05/documents/pfos_health_advisory_final_508.pdf

21 Health Canada. Guidelines for Canadian Drinking Water Quality: Guideline Technical Document — Perfluorooctane Sulfonate (PFOS). Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch. Ottawa, Ontario. Catalogue No. H144-13/9-2018E-PDF. (2018). https://www.canada.ca/content/dam/canada/health-canada/migration/healthy-canadians/publications/healthy-living-vie-saine/guidelines-canadian-drinking-water-quality-guideline-technical-document-perfluorooctane-sulfonate/PFOS%202018-1130%20ENG.pdf

22 Ibid, at 49.

23 Lefebvre DE *et al.* Immunomodulatory effects of dietary potassium perfluorooctane sulfonate (PFOS) exposure in adult Sprague -Dawley rats. *J Toxicol Environ Health A* 71:1516-1525 (2008).

24 Peden-Adams MM *et al.* Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol Sci* 104(1): 144–154 (2008).

25 Dong GH *et al.* Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. *Arch Toxicol* 83(9): 805–815 (2009).

days vs. 60 days). Moreover, these effects were observed at lower levels in males than in females. However, there are no indications that prenatally exposed mice are more sensitive to immunological effects than adults, as changes in SRBC-specific IgM response were not observed at ≤1 milligrams per kilogram (mg/kg) per day in male mice exposed *in utero* on GD 1–17,[26] whereas LOAELs for these effects were <0.1 mg/kg per day in the studies in adult mice.

Although the studies reported immune effects, USEPA concluded that the differences in the levels at which effects were reported (and conflicts in the direction of the effects) "highlight the need for additional research to confirm the NOAEL and LOAEL for the immunological endpoints."[27] Health Canada reached a similar conclusion noting that "[f]urther exploration should be performed to address the nearly two orders of magnitude difference in LOAELs in the studies before these endpoints can be reliably considered as a basis for risk assessment."[28]

The National Toxicology Program's systematic review of the animal immunotoxicity data concluded that it cannot be confident in the outcome assessment of the Dong *et al.* study that is the basis for the proposed SAL.[29] NTP's lack of confidence is supported by the inability of benchmark dose (BMD) modeling of the plaque-forming cell response data to provide an acceptable fit to any of the dose-response models included in USEPA's BMD software. The inability of BMD modeling to yield a valid point of departure suggests that the response data reported by Dong *et al.* are not sufficiently robust to use for risk assessment.

As with the animal data, the human immunotoxicity data are inconsistent, as noted by Health Canada which concluded that "associations are observed between PFOS levels and decreases in antibodies against some (but not all) illnesses and the influence of PFOS exposure on clinical immunosuppression (i.e., incidence of illnesses) appears to be more tenuous."[30] Health Canada further noted that, while the available animal and human data may indicate immune system changes, "it is unclear whether small variations in these measures are sufficient to result in adverse health effects in humans."

A study in children of the Faroe Islands found an inverse relationship in immune response with exposure to perfluorinated alkyl acids (Grandjean *et al.* 2012, Grandjean and

---

[26] Keil DE *et al.* Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. *Toxicol Sci* 103(1): 77–85 (2008).

[27] USEPA. Health Effects Support Document for Perfluorooctane Sulfonate (PFOS). EPA 822-R-16-202 (May 2016), at 4-7.

[28] Health Canada. Guidelines for Canadian drinking water quality - PFOS (2018), at 69.

[29] NTP. Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic acid (PFOA) or Perfluorooctanoic Sulfonate (PFOS). Office of Health Assessment and Translation. (September 2016). Monograph: Perfluorooctanoic Acid or Perfluorooctane Sulfonate; Sept. 2016 (nih.gov)

[30] Health Canada. Guidelines for Canadian drinking water quality - PFOS (2018), at 69.

Budtz-Jørgensen 2013),[31,32] with maternal cord PFOS levels negatively correlated with anti-diphtheria antibody concentration at 5 years. Children in this population demonstrated increased odds of not reaching protective antibody levels for diphtheria after vaccination at 7 years old (Grandjean *et al.* 2012). The relevance of these findings to other populations is questionable, however, due to the unique genetics and diet of the Faroe Island population, and the fact that increased exposure to other potential immunosuppressants was not accounted for in the study.

Increased PFOS exposure was associated with decreased antibodies against rubella in children from a prospective birth cohort of pregnant women from Norway in a 2013 study by Granum *et al.* 2013.[33] In contrast, prenatal exposure to PFOS was not associated with hospitalizations for infections in a 2010 Danish cohort study by Fei *et al.*,[34] nor with episodes of common cold, gastroenteritis, eczema or asthma in the Norwegian cohort (Granum *et al.* 2013). In a Taiwanese cohort study, the median serum PFOS concentration was significantly higher in asthmatic children (Dong *et al.* 2013),[35] and prenatal exposure to PFOS was positively correlated with cord blood Immunoglobulin E (IgE) levels, particularly in male children. However, Wang *et al.* (2011)[36] found no association with atopic dermatitis. Cord blood IgE levels, food allergy, eczema, wheezing, or otitis media were not associated with maternal PFOS in female infants in a prospective cohort study of pregnant women in Japan (Okada *et al.* 2012).[37]

Interpretation of a recent report of an association between PFOS exposure and hospitalizations for lower respiratory tract infections (LRTI) among children by Dalsager *et al.*

---

[31] Grandjean *et al.* Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *J Am Med Assoc* 307(4): 391–397. Comment in: *J Am Med Assoc* 307(18): 1910; author reply 1910–1. Erratum in: *J Am Med Assoc* 307(11): 1142 (2012).

[32] Grandjean P and Budtz-Jørgensen E. Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. *Environ. Health*, 12:35 (2013).

[33] Granum B *et al.* Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotox* 10(4): 373–379 (2013).

[34] Fei *et al.* Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. *Environ Res* 110: 773–777 (2010).

[35] Dong *et al.* Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case–control study of Taiwanese children. *Environ Health Perspect* 121(4): 507–513 (2013).

[36] Wang Y *et al.* Modulation of dietary fat on the toxicological effects in thymus and spleen in BALB/c mice exposed to perfluorooctane sulfonate. *Toxicol Lett* 204(2–3): 174–182 (2011).

[37] Okada E *et al.* Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. *Environ Res* 112: 118–125 (2012).

(2021)[38] is complicated by the fact that the authors used maternal PFOS concentrations. The available data suggest that in utero exposures likely are not an accurate predictor of exposure in infants and children (**see Figure 1**). In addition, the authors only report statistics for total LRTI hospitalizations, even though some of the children made multiple hospital visits - accounting for one-third of the total LRTI visits.

Finally, a cohort of 411 adult members of the C8 Health Project in West Virginia was evaluated to determine whether there was an association between serum PFOS levels and antibody response following vaccination with an inactivated trivalent influenza vaccine (Looker *et al.* 2014).[39] Vaccine response, as measured by geometric mean antibody titer rise, was not affected by PFOS exposure. A recent study in the Faroe Islands, moreover, did not report an association between PFOS levels measured at birth and at ages 7, 14, 22, and 28 and hepatitis type A and B, diphtheria, or tetanus antibody concentrations.[40]

After reviewing the available human data, Health Canada concluded –

> Although some effects on the antibody response have been observed, conflicting results were common in the dataset, which remains relatively small. A low level of consistency was observed across studies, with variations between genders, specific microbial immunoglobins, infections, mother vs. child exposure, and child years, amongst other characteristics. Moreover, the risk of residual confounding, bias, and chance cannot be discarded. These flaws impede concluding on a causative mechanism, and the nature of the association remains unclear.[41]

In support of the proposed SAL, the Health Department assumes the relevance of reduced SRBC response observed in mice to reduced resistance to infection in humans. Yet, the human studies generally report no increase in infection in children or adults, and both USEPA and Health Canada have questioned whether the small variations in the antibodies observed in the available studies are sufficient to result in adverse health effects in humans. These agencies

---

[38] Dalsager L *et al.* Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Childe Cohort. *Environ Intl* 149:106395 (2021). https:/www.doi.org/10.1016/j.envint.2021.106395

[39] Looker C *et al.* Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. *Toxicol Sci* 138: 76–88 (2014).

[40] Shih YH *et al.* Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substance: A birth cohort in the Faroe Islands. *J Immunotox* 18(1):85-92 (2021). https://www.doi.org/10.1080/1547691X.2021.1922957

[41] Health Canada. Guidelines for Canadian drinking water quality - PFOS (2018), at 40.

instead focused on the more robust data available for developmental (USEPA) and liver (Health Canada) effects.

Perfluorononanoic Acid (PFNA)

In the 2021 ATSDR assessment of PFNA that is the basis of the proposed SAL, ATSDR identified only three available animal studies. Significantly, one of these studies reported that the developmental effects observed in offspring of mice exposed to PFNA required activation of the peroxisome proliferator activated receptors (PPARα) that is of questionable relevance to humans.[42] The decreased body weight gain and development delays reported in the offspring of mice administered PFNA via gavage on GDs 1-17 that are the basis of the SAL occurred concomitant with maternal toxicity and therefore, should not be used as the critical effect.[43] Moreover, alterations in pup body weight or postnatal development in PPARα knockout mice at 2 mg/kg-day, suggesting that these effects are rodent-specific responses to PFNA.[44]

Of greater significance than the selection of the key study is the inclusion of a database uncertainty factor (UF_D) of 10 for the lack of chronic toxicity testing and emerging evidence of male reproductive toxicity. Database uncertainty factors are typically and properly applied in the absence of reproductive and developmental information.[45] In the case of PFNA, developmental toxicity data do exist that suggest that the observed effects are the result of PPARα activation. As previously discussed, the PPARα relevance to a human response to PFNA is not clear: a lower number of PPARα receptors present in humans versus rodents suggests an obvious difference between animals and humans that logically should result in *less* concern for PPARα-activated pathways in humans than results might suggest in animal studies.

Although recent studies have reported reductions of testosterone in animal studies, the effects do not appear to have impacted fertility. Moreover, it is not clear that a lowering of testosterone levels is a more sensitive endpoint than the liver and developmental effects reported in other studies as the NOAELs and LOAELs are similar or higher.

---

[42] Wolf CJ *et al.* 2010. Developmental effects of perfluorononanoic Acid in the mouse are dependent on peroxisome proliferator-activated receptor-alpha. *PPAR Res* 2010:282896 (2010). Although the authors did not rule out the possibility of PPARα relevance to a human response to PFNA, they noted the lower number of these receptors in the liver of humans versus mice.

[43] Das KP *et al.* 2015. Developmental toxicity of perfluorononanoic acid in mice. *Reprod Toxicol* 51:133-144 (2015)

[44] Wolf *et al.* 2010.

[45] EPA Risk Assessment Forum. A review of the reference dose and reference concentration processes. EPA/630/P-02/002F (December 2002). https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf

ACC recommends that the Department defer the development of an SAL for PFNA pending the review of the substance under USEPA's Integrated Risk Information System (IRIS) program. The IRIS program has released a protocol for the assessment of several PFAS, including PFNA,[46] and has indicated that it will issue a draft assessment within the next year.

Perfluorohexane Sulfonate (PFHxS)

The data selected by the Health Department to derive the SAL proposal come from the results of a 28-day toxicity study conducted by the federal National Toxicology Program (NTP). ACC agrees with the Department that the results of the chronic study conducted by Chang *et al.* 2018 do not represent a significant health effect,[47] but questions why the analysis does not consider the study by Butenhoff *et al.* (2009) which has been used by other groups for assessing the health effects of PFHxS.[48] The Department's supporting document also does not address the suggestion by Butenhoff *et al* that thyroid effects (such as those reported in the NTP study) may be related to hepatocellular hypertrophy caused by PPARα activation leading to hyperplasia of the thyroid that is likely not relevant to human health risk.[49]

Before committing to an onerous SAL based on thyroid effects, the Department should carefully review interspecies differences and human study data on the relevance of thyroid effects and the variability of thyroid hormones across life. A recent French study reports that PFAS levels at birth were not associated with TSH levels later in life,[50] and similar studies are underway to continue to add to evaluate the potential significance of TSH variance. Previous study data show a lack of strong evidence to suggest PFAS are associated with overall TSH and free T4, and even at the highest levels, any statistical variance in TSH-PFAS concentration correlations does not persist in humans beyond gestational week 10.[51] This would suggest that, even if a potential mechanism of action included possible competition with T4 for binding to transthyretin (a main carrier protein of thyroid hormone in mammals), observational

---

46 https://www.epa.gov/system/files/documents/2021-07/iris-program-outlook-june-2021.pdf

47 Chang S *et al.* Reproductive and developmental toxicity of potassium perfluorohexanesulfonate in CD-1 mice. *Reprod Toxicol* 78:150-168 (2018).

48 Butenhoff JL *et al.* 2009. Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats. *Reprod Toxicol* 27(3-4):331-341 (2009).

49 Wu KM Farrelly JG. Preclinical development of new drugs that enhance thyroid hormone metabolism and clearance: inadequacy of using rats as an animal model for predicting human risks in an IND and NDA. *Am J Ther* 13(2):141-44 (2006). https://www.doi.org/10.1097/01.mjt.0000209673.01885.b0

50 Dufour P *et al.* Association between exposure to persistent organic pollutants during pregnancy and thyroid function during childhood: a pilot longitudinal study and literature review. *Rev Med Liege* 75:37-42 (2020). https://www.rmlg.ulg.ac.be/

51 Inoue K *et al.* PFAS and maternal thyroid hormones in early pregnancy: Findings in the Danish National Birth Cohort. *Environ Health Persp* 127:117002 (2019)

(community epidemiology) studies do not suggest this effect occurs at relevant human exposures , either in the mother or infant.

The decision to focus on a short-term study for deriving the proposed MCL reflects the limited amount of toxicity data available for PFHxS. This paucity of data is further amplified by the application of a 10-fold data base uncertainty factor "penalty" based on unspecified concerns about early life sensitivity and the lack of two-generation and immunotoxicity studies. The lack of a two-generation study would justify the use of a 3-fold uncertainty factor, based on USEPA guidance. Concern about early-life sensitivity is addressed by Chang *et al.* who reported no treatment-related effects on postnatal survival of development in offspring exposed *in utero* through PND 36. Although limited, Butenhoff *et al.* did not find evidence of immunotoxicity in rats exposed to up to 10 mg/kg per day by gavage for up to 56 days.

ACC's concerns about using the NTP study results, notwithstanding, the calculation on which the Department rely inappropriately uses a benchmark response (BMR) of 20 percent rather than a BMR of one standard deviation directly observed from study results as advised by USEPA's benchmark dose (BMD) modeling guidance.[52] Although the Minnesota Department of Health has indicated that use of a BMR_{20} provides a more reliable result, that analysis has not been made available for review by external scientists and other stakeholders.

If the Department does not feel that published reports on PFHxS provide a sufficient basis for developing an MCL, the Department should defer establishing standards until more data on chronic effects are available. As noted above, an IRIS assessment for several PFAS, including PFHxS, is scheduled to be available within the next year.

Perfluorobutane Sulfonate (PFBS)

The database for PFBS includes multiple short-term and sub chronic-duration toxicity studies of laboratory animals, multiple developmental toxicity studies with mice and rats, and a two-generation reproductive toxicity study with rats. The proposed SAL for PFBS is based on the recent review of the substance by USEPA[53] which based its toxicity value on a decrease in thyroid hormones in pregnant mice and their female offspring following gavage exposure to 200 mg/kg per day from GD1 to 20.[54]

---

[52] USEPA. Benchmark Dose Technical Guidance. Risk Assessment Forum. Washington, DC. EPA/100/R-12/001 (June 2012). https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf

[53] USEPA. Human Health Toxicity Value for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3). EPA/600/R-20/345F. Office of Research and Development (April 2021).

[54] Feng X *et al.* Exposure of pregnant mice to perfluorobutanesulfonate causes hypothryoxinemia and developmental abnormalities in female offspring. *Toxicol Sci* 155: 409-419 (2017)

For short-chain PFAS like PFBS, use of the default approach of body-weight scaling to estimate the human equivalent dose is consistent with USEPA guidance[55] and the state of the science in the use of body weight allometric scaling.[56] Although the data may not be sufficient to model external dose and clearance in humans, the information available for the substance suggests that it is eliminated relatively rapidly and thus will not accumulate -- in contrast to the other four PFAS for which SALs are proposed.[57] As a result, body-weight scaling is the most appropriate approach to estimating the human equivalent dose – rather than the serum elimination half-life adjusted approach used by USEPA.[58]

In calculating the toxicity value for PFBS, USEPA included a UF_D of 10 for the chronic values. This decision was based on a lack of information on neurodevelopmental and immunotoxicity effects. For PFBS, however, robust data are available on reproductive and developmental effects, including both a mammalian prenatal toxicity study and a two-generation reproduction study. USEPA notes, moreover, that developmental effects appear to be "less sensitive than thyroid hormone perturbations in developing mice."

Consequently, a toxicity value that protects against effects on thyroid hormones also will protect against developmental effects, particularly effects on mammalian neurodevelopment since the identified rationale is that perturbations in thyroid hormones may trigger neurodevelopmental effects. After pointing out the connection between thyroid hormones and neurodevelopment, USEPA provides no rationale for why neurodevelopmental effects should then be considered separately. Consequently, inclusion of an uncertainty factor for neurodevelopmental effects is inappropriate.

Similarly, addition of an uncertainty factor for a lack of data on immunotoxicity is inappropriate. The concern about the potential immunotoxicity of PFBS is based entirely on suggestions of immunotoxicity for other PFAS. In explaining the addition of the UF_D, USEPA suggests that "immunotoxicity is an effect of increasing concern across several members of the larger PFAS family." In fact, to date, the Agency has critically evaluated the immunotoxicity data for only two PFAS. In each case, it has concluded that the available data did not suggest that immune effects are a particularly sensitive health endpoint. ACC-CPTD is not aware of

---

55 USEPA. Recommended Use of Body Weight ¾ as the Default Method in Derivation of the Oral Reference Dose. Office of the Science Advisor. Risk Assessment Forum. Washington, DC. EPA/100.R11/001 (2011). https://www.epa.gov/risk/recommended-use-body-weight-34-default-method-derivation-oral-reference-dose

56 Sharma V and McNeill JH. To scale or not to scale: the principles of dose extrapolation. *Brit J of Pharma* 157(6):907-921 (2009).

57 Xu Y et al. 2020. Serum half-lives for short- and long-chain perfluoroalkyl acids after ceasing exposure from drinking water contaminated by firefighting foam. *Environ Health Persp* 128:7 (2020). https://doi.org/10.1289/EHP6785.

58 USEPA's approach assumes that the clearance value for PFBS in humans is equivalent to that in rodents.

other data that would suggest that immunotoxicity is a concern for PFBS, which -- as clearly demonstrated by USEPA's analysis -- exhibits dramatically different properties than the two PFAS previously evaluated.

ACC/CPTD appreciates the opportunity to submit comments on the proposed SALs for the five PFAS. Please do not hesitate to contact me at srisotto@americnchemistry.com or at 202-249-6727 if you would like to discuss the information provided above.

Sincerely,

Steve Risotto

Stephen P. Risotto
Senior Director

September 1, 2021

Jocelyn W. Jones
Washington State Department of Health Office of Drinking Water
P.O. Box 47823, Olympia, WA 98504-7823
PFAS1@doh.wa.gov

RE: PFAS Rulemaking Comments

The Per- and Polyfluoroalkyl Substances (PFAS) rulemaking and the assurance of providing a safe water supply are extremely important to the Vancouver Water Utility, the third largest provider of drinking water in the state of Washington with a water system that is exclusively supplied from groundwater. With that understanding, we would like to offer the following comments for Washington State Department of Health (WDOH) consideration:

The City of Vancouver agrees that setting a State Action Level (SAL) instead of a Maximum Contaminant Level (MCL) for PFAS is the least burdensome alternative that meets the general goals and specific objectives of public health. However, there are elements associated with this specific rulemaking effort that are concerning. Specifically, the process outlined to establish an SAL and required response actions for SAL exceedances are not consistent with the Safe Drinking Water Act rules that the federal government follows for a Maximum Contaminant Level Goal (MCLG), of which SALs have been repeatedly compared.

Based on a review of available information at state and federal levels, the City of Vancouver Water Utility is concerned that the WDOH is proceeding with rulemaking without sufficient data and a full understanding of potential impacts to utilities statewide. It has been stated numerous times by WDOH staff and within the rulemaking documents that one of the reasons to create the PFAS SAL is that "monitoring for these proposed contaminants will help us identify PFAS contamination in Group A water systems across our state." (**pg. 4 of the Significant Legislative Rule Analysis**). This is contradictory to the EPA standard of first gathering data to identify prevalence and evaluating impacts prior to rule establishment. Vancouver would prefer to see the state establish a process similar to the EPA's Unregulated Contaminant Monitoring Rule (UCMR) process. This process ensures necessary scientific data is gathered first to provide the relevant data and in-depth analysis to support a SAL , before a rule is made that impacts public perception of drinking water safety, and assures customer confidence is maintained. Ultimately, this protects public health with scientifically relevant data and in-depth analysis.

Some have argued that UCMR 3 gathered data on PFAS prevalence. Unfortunately, in the case of three PFAS included in the proposed rule, the reporting levels used during the UCMR 3 testing were higher than the proposed SAL planned by WDOH. This increases the risk of wide gaps in the understanding and

knowledge of prevalence. As a result, it is highly likely that water utilities statewide will be completely unaware of the presence of PFAS in water supplies potentially exceeding the proposed SALs, until after the rule goes into effect and testing begins. Many utilities will be required to quickly initiate public outreach and action plans in response to the test results and their customers will be caught off guard. It is vital for public trust, health and safety that the data gathered to support the SAL be clear, scientifically relevant and gathered in advance of adoption of the SAL.

Pg. 13 of the Significant Legislative Rule Analysis, states "The SALs define a level in daily drinking water expected to be without appreciable health effects even in sensitive populations and life stages. They are comparable to a health advisory level or maximum contaminant level goal (MCLG) in the federal Safe Drinking Water Act." Although WDOH staff have repeatedly indicated that an SAL is set at a level comparable to a MCLG, the rule as written requires utilities to take actions for repeated sampling, public notification, and 24-hour WDOH notification (246-290-480(2)) similar to that of an MCL at the federal level per 40.CFR.141.203. Why treat an SAL exceedance as if it is an MCL violation if the levels are set as low as what would be considered an MCLG? There is no notification required for and MCLG exceedance outside of the Consumer Confidence Report unless the MCLG is set at the same level as the MCL. Such 24-hour notification is not warranted for an SAL violation. Treating a contaminant with long-term health effects similar to a pathogen erodes public confidence and raises questions in the public as to why all MCLG exceedances would not be treated in the same manner.

246-290-010(170) - Aqueous film forming "form" should be "foam" instead of form.

246-290-130(4)g(vi) is out of place. 246-290-130(4)g references 246-290-310 dealing with MCLs, not SALs, yet 246-290-130(4)g(vi) indicates that the SALs need to be met for source approval. It appears that the intent is to require new sources to meet the water SAL water quality standards. This is not appropriate given that existing sources are required to meet MCLs, not SALs or MCLGs. Per the proposed regulations, utilities are not even required to meet an SAL with their existing sources, so how is it appropriate to require new sources to meet these requirements? At the very least this section is not clear on if sources can be approved if they exceed an SAL. It is also unclear if a source that exceeds an SAL can install treatment to mitigate and still obtain source approval.

246-290-320(1)d(v) – Take action as directed by the department.
Although this is already language in the WAC, including this action as it relates to an SAL is unclear as to intent. It is also unclear what is expected of utilities if they exceed one or more SAL. Will treatment be expected if one SAL is minimally exceeded or when will the trigger occur to "take actions as directed by the department?" The potential for erosion of consumer confidence may direct many utilities to spend millions of ratepayer dollars on treatment systems that may not necessarily be warranted if the exceedance is only minimally above one of the limits. An exceeded SAL will create a perception of mistrust and a lack of faith in the quality of drinking water systems, as well as misunderstanding since treatment can't be required with an SAL.

246-290-320(8) - It is currently unclear why it is beneficial and necessary to sample four times per year. There is no indication that PFAS in groundwater supplies change rapidly enough to warrant such a high frequency of sampling. If there is no anticipated significant change in concentrations, then how are repeated samples warranted? Sampling will be a significant cost to the utility rate payers in areas where PFAS concentrations are greater than 80% of the SAL or over the SAL.

246-290-71006 and pg. 20 of the Significant Legislative Rule Analysis - It is required that a notification be sent out every quarter to customers for as long as an exceedance exists. For an integrated water system the size of Vancouver, this would be at an estimated cost of over $100,000.00 per year in direct mailing costs in order to reach all customers, not just billed accounts. In addition to these high costs, such frequent and repetitive notification, even in the face of no changes, is logistically complex for large systems and will not significantly impact public health. PFAS information will already be included in the annual consumer confidence report (CCR), which is required to be available to all customers throughout the year. In addition to CCR reporting, notifying all customers upon first identifying an exceedance, notifying new water customers at the time of initiating service and up to one additional notification per year would be adequate to assure that affected customers throughout the state are aware and informed. Contrary to the intent, constant repeated notifications, particularly without new information, can create confusion, clutter and loss of audience attention.

On pg. 7 of the Proposed Rule Making document and pg. 5 of the Significant Legislative Rule Analysis, it states "PFAS contamination of groundwater is likely to be a localized problem" is an assumed statement based on limited sampling around the state and should be removed. Appendix 5,Environmental Occurrence of the Washington State Department of Ecology PFAS Chemical Action Plan (*Per- and Polyfluoroalkyl Substances Draft Chemical Action Plan*, WDOE & WDOH, October 2020, Publication 20-04-035) details that wherever the compounds were investigated - - surface waters, sediments, fish, etc. - - they were found. The groundwater monitoring section, Section 5.1.3, included minimal information when compared to the surface water monitoring section and detailed that Ecology did not identify any ambient groundwater monitoring for PFAS in Washington.

On pg. 7 of the Proposed Rule Making document and pg. 4 of the Significant Legislative Rule Analysis , it states " There may be individual situation where a water system's PFAS results are very high and pose an immediate public health threat." Is there a definition of "very high" or will this be left to be administratively determined by WDOH? If the latter, please reference what data and scientific findings will be the basis for determinations.

Pg. 9 of the Significant Legislative Rule Analysis states "the PFAS SALs will result in the state knowing more about the extent, locations, and severity of PFAS contamination across the state, a clear public health benefit as a first step in addressing the sources and health risks associated with PFAS contaminated drinking water. The proposed rule changes also create a clear framework for how the board may address unregulated contaminants in the future." The City of Vancouver does not agree that the proposed rule changes create a clear framework. A clear framework would include statewide testing to understand the prevalence and occurrence of a contaminant prior to implementing a rule that will erode public confidence. A UCMR-like sampling process is necessary to establish a clear framework. The previous UCMR3 sampling was not effective in identifying contamination at the SAL levels proposed by the current PFAS Rulemaking.

Pg. 16 of the Significant Legislative Rule Analysis states "The proposed amendments to this section establish actions a Group A water system must take if there is an exceedance of a contaminant's SAL. These proposed amendments are like those for other federally regulated contaminants, including that the Group A water system must notify the department, notify Group A water system users, and owners and operators of any consecutive systems." This is not a true statement. The actions and notification being required for an SAL exceedance are like those of an MCL exceedance for federal regulated contaminants, not an MCLG exceedance. There are no specific actions required for an MCLG

exceedance. It is recommended that WDOH not compare an SAL to an MCLG or change the requirements for an SAL exceedance to be similar to an MCLG exceedance at the federal level.

Pg. 27 of the Significant Legislative Rule Analysis repeats language from the State Chemical Action Plan that "State agencies, the Washington State Legislature, and local water systems should work together to fund PFAS drinking water mitigation. These costs should be reimbursed by responsible parties under applicable laws." Vancouver is concerned that there is currently no specific source of funding for PFAS mitigation, and that this rulemaking may initiate significant capital and operational costs for treatment and that this cost burden will fall on utility ratepayers. Identifying and documenting legally responsible parties in cases of direct source contamination is typically a long and costly process. In many cases, and because of the historic widespread use of PFAS, it will be difficult to determine responsible parties for contamination and ultimately for any cost reimbursement.

While we support taking action to reduce the prevalence of PFAS in the environment, this rule would run counter to the important "polluter pays" principle that guides Superfund site cleanups under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), and would step back from the transparent, science-based process of regulating drinking water contaminants under the Safe Drinking Water Act (SDWA)

General Rule Comments:
This rule would undermine the development of transparent, science-based drinking water standards, and would place undue cost burdens on our communities and ratepayers while leading to premature regulatory decisions that lack public review and scientific validity. The rulemaking process that EPA follows in establishing limits for contaminates in drinking water is not only clear, but also a well-established process. The establishment of an SAL at the state level in Washington is not clear nor is it established. It is recommended that WDOH delay the rulemaking further and develop a process similar to a UCMR testing process so not only PFAS, but also future contaminants of concern can be evaluated to determine contaminant prevalence to aid in the rulemaking. This will also give WDOH and utilities enough time to prepare for potential consumer confidence issues that will come with the presence of any contaminant. The rule as currently written requires immediate testing and public notification without any previous knowledge of contaminant presence.

The US House recently passed H.R. 2467 titled "PFAS Action Act of 2021" that requires the EPA to establish a national drinking water standard for PFOA and PFOS within two years. The state's proposed rulemaking along with different PFAS action limits and MCLs set by other states creates ambiguity as we wait for federal regulations. Which state is correct? What level is the correct level? We urge the state to allow EPA to complete the rulemaking process to determine an MCL.

The majority of public water systems make the provision of clean, safe water to their customers their number one priority. This rulemaking process and lack of uncertainty to the data and results can only create mistrust around the state as water utilities struggle to explain sample results to their customers amid substantial differences between levels set by the federal and state agencies. A period of information gathering, distribution and community preparation provides regulators, purveyors, and consumers the best path to understanding, addressing and communicating risks.

Thank you for your consideration of these comments.

Sincerely,

Tyler Clary
Water Engineering Program Manager
City of Vancouver

cc: Jennifer Belknap Williamson
Dan Swensen
Brian Wilson
Patrick Craney
Loretta Callahan
Tim Buck

From: Deborah Crosetto
To: DOH EPH DW PFAS
Subject: Comment on Group A Rule of PFAS Proposal
Date: Thursday, August 12, 2021 1:23:22 PM

External Email

To the Washington State Department of Health:

Consumer Reports Special report on drinking water entitled "How Safe is Our Drinking Water" in the May 2021 issue of Consumer Reports Magazine explained the testing Consumer Reports did across the nation of public water. Their research yielded their recommendations for PFAS: 5 PPT for any one PFAS chemical and 10 PPT for two or more.
In addition, the Environmental Working Group 2019 update on tap water contaminants has also given their recommended limits for PFAS as follows: 0.001 PPB. Please see link below:
ewg.org/tapwater/ewg-standards.php

I would strongly urge that as a precautionary rule, Washington state follow these guidelines as recommended for the best-known-at-this time health standards for our citizens.
Thank you for your consideration,
Deborah Clark Crosetto
1652 25th Pl NE, Issaquah, WA 98029

From:	Mehinagic, Denis
To:	DOH EPH DW PFAS
Cc:	Barfuss, Brad C.; Ramos, Mary Joy.; McDaniel, Codee L.
Subject:	PFAS Rule Comments
Date:	Wednesday, September 1, 2021 4:29:33 PM

External Email

To whom it may concern,

Thank you for the opportunity to provide an educational statement on the proposed changes to WAC 246-290 and WAC 246-390 pertaining to per-and polyfluoroalkyl substances (PFAS) in drinking water. While agreeing with the overall premise of the rule change, it appears the rule does not properly exclude water systems whose primary and only source of water is a surface water source. This puts an undue burden of sampling and monitoring on those systems. Over the years, most, if not all the PFAS contamination found in Washington State drinking water systems have been in ground water wells. Therefore, the proposed rule change should contemplate the exclusion of water systems whose primary source and only source of drinking water, is surface water.

Thank you,

Denis Mehinagic | Environmental Scientist, Sr.
Energy Northwest
Environmental & Regulatory Programs
509-372-5768 | dmehinagic@energy-northwest.com

From: Elizabeth Maupin
To: DOH EPH DW PFAS
Subject: PFAS
Date: Thursday, September 2, 2021 3:45:07 PM

External Email

I live in Issaquah. The tap water foams. It tastes different and I'm not sure that Britta filters remove all this stuff. Do you know?

The current proposal only sets standards for the 5 most common types of PFAS chemicals, instead of dealing with them as an entire class. If PFAS is detected above a state action level there are not any set requirements to clean up the contamination. I would prefer that PFAS be addressed comprehensively as a class, and that regulations outline requirements to get PFAS concentrations back below the standard in the case of testing results above the limits set.

Thank you!

Elizabeth Maupin, M.Div.
www.facebook.com/IssaquahSammamishInterfaithCoalition
425 677 8043 (home phone), 206 478 3899 (cell)
eli410maupin@gmail.com

From: LARA LORENZ
To: DOH EPH DW PFAS
Subject: Washington State Must Act Now to Adopt PFAS Drinking Water Standards
Date: Thursday, September 2, 2021 11:37:26 AM

External Email

Dear Jocelyn Jones,

Thank you for working to adopt standards that address PFAS in Washington State's drinking water and for improving the draft state action levels for PFAS.

While the proposed rules are an important step in protecting residents from PFAS exposure, the rules must be strengthened in the following ways:

1. Address PFAS as a class: There are more than 5,000 PFAS in the class of PFAS and the Department of Health is proposing to address five. Testing for all PFAS and requiring mitigation for the entire class is critical to protecting public health.

2. When state action levels (SALs) are exceeded, it should be clear that mitigation is required to meet the SAL. The rule should more clearly require PFAS to be mitigated so the SALs are not exceeded.

3. Do not delay testing or rule implementation until 2023: PFAS contamination is a serious health threat. There is no reason for such a delay and water systems have known this rule would be adopted since 2017.

4. Require PFAS testing for transient noncommunity systems once every three years. For transient noncommunity systems, only those transient systems determined by the department to be at risk are required to conduct analysis. Transient noncommunity systems include several categories that often serve individuals for an extended period: motels, restaurants, churches, and farmworker housing. We urge the department to include transient noncommunity systems in the full monitoring requirement in which analysis is required once every three years.

Please adopt these rules with the above modifications and without delay so that Washington residents can be confident that their drinking water is safe from toxic PFAS contamination.

Sincerely,
LARA LORENZ
8312 21st Ave NW
Seattle, WA 98117

From: GlenAnderson@everyactioncustom.com on behalf of Glen Anderson
To: DOH EPH DW PFAS
Subject: VOTERS DEMAND VERY STRONG standards to protect our drinking water from PFAS !!!
Date: Wednesday, August 25, 2021 1:49:35 PM

External Email

Dear WA Department of Health,

I appreciate you taking action to protect the health of our communities and ecosystems by setting State Action Levels (SALs) for five different PFAS chemicals in drinking water. As we have seen in communities like Coupeville, DuPont, Airway Heights, and Issaquah, the consequences of drinking water contaminated with PFAS are serious and we have a moral obligation to address PFAS pollution from the source.

I'm a 72-year-old person of faith who STRONGLY SUPPORTS a clean environment, public health, and STRONG GOVERNMENTAL PROTECTIONS FROM POLLUTION, including PFAS chemicals!!!!!

VOTERS DEMAND YOU DO YOUR JOB and PROTECT US VIGOROUSLY from PFAS chemicals and other pollution that hurts our environment and our health!!!!!

Thank you for listening to faith communities and other stakeholders and editing the draft standard to require that important transient non-community (TNC) water systems near known PFAS contamination follow the same testing guidelines as other large Group A water systems.

In absence of a federal EPA standard, I'm grateful that the Department of Health is taking action on PFAS, but I would like to see more comprehensive action. I am concerned that the state SALs only cover five different PFAS chemicals but there are about 5,000 PFAS in the class. I urge the Department to require additional monitoring for total PFAS and implement a limit on all PFAS to protect drinking water and human health.

Under the current draft rule, if a water system exceeds the proposed SALs for PFAS, further testing/monitoring and public notification is mandated, but action to address the contamination, including clean-up, is not specified. I ask that the rule clearly articulate that if a PFAS SAL is exceeded, clean-up and/or other actions must be taken to return the drinking water supply to concentrations of PFAS below the SAL.

Thank you for your work to protect our communities from toxic PFAS.

Sincerely,
Mr. Glen Anderson
5015 15th Ave SE Lacey, WA 98503-2723
GlenAnderson@integra.net

From: IRRIGATION DIST,ORCHARD AV IRRIGATION DIST
To: DOH EPH DW PFAS
Subject: PFAS Rule Proposal
Date: Friday, August 6, 2021 11:30:56 AM

External Email

Good morning,
We would like to comment on the new PFAS Rule Proposal. We feel as if this area is not affected by this chemical. The airports are all down stream from us.
Thank you,
Greg

Washington Association of Sewer & Water Districts
EDUCATE ▪ ADVOCATE ▪ COLLABORATE

September 3, 2021

Jocelyn Jones
WA Department of Health
Office of Drinking Water
Tumwater, WA

RE: Comments on proposed changes to 246-290 WAC regarding PFAS and unregulated contaminants

Dear Ms. Jones:

The Washington Association of Sewer **&** Water Districts (WASWD) appreciate the opportunity to comment on the proposed rule changes to 246-290 WAC during the formal public comment period. WASWD represents the approximately 180 public sewer and water districts in the state, serving nearly 25% of the state's population ranging from the largest population centers to the smallest rural communities. Clean, safe water is top priority for WASWD members and the customers they serve. The situation with per- and polyfluoroalkyl substances (PFAS) is particularly alarming given the longevity and ease of travel of these compounds. Further, potential contamination with PFAS is especially a concern since beyond wellheads and collection points districts have no control over what is sprayed, injected, discharged, or built near drinking water facilities.

We support the State Department of Health (DOH) acting now on PFAS to protect human health, and to assist those water suppliers that are affected through no fault of their own, rather than waiting for the Environmental Protection Agency to take action. The limits you have set appear to be consistent with those developed in other states.

We also appreciate how the regulation gives DOH the ability to respond to future contaminants, not just PFAS. Being poised and ready to respond to the next contaminant of concern will allow DOH to be more responsive to human health concerns and will give utilities greater certainty and access to assistance. The addition of new Section 315 explaining the process and criteria for proceeding with establishment of state action levels and maximum contaminant levels is a welcome clarification from previous drafts.

As the proposed rule goes into effect, communicating with the public will be important and ideally include a well-coordinated message from DOH and water systems throughout the state. If possible, we would request informational handouts or a FAQ document be made available from DOH. These documents could be referenced or distributed by water systems to customers who have questions about the reason for establishing a State Action Level instead of MCL for PFAS.

PFAS has contaminated drinking water wells around the country from surface application. Providing a more direct path to aquifers via injection wells for disposing of stormwater makes drinking water supplies at risk of contamination from PFAS as well as the next contaminant of concern. We appreciate the work DOH and Department of Ecology (Ecology) are doing with WASWD to keep aquifers safe.

One possible consequence of PFAS contamination of drinking water wells is jeopardizing water rights of affected utilities. When wells are taken offline due to contamination while waiting for construction of facilities to remediate the contamination, or in switching to other wells to continue supplying the public, water is not being pumped. This would result in no average daily consumption to be reported to maintain water rights. We suggest that DOH and Ecology work together now to address this problem, especially if it will require legislation.

Funding to address PFAS contamination of drinking water sources is of great concern to us. We appreciate that some money has been utilized to assist some of the affected utilities in our state, but it is evident that water rate payers are going to be paying much more for a problem not of their making. Areas with a sole source for water supply will not necessarily have the option of switching to different water sources if their source is contaminated, meaning that expensive treatment may be their only option. It will be vitally important for the state to make robust funding available, particularly in the form of grants, to ensure communities faced with cleanup of PFAS contamination continue to have access to safe drinking water without being burdened by costs to address a problem thrust upon them. The state should also be actively working with federal representatives and the Department of Defense for relief and funding in those instances where military bases or activities caused the contamination.

In addition, we request that costs associated with PFAS not be borne by the water utilities. The draft rules pose a number of very costly fiscal impacts to water utilities, including environmental analysis to find the source of release and treatment for removal. Drinking water providers won't be the party who caused the contamination, but are tapped for significant costs and responsibilities to address the contamination.

We recognize and appreciate the efforts made by DOH staff to examine and incorporate the best available science in developing this regulation. We look forward to working with you further to keep our drinking water safe.

Sincerely,

Judi Gladstone
Executive Director

From: Twila Fluaitte on behalf of Judi Gladstone
To: DOH EPH DW PFAS
Cc: Judi Gladstone; Heather Kibbey; WASWD Staff
Subject: Comments on proposed changes to 246-290 WAC regarding PFAS and unregulated contaminants
Date: Friday, September 3, 2021 8:52:20 AM
Attachments: PFAS comment letter September 2021.pdf

Jocelyn Jones
WA Department of Health
Office of Drinking Water
Tumwater, WA

RE: Comments on proposed changes to 246-290 WAC regarding PFAS and unregulated contaminants

Dear Ms. Jones:

The Washington Association of Sewer **&** Water Districts (WASWD) appreciate the opportunity to comment on the proposed rule changes to 246-290 WAC during the formal public comment period. WASWD represents the approximately 180 public sewer and water districts in the state, serving nearly 25% of the state's population ranging from the largest population centers to the smallest rural communities. Clean, safe water is top priority for WASWD members and the customers they serve. The situation with per- and polyfluoroalkyl substances (PFAS) is particularly alarming given the longevity and ease of travel of these compounds. Further, potential contamination with PFAS is especially a concern since beyond wellheads and collection points districts have no control over what is sprayed, injected, discharged, or built near drinking water facilities.

We support the State Department of Health (DOH) acting now on PFAS to protect human health, and to assist those water suppliers that are affected through no fault of their own, rather than waiting for the Environmental Protection Agency to take action. The limits you have set appear to be consistent with those developed in other states.

We also appreciate how the regulation gives DOH the ability to respond to future contaminants, not just PFAS. Being poised and ready to respond to the next contaminant of concern will allow DOH to be more responsive to human health concerns and will give utilities greater certainty and access to assistance. The addition of new Section 315 explaining the process and criteria for proceeding with establishment of state action levels and maximum contaminant levels is a welcome clarification from previous drafts.

As the proposed rule goes into effect, communicating with the public will be important and ideally include a well-coordinated message from DOH and water systems throughout the state. If possible, we would request informational handouts or a FAQ document be made available from DOH. These documents could be referenced or distributed by water systems to customers who have questions about the reason for establishing a State Action Level instead of MCL for PFAS.

PFAS has contaminated drinking water wells around the country from surface application. Providing a more direct path to aquifers via injection wells for disposing of stormwater makes drinking water supplies at risk of contamination from PFAS as well as the next contaminant of concern. We appreciate the work DOH and Department of Ecology (Ecology) are doing with WASWD to keep aquifers safe.

One possible consequence of PFAS contamination of drinking water wells is jeopardizing water rights of affected utilities. When wells are taken offline due to contamination while waiting for construction of facilities to remediate the contamination, or in switching to other wells to continue supplying the public, water is not being pumped. This would result in no average daily consumption to be reported to maintain water rights. We suggest that DOH and Ecology work together now to address this problem, especially if it will require legislation.

Funding to address PFAS contamination of drinking water sources is of great concern to us. We appreciate that some money has been utilized to assist some of the affected utilities in our state, but it is evident that water rate payers are going to be paying much more for a problem not of their making. Areas with a sole source for water supply will not necessarily have the option of switching to different water sources if their source is contaminated, meaning that expensive treatment may be their only option. It will be vitally important for the state to make robust funding available, particularly in the form of grants, to ensure communities faced with cleanup of PFAS contamination continue to have access to safe drinking water without being burdened by costs to address a problem thrust upon them. The state should also be actively working with federal representatives and the Department of Defense for relief and funding in those instances where military bases or activities caused the contamination.

In addition, we request that costs associated with PFAS not be borne by the water utilities. The draft rules pose a number of very costly fiscal impacts to water utilities, including environmental analysis to find the source of release and treatment for removal. Drinking water providers won't be the party who caused the contamination, but are tapped for significant costs and responsibilities to address the contamination.

We recognize and appreciate the efforts made by DOH staff to examine and incorporate the best available science in developing this regulation. We look forward to working with you further to keep our drinking water safe.

Sincerely,

Judi Gladstone
Executive Director

Washington Association of Sewer & Water Districts
900 SW 16th Street, Suite 305 ▪ Renton, Washington 98057
206.246.1299 ▪ 800.244.0124 ▪ fax: 206.246.1323
www.waswd.org

From: Loren Howell
To: DOH EPH DW PFAS
Subject: PFAS Rule Proposal
Date: Thursday, August 5, 2021 6:54:54 AM

External Email

I'm the water manager for the City of Okanogan. My question is, in an area that has not used those chemicals for fire fighting will the purveyors of water systems need to test for PFAS?

September 2, 2021

Dear Washington Department of Health Office of Drinking Water,

Earth Ministry/Washington Interfaith Power & Light represents over 3,700 people of faith across Washington state. Together, we transform faith into action for the well-being of communities and the environment. We organize people of faith to advocate for strong environmental policies and provide strategic guidance to religious communities working toward environmental justice.

The faith community appreciates the Department of Health's action to protect the health of our communities and ecosystems. As we have seen in communities like Coupeville, DuPont, Airway Heights, and Issaquah, the consequences of drinking water contaminated with PFAS are serious and we have a moral obligation to address PFAS pollution from the source. Thank you for working to protect Washingtonians in absence of federal action.

We appreciate you responding to input from faith communities and other stakeholders by editing the draft standard to require that important transient non-community (TNC) water systems near known PFAS contamination follow the same testing guidelines as other large Group A water systems. This is a good step towards eventually requiring all TNC water systems to test for PFAS. TNC systems often provide individuals with water for long periods of time, and these systems often include houses of worship, hotels, and farmworker housing. Clean water should be a right for all, not just those that get 100% of their water from Group A systems.

Looking at the current draft rule, we are concerned that it only focuses on five different types of PFAS instead of the entire class of 5000+ PFAS chemicals. We urge the Department to require additional monitoring for total PFAS and implement a comprehensive limit on all PFAS to protect drinking water and human health.

Also, the draft currently does not specify action to address contamination if a water system exceeds a proposed SAL for PFAS. We appreciate that further testing/monitoring and public notification is mandated, but also want to see requirements for swift action that addresses polluted water sources. Please edit the rule to clearly state that if a PFAS SAL is exceeded, clean-up and/or other actions must be taken to return the drinking water supply to concentrations of PFAS below the SAL.

Finally, we ask that implementation of the rule be sped up from the current 2023 timetable. Many water systems are already testing for PFAS as public knowledge about these toxic chemicals continues to increase. Water systems have known about the process to adopt this PFAS rule for several years. Now that these standards are close to being finalized, the Department of Health has a moral obligation to implement the final rule as quickly as possible.

Thanks again for your hard work on this process, especially during the pandemic.

Sincerely,

Jessica Zimmerle

Jessica Zimmerle
Program & Outreach Director
Earth Ministry/Washington Interfaith Power & Light

From: Michelle Anderson
To: DOH EPH DW PFAS
Subject: PFAS Rule Proposal
Date: Friday, August 13, 2021 8:46:51 AM

External Email

We the people of Spokane county have repeatedly voted AGAINST ANY FLORIDATION INTO OUR WATER SUPPLY!!
WE DO NOT WANT THIS!

WE DO NOT WANT ANY FLORIDATION IN OUR WATER!!

HOW CAN YOU TRY to pass something the people have already said over and over that they don't want!
This is not a dictatorship!! We have already voted!!
Do we need to take this to court?? Set a new president for what governments cannot do?

WE DO NOT WANT ANY FLORIDATION IN OUR WATER SUPPLY!!

From:	jeffrey johnson
To:	Larry Jones
Cc:	DOH EPH DW PFAS; Jones, Jocelyn W (DOH); Helpling, Nina D (DOH); Chris McMeen; Craig Downs; Steve Sacksteder
Subject:	Re: Request for an extension of the WDOH PFAS Rule Proposals (Chapter 246-290 and 246-390 WAC)
Date:	Thursday, August 26, 2021 2:07:45 PM

External Email

I vote yes.

On Wed, Aug 25, 2021 at 11:08 AM Larry Jones <LJones@firgrove.org> wrote:

> Good morning,
>
> This message was prepared by and is submitted on behalf of the **Regional Water Cooperative of Pierce County,** an organization serving drinking water to over a half million Washingtonians in multiple counties.
>
> The Washington Department of Health advertised Proposed Rule revisions to Chapters 246-290 and 246-390 WAC related to PFAS and associated Lab monitoring on 8/13/21 (*Department of Health Code Reviser Filings from July 21, 2021- August 4, 2021*). The included CR 102 forms for each identified a deadline of September 3, 2021, for comments to the proposed rules. We recognize this is a significant rulemaking effort to an emerging environmental challenge. Given the complexity of the issues, and the very substantial impact this proposed rule may have on drinking water utilities and the people they serve, we request an approximately one-month time extension for comments to these proposals until October 4, 2021.
>
> Sincerely,
>
> Larry D. Jones
>
> President
>
> 253-845-1542
>
> Regional Water Cooperative of Pierce County

From:	Larry Jones
To:	DOH EPH DW PFAS; Jones, Jocelyn W (DOH); Helpling, Nina D (DOH)
Cc:	Chris McMeen; Craig Downs; Steve Sacksteder; Jeff Johnson
Subject:	Request for an extension of the WDOH PFAS Rule Proposals (Chapter 246-290 and 246-390 WAC)
Date:	Wednesday, August 25, 2021 11:10:07 AM

External Email

Good morning,

This message was prepared by and is submitted on behalf of the **Regional Water Cooperative of Pierce County,** an organization serving drinking water to over a half million Washingtonians in multiple counties.

The Washington Department of Health advertised Proposed Rule revisions to Chapters 246-290 and 246-390 WAC related to PFAS and associated Lab monitoring on 8/13/21 (*Department of Health Code Reviser Filings from July 21, 2021- August 4, 2021*). The included CR 102 forms for each identified a deadline of September 3, 2021, for comments to the proposed rules. We recognize this is a significant rulemaking effort to an emerging environmental challenge. Given the complexity of the issues, and the very substantial impact this proposed rule may have on drinking water utilities and the people they serve, we request an approximately one-month time extension for comments to these proposals until October 4, 2021.

Sincerely,

Larry D. Jones
President
253-845-1542

Regional Water Cooperative of Pierce County

Regional Water Cooperative of Pierce County (RWCPC) Review

Chapter 246-290 WAC and CR 102 Document

#	Page Number	Section	Review Comment	Recommended Language or Action (if applicable)	Reviewer
1	General	N/A	Members of the Regional Cooperative of Pierce County represent over 20 public water systems in Washington State, serving over a half million drinking water customers. We take our mission to provide safe drinking water very seriously. Although not a problem created by water utilities, we support the work of characterizing PFAS occurrence in drinking water, and the science of understanding the public health significance of that occurrence against the backdrop of the many modes of exposure in our communities. Our comments generally are intended to improve the clarity and accuracy of risk understanding and communication, and the rational, clear, science-based development of regulations to protect people.		RWCPC
2	CR102 P.2 246-290-71006, p. 83	Statement: "Group A water systems with an exceedance of any PFAS SAL must notify their customers so they can make more informed decisions about their health and the health of their families." 246-290-71006	Accurately Informed customers is important. The challenge is that the information required to accurately inform customers does not appear in the proposal or supporting documentation. If a SAL is 10 ng/L, but a water system measures 12 ng/L, what is the appropriate message to help customers be "more informed about their health..."? Is the water unsafe for all people, for a subset of people (a most vulnerable population)?	Please develop clear, fact-based messaging for PWS to use across the array of potential sampling results. In plain language, clearly identify the process and assumptions (Subpopulation most at risk, RfD development, application of uncertainty factors, etc.) used in deriving SALs. Please provide water systems with consistent language and guidance for PFAS-related public notice. Provide different notice language based on the range and relative health risk of PFAS measured in the water source. Prior to requiring water systems to provide public notice regarding PFAS in drinking water, please provide relevant communication and messaging to healthcare providers in Washington State so that they may appropriately respond to potential patient concerns following notice to the public.	RWCPC
3	CR102 P.6	Statement "In this rulemaking, the board and the department considered setting a state maximum contaminant level (MCL) for PFAS but ultimately the board directed the department to develop a "state advisory level", which is undergoing a concurrent name change in this proposal to “state action level (SAL)."	Different toxicologists (Federal and State) have used different analyses (toxicological endpoints of concern, points of departure, reference doses, water consumption, bioaccumulation in serum, serum half-life relative source contributions, application of uncertainty factors, etc.). These considerations and their application result in a range of action or maximum contaminant levels for the selected PFAS, all of which are deemed by the respective toxicologist as "safe". It is very difficult to explain to customers the variation in these analyses, the inherent uncertainties of toxicological assessments, and layers of conservatism applied. The supporting document "PFAS Toxicological Assessment", which forms the underlying basis for the SALs, equates SALs to maximum contaminant level goals (MCLGs) under the Safe Drinking Water Act, representing the "maximum level in tap water that we consider to be without health concern for long-term consumption in daily drinking water." However, there are no requirements for enforcement or public notification for MCLGs, which makes the SAL thresholds much more complex to explain to water system customers. If public notification is required for SAL exceedances, SAL development should include a cost-benefit analysis similar to what is required for setting MCLs. We recommend that the more rigorous development of a maximum contaminant level (MCL) be completed. This must weigh the totality of expected benefits across the totality of costs, which is a more appropriate approach to addressing this emerging and rapidly evolving concern. This allows optimized risk reduction solutions for a community facing a range of resource constraints.	Continue forward with a requirement to monitor (to develop occurrence data), similarly to the approach used by EPA in developing new regulatory determinations. Develop supporting toxicological assessments applicable to all people in a community. This will enable development of applicable risk communication materials for all community members, and support informed decisions regarding the removal of a water source from use, or investment in treatment, if feasible. The Department, through this SAL approach, is placing very difficult public health analysis, risk assessment and decision-making on utilities and customers that often do not have the training or background needed to fully assess options. Perform the necessary analyses including a rigorous cost: benefit model to develop enforceable maximum contaminant levels (MCLs). Promulgate appropriate MCLs with associated required action.	RWCPC

#	Page Number	Section	Review Comment	Recommended Language or Action (if applicable)	Reviewer
4	246-290-**010** Definitions, p 5	(44) Confirmation means to demonstrate the accuracy of results of a sample by analyzing another sample from the same location within a reasonable period of time, generally not to exceed two weeks. Confirmation is when analysis results fall within plus or minus thirty percent of the original sample results.	Suggest that the definition be "Confirmation sample" rather than "Confirmation". "Confirmation sample" is how it is used throughout the monitoring and follow up actions sections. A confirmation sample does not demonstrate the accuracy of results. Accuracy is not the correct word to use here. A second sample collected on a different day under different conditions cannot demonstrate "accuracy" of the initial sample result. They are two different samples. The accuracy of a sample result is confirmed only by the QA/QC performed by the lab at the same time that the sample is run, and/or by analyzing a second aliquot of the very sample (leftover sample) and obtaining the same result (+/- allowable limits). A confirmation sample is a second sample from the same location collected at a later date (generally within two weeks) and analyzed to confirm with confidence that the earlier detection/presence in the initial sample is real and valid and representative of that sampling location's source water concentration.	Should be able to get the official lab definition from Ecology's environmental lab accreditation unit or an accredited lab. But something like below (this is a difficult one to word): A confirmation sample is a second sample from the same location collected at a later date (generally within two weeks*) and analyzed to confirm with confidence that the earlier detection/presence in the initial sample is real and valid and representative of that sampling location's source water concentration. (*generally not to exceed two weeks following initial sample collection date, or within 10 business days of receiving initial sample results from the lab, whichever is greater)	RWCPC
5	246-290-010 Definitions, p. 14	(170) "PFAS"	In the definition of "PFAS", the word "form" should be "foam".	Change "form" to "foam".	RWCPC
6	246-290-**010** Definitions, p 16	(214) Running annual average (RAA)	Thank you for adding this definition. All is clear, as written. An addition is suggested. There is another scenario where zero may be used to calculate the RAA, and that is when the source(s) with >MCL concentration of the chronic contaminant is out of service the entire quarter: 1) intentionally removed from service for mitigation purposes,i.e., to reduce customer exposure to the chronic contaminant and to ensure compliance with the RAA-based MCL, or 2) out of service for operational or other reasons. EXAMPLE of #1: Arsenic at >10 ppb, when blending of sources to <10 ppb prior to entry is not yet an option, for example during treatment design.	"If source(s) with ≥MCL concentration of a chronic contaminant are not in service the entire quarter, and therefore not being served to customers, zero may be used for that quarter to calculate the RAA." Probably not appropriate for the Definition section, but: DOH may want to add that "water system shall let department know if/when the source is being removed from service and when it is returned to service".	RWCPC
7	246-290-**010** Definitions, p 18	(238) State action level (SAL)	Change "triggers actions a purveyor takes" to "triggers actions a purveyor **must** take" (per 246-290-320 - Follow up action, and consistency with Summary of Changes wording for -320)	see at left	RWCPC
8	246-290-**130** Source Approval, p 28	(4)(g)(ii), or (vii)	Add corrosion WQPs to initial water quality analysis (at minimum, alkalinity and calcium). Even better, add these tests to all routine compliance IOCs. In addition to the complete IOC for initial analysis (which already includes hardness and conductivity), please also require alkalinity & calcium (tested by lab) and field pH and temperature measured by qualified trained operator/sampler. With the increased requirements of the revised LCR to ensure corrosion in the system is controlled/optimized, DOH Regional Engineers are now expecting the water system to evaluate in the project report the impact the new source may have to the overall water quality on the system. These corrosion WQPs inform a better assessment; please require them upfront for new source approval. Better yet, they should be required in every routine IOC, as another indicator of water quality stability. It's more effective to just add these tests to the lab template IOC report and require them up front than it is to expect water systems to do them voluntarily (at least alkalinity and calcium; We realize field measurements like pH and temp can be difficult to require via a lab template). Thanks for considering.	Add corrosion WQPs to (4)(g)(ii) or (vii).	RWCPC
9	246-290-**130** Source Approval, p 29	(4)(g)(vi)	Re-word sentence slightly for clarity. First state where contaminants with SALs can be found, then state the exceptions and where those exceptions can be found.	(vi) Contaminants with a SAL under WAC 246-290-315, Table 9, except where waived or not applicable under WAC 246-290-300 (10)	RWCPC
10	246-290-**130** Source Approval, p 29	(4)(h)	Contradicts (4)(g)(i), which states raw water coliform source sample must be satisfactory	If unsatisfactory raw water coliform sample may be approved if treatment is provided, add "unless approved disinfection treatment is provided" to end of sentence in (4)(g)(i).	RWCPC

#	Page Number	Section	Review Comment	Recommended Language or Action (if applicable)	Reviewer
11	246-290-**300** Monitoring Requirements, p 30	(1)(c)	This sentence needs clarification. "The analyses must be performed by a laboratory accredited by the state using EPA-approved methods or other department-approved methods"	By definition, "department" refers to the Department of Health. Does DOH approve methods used by accredited labs for analyses? (if so, how?) By "department-approved", do we actually mean Ecology's environmental lab accreditation unit? Or does the "using EPA approved methods" portion of this sentence apply only to the accredited lab, and the "or other department-approved methods" apply to the tests that are mentioned in the next sentence that can be performed by the various DOH-approved parties? If so, please split this sentence up for clarity.	RWCPC
12	246-290-**300** Monitoring Requirements, pp 43-44	(10)(a)	1. First sentence: "Purveyors shall monitor for contaminants with an SAL in accordance with Tables 3 and 4 of this section." The word "monitor" in this sentence is too general. Tables 3 and 4 specifically address frequency and location for sampling. Replace the word "monitor" with "sample". 2. Second sentence: "Source sample locations and blended samples are allowed as consistent with other federally regulated organic contaminants referenced in subsection (7)(b) of this section". "Other" as it is used in this sentence could mistakenly imply that, being an organic with source sample locations and blended samples also being allowed as consistent with those "other" federally regulated organic contaminants, that PFAS contaminants are also federally regulated. But they are not federally regulated; they are state regulated.	1. "Purveyors shall sample for contaminants with an SAL in accordance with Tables 3 and 4 of this section." 2. Remove the word "other" from the sentence. Not needed. "Source sample locations and blended samples are allowed as consistent with federally regulated organic contaminants referenced in subsection (7)(b) of this section".	RWCPC
13	246-290-**300** Monitoring Requirements, pp 43-44	(10)(a), Table 3	1. Table 3 heading - "SAL Monitoring" SALs are not monitored. Contaminants are monitored. 2. Table 3 addresses sampling requirements for contaminants with SALs. First column heading "Per and polyfluoroalkyl substances (PFAS)" is too general. Not all PFAS chemicals have SALs. 3. Table column headed "Initial Sampling": "One sample on or before December 31, 2025". The "one sample" is misleading. If one were to not read beyond Table 3, one would not be aware that additional sample(s) are required to confirm the presence and concentration of a detection in an initial sample within a certain time frame (two weeks?), in order to determine required follow up action and future sampling frequency. 4. Table column headed "Routine Sampling Frequency": "Once every three years". Without further elaboration of what routine means, this could be misleading.	1. Change heading to "Monitoring for Contaminants with SALs". 2. Only the specific contaminants with SALs should be listed in this column. Column heading should be "Contaminant". Remove the words "or Groups of Contaminants". 3. "One sample on or before December 31, 2025". (unchanged) Footnote needed below table: "Additional quarterly sample(s) is/are required if there is a detection of any PFAS contaminant tested, and if there is an exceedance of any PFAS SAL. This is to confirm the presence and concentration of PFAS. Number of required quarterly samples is based on concentration in the initial sample (see the appropriate section for low, med, high % of the SAL, and exceedance of the SAL)". 4. "Once every three years. Add footnote: "If no PFAS contaminants tested are detected during initial sampling".	RWCPC

#	Page Number	Section	Review Comment	Recommended Language or Action (if applicable)	Reviewer
14	246-290-**300** Monitoring Requirements, p 44	(10)(b)	"Purveyors shall monitor for the PFAS contaminants listed in Table 7 under WAC 246-390-075". (Lab Rule -390, different chapter of the WAC than -290) Each of the two currently approved EPA test methods requires a list of 18 or 25 specific PFAS contaminants that are specific to that test method. Method 537.1 requires testing for 18 PFAS contaminants. Method 533 requires testing for 25. Most overlap between the two methods but a few do not. All 18 or 25 contaminants must be tested by the lab - not just the five PFAS contaminants with SALs - and reported to DOH in order for the water system to qualify for a monitoring waiver at a later date (once waiver model is developed). The lab cost per sample can also vary for each of the test methods used. The purveyor's lab may give the purveyor the option of choosing which test method they would like the lab to use. Because WAC 246-290-300(10)(b) refers out to a different chapter (the Lab Rule -390), and there is no mention in -300 (10)(b) of the differences between the two available methods, there should be additional information provided in -300(10(b) on all of this.	Please clarify if it is the department's intent that every system required to monitor for a SAL must have each sample analyzed using both EPA Method 533 and EPA Method 537.1 in order to test for <u>all 29 analytes</u> listed in Table 7 (WAC-246-390-075) as seemingly required by WAC 246-290-300(10)(b). Or, can either method be used, with analyses completed only for PFAS for which there is a SAL? <u>Recommendations:</u> "Purveyors shall monitor for the PFAS contaminants listed in Table 7 under WAC 246-390-075. The total number of contaminants required to be tested, and the specific contaminants required to be tested, is specific to the test method used. All contaminants required by each method must be tested and reported to the department in order for the water system to qualify for a monitoring waiver." 1. Change heading to "Monitoring for Contaminants with SALs". 2. Only the specific contaminants with SALs should be listed in this column. Column heading should be "Contaminant". Remove the words "or Groups of Contaminants". 3. "One sample on or before December 31, 2025". (unchanged) Footnote needed below table: "Additional quarterly sample(s) is/are required if there is a detection of any PFAS contaminant tested, and if there is an exceedance of any PFAS SAL. This is to confirm the presence and concentration of PFAS. Number of required quarterly samples is based on concentration in the initial sample (see the appropriate section for low, med, high % of the SAL, and exceedance of the SAL)". 4. "Once every three years. Add footnote: "If no PFAS contaminants tested are detected during initial sampling".	RWCPC
15	246-290-**300** Monitoring Requirements, p 44	(10)(b)(ii)	"Initial PFAS sampling prioritization and scheduling is based on the following criteria:..." Just as (10)(b)(iii) states specifically that at-risk TNC systems must sample as directed by the department), (ii) should also state something similar for Group A community and NTNC systems. It is not clear, as currently written in (i) and (ii), that the department prioritizes which sources are to be scheduled during initial sampling and/or if the water system can prioritize the sampling themselves using the criteria.	(ii) "Initial PFAS sampling prioritization and scheduling, as determined by the department, is based on the following criteria".	RWCPC
16	246-290-**300** Monitoring Requirements, p 44	(10)(b)(ii) and (ii)(A),(B, and)(C)	Consistent with at-risk TNC systems in (iii), sampling prioritization and scheduling criteria used by the department for community and NTNC systems should include "due to proximity of the system's water supply to known PFAS contamination". Should also define what the proximity criterion is ("within 2 miles"?). This could be listed under (B) Vulnerability of the source to PFAS contamination. "(A) Susceptibility of the source water to contamination by surface activities due to physical attributes of the source". "(B) Vulnerability of the source water to PFAS contamination".	List examples of the source physical attributes that contribute to this susceptibility and that DOH will use to prioritize and schedule. List examples of vulnerability. Proximity and relative location to a known source of contamination could be listed here. Groundwater flow in the area, and the source being downgradient of a known contaminated source. The above will help water systems better understand source vulnerability and susceptibility, source protection, and how to prioritize their source sampling. In turn, water systems can share their intimate knowledge of their systems/sources with DOH to assist with accurately assessing and prioritizing susceptibility and vulnerability.	RWCPC
17	60	246-290-455(2)	This section reads that "Purveyors using treatment <u>or blending</u> to remove or reduce a contaminant with a SAL" shall collect finished drinking water samples on a quarterly basis. With the inclusion of blending in this section, any system that blends sources prior to the entry point to the distribution system and has some detection of PFAS in any of those sources would inherently have to monitor quarterly. WAC 246-290-300(10)(a) indicates that blended samples are allowed. Please clarify in what cases blending is considered treatment for PFAS and requires quarterly monitoring.	Remove the words "or blending" from the section, or clarify that the quarterly monitoring requirement only applies when initial blended sample results are greater than a SAL and changes in blending operations are used to reduce the concentrations below that SAL.	RWCPC

#	Page Number	Section	Review Comment	Recommended Language or Action (if applicable)	Reviewer
18	Page 18 & Page 85	246-290-010 Definitions 246-290-72004(5) Contents Definitions	Definition of SAL is not consistent; if SAL is exceeded one indicates "actions a purveyor takes", while the other indicates, "actions a water system **must** take" (emphasis added). The first allows ambiguity (compulsory vs. voluntary?), the second does not.	Adopt uniform definition language that is clear about requirements.	RWCPC
19	95-96	WAC 246-290-72012 Regulated contaminants.	The co-mingling of SALs and MCLs in this table, alongside MCLGs may be misleading to some readers. The development document for the PFAS SALs explicitly states that the derived values are based on the MCLG model. It would therefore be more transparent and accurate to list SALs with MCLGs than with MCLs. The prescribed health effects language is challenging. All possible adverse health impacts are listed, but the derived SAL is not based on all those health effects. As well, terms like "much higher than" are ambiguous, and may cause confusion.	Move the SALs to the same column as MCLGs, or differentiate with their own column. Develop comprehensive health effects communication tools, and cite them as references here. At our current state of knowledge, the varying potential impacts of PFAS across populations and exposure levels do not lend themselves to be effectively reduced to two sentences. EPA's current Health Advisory Fact Sheet is 5 pages long, and its document titled "Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA)" is over 100 pages long. A balance of clarity and depth must be struck, but these two sentences as mandatory health effects language, at our current state of knowledge and national consensus, may be insufficient.	RWCPC
20	95	246-290-71006	Table 17 includes DCPA acid metabolites but with an assigned tier level but it is not included with an established SAL under table 9 of 246-290-315(4)a.	Clarify monitoring/SAL requirements for DCPA acid metabolites	RWCPC
21	99	246-290-72012	Under Treatment Technique Violations, the added lines for Acrylamide and Epichlorohydrin do not identify their use as drinking water treatment chemicals in the "Major Sources in Drinking Water" column.	Include "Added to water during water treatment" to both lines.	RWCPC
22	Recommended State Action Levels for Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water: Approach, Methods, and Supporting Information - Page 4	Glossary of Toxicological Assessment	Gives m/L instead of mg/L; (typo).	Use mg/L	RWCPC

Sammamish Plateau Water

1510 228th Avenue SE
Sammamish, WA 98075
Main: 425.392.6256
Fax: 425.391.5389
www.spwater.org

VIA EMAIL (PDF) TO PFAS1@DOH.WA.GOV

September 2, 2021

Jocelyn W. Jones
Department of Health
Office of the Assistant Secretary
PO Box 47820-7820
Olympia, WA 98504-7820

Re: Comments on Chapter 246-290 WAC, Group A rule proposal (PFAS)

Sammamish Plateau Water and Sewer District (District) has reviewed and considered the Group A rule proposal regarding regulation of per- and polyfluoroalkyl substances (PFAS) in public drinking water sources, and is offering the following comments regarding sections cited as part of the formal public comment period:

WAC 246-290-300 (1)(c)
Water purveyors often have wells in shared aquifers. As a matter of testing correlation and consistency, the District recommends requiring testing methods for shared aquifers which would be consistent among purveyors who share an aquifer, rather than provide for alternative methods that may results in non-congruent results from agencies whom share the aquifer. Where purveyors share an aquifer, they should be required to use the same lab and consistent laboratory methods for testing and monitoring.

Additionally, for purveyors with shared systems and/or interties, the District recommends that systems exchanging water should be required to use the same lab and consistent laboratory methods to avoid dis-alignment of detection levels and analytes.

WAC 246-290-300 (10)(b)(ii)(A)
When the Department of Health identifies and prioritizes agencies for scheduling sampling, the District suggests the Department of Health take into consideration and prioritize those purveyors whose sources which are susceptible to contamination as a result of storm water injection (UICs) which is permitted or rule authorized by the Department of Ecology or local storm water utilities operating under an NPDES permit.

WAC 246-290-300 (10)(e)
The District supports confirming samples, but questions the logic and validity behind averaging test results when the lab results should speak for themselves.

WAC 246-290-300 (10)(g)(i) and (ii)
The language in these sections are unclear and confusing. It does not define who's MRL is being referenced (is it the labs, EPAs, DOHs?). Additionally, if a lab test result is found to exceed an SAL, regardless whether the sample was related to UCRM5 or proposed rules, an exceedance of the SAL should not be reported as a nondetect.

To eliminate contradictions and provide clarity, the District suggests separating the proposed state rules from federal UCMR5 requirements. The Department of Health (DOH) rules should be applied using the State's standards for ease of understanding and administration.

If a water purveyor uses a lab with a detection limit that can report to the SAL level, a nondetect result should not be reported. The District suggests the State require purveyors use a lab that will meet both the State's and federals requirements.

WAC 246-290-315 (8)
Current proposals to establish an SAL, rather than an MCL, which will default in the future to a federal established MCL create significant operational and financial uncertainty for water purveyors. The current proposed SAL may result in circumstances where purveyors could be required to construct and operate PFAS removal treatment plants which at a future date may be rendered unnecessary as a result of the proposed rules defaulting to a federal MCL.

In the case of Sammamish Plateau Water and Sewer District, we currently exceed the proposed State SAL for PFOS for two wells which have been taken out of service. The District is currently proactively designing a PFAS removal treatment plant to comply with the proposed rules so the wells could be returned to service. The proposed treatment plant is estimated to cost $15-20 million dollars to construct. However, the proposal to default to a future unknown federal MCL, or undefined State MCL, may render this investment unnecessary and a waste of our ratepayer’s resources.

WAC 246-290-320 (1)(b)(iii) and WAC 246-290-320 (1)(d)(iv)
It is unlikely a purveyor will ever be the party responsible for the cause of the contamination, as it is primarily attributable to the use of AFFF. In order to conduct environmental analysis of the contamination, a purveyor would require prior knowledge of sources which may have existed and/or where fire agencies have used AFFF during the course of operations or training. In the District's experience, fire agencies are guarded/reluctant to disclose where AFFF was used, and environmental analysis is costly and time consuming. We recommend that the responsibility for investigation of

the cause of contamination be assigned to the Department of Health or Department of Ecology. Water purveyors should not be required to bear the cost or responsibility. Note that the Department of Ecology is also engaged in rulemaking regarding PFAS Chemical Action Plans. As such, the District recommends that this requirement be considered for assignment to the Department of Ecology rather than the Department of Health.

WAC 246-290-320 (8)(b) Table 10
We recommend simplifying the proposed monitoring requirements under Table 10 and establishing clear parameters for follow up based on positive or negative sample results as opposed to using SAL ranges.

WAC 246-290-320 (9)(a)
The proposed rule does not adequately define the term "sampling point."

WAC 246-290-71006 (2)(b)
The language in WAC 246-290-320 (9)(a) requiring quarterly samples is logistically dis-aligned with this WAC subsection. In other words, purveyors may be required to issue a notice under this WAC subsection without having the benefit of the additional quarterly sampling result required under WAC 246-290-320 (9)(a).

Thank you for taking our comments into consideration and including them as part of the record for the formal public comment period.

Sincerely,

John C. Krauss
General Manager

cc: Sammamish Plateau Water Board of Commissioners
Judi Gladstone, Washington Association of Sewer and Water Districts

TACOMA WATER
TACOMA PUBLIC UTILITIES

3628 South 35th Street
Tacoma, Washington 98409-3192

TACOMA PUBLIC UTILITIES

September 2, 2021

Jocelyn W. Jones
Department of Health
Office of the Assistant Secretary
PO Box 47820-7820
Olympia, WA 98504-7820
PFAS1@doh.wa.gov

RE: Comments on Proposed PFAS Rule

Dear Ms. Jones:

Tacoma Water is appreciative of the efforts that the Department of Health has taken to obtain and incorporate feedback on the PFAS rule. We have reviewed the current language of the PFAS rule and the proposed changes to WAC 246-290 and would like to provide the following comments:

- WAC 246-290-455(2) states that "Purveyors using treatment or blending to remove or reduce a contaminant with a SAL" shall collect finished drinking water samples on a quarterly basis. With the inclusion of blending in this section, any system that blends sources prior to the entry point to the distribution system and has some detection of PFAS in any of those sources would inherently have to monitor quarterly. Please remove the words "or blending" from the section, or clarify that the quarterly monitoring requirement only applies when initial blended sample results are greater than a SAL and changes in blending operations are used to reduce the concentrations below that SAL.

- WAC 246-290-71006 Table 17 includes DCPA acid metabolites as contaminants with a SAL requiring Tier 2 public notice designation; however, there is no SAL value established for DCPA acid metabolites in WAC 246-290-315(4)a Table 9. Please remove DCPA acid metabolites from Table 17 or clarify what SAL value and monitoring requirements are proposed for DCPA acid metabolites.

- WAC 246-290-72004(5) provides a required definition of a SAL as "the concentration of a contaminant in drinking water established to protect public health and which, if exceeded, triggers action a water system must take." This definition is inconsistent with the definition in WAC 246-290-010(238), which includes the language, "…triggers actions a purveyor takes". Please ensure clear and consistent definitions.

- WAC 246-290-72012 adds lines for Acrylamide and Epichlorohydrin under Treatment Technique Violations, but their use as drinking water treatment chemicals is not identified in the "Major Sources in Drinking Water" column of the table. Please include "added to water during water treatment" to both lines.

- WAC 290-72012 includes SALs in the same column as maximum contaminant levels (MCLs) in the table, yet the Toxicological Assessment equates SALs to maximum contaminant level goals (MCLGs) under the Safe Drinking Water Act. If SALs are calculated based on the model for MCLGs, SALs should be included in the MCLG column of the table rather than the MCL column.

- The Toxicological Assessment compares SALs to MCLGs; however, there are no requirements for enforcement or public notification for MCLGs, which makes the SAL thresholds much more complex to explain to water system customers. If public notification is required for SAL exceedances, SAL development should include a cost-benefit analysis similar to what is required for setting maximum contaminant levels (MCLs).

- Please provide water systems with consistent language and guidance for PFAS-related public notice. Provide different notice language based on the range and relative health risk of PFAS measured in the water source.

- Prior to requiring water systems to provide public notice regarding PFAS in drinking water, please provide relevant communication and messaging to healthcare providers in Washington State so that they may appropriately respond to potential patient concerns following notice to the public.

If there are any questions regarding these comments, please contact Craig Downs at 253-318-6695.

Sincerely,

Scott Dewhirst
Water Superintendent

cc: File

SIERRA CLUB
WASHINGTON STATE

September 3, 2021

Jocelyn Jones
Policy Planner and Project Manager
Washington State Department of Health

Nina Helpling
Policy and Rules Coordinator
Washington State Department of Health

Dear Ms. Jones and Ms. Helpling:

The Sierra Club Washington Chapter is writing to urge the Department of Health to take strong and immediate action to protect state residents from toxic per- and poly-fluoroalkyl substances (PFAS) chemicals in drinking water. The state is leading national efforts to curtail the unnecessary use of PFAS in new products including food packaging and textiles, yet communities around Washington have significant exposure to PFAS in drinking water. The state should act with urgency to avert these exposures.

We applaud the proposed water guidelines as a first step toward community protection and suggest ways the Department of Health can modify and extend this proposed action to ensure the most meaningful and timely protection for state residents who have been exposed to harmful amounts of PFAS in drinking water, sometimes for decades.

Proposed SALs - The proposed State Action Levels (SALs) for drinking water are a useful way to identify water sources that contribute to excessive exposures for residents of the state. However, State Action Levels are not binding. Washington should explore provisioning state funding and technical support for water systems and well owners with water levels that exceed the SALs. Otherwise, potentially only larger and more affluent cities/water systems will enact costly treatment which could result in inequitable protection from contaminated water across the state.

We encourage the Department of Health to enact Maximum Contaminant Levels (MCLs) as the next step to ensuring lasting protection of drinking water sources. MCLs are legal limits for pollutants, and grant the state enforcement authority for non-compliance. MCLs also give additional legal protection to communities impacted

by military contamination or other industrial sources. Presently, the military is only extending water filtration to communities where PFAS levels exceed the weak and non-protective federal health advisory of 70 parts per trillion for the combined PFOS + PFOA. We also support provisions in WAC 246-290-315(8) (8) that clarify that future federal MCLs for contaminants will superseded state SALs or less protective state MCLs.

Ensuring health protective guidelines - While the proposed SALs are stronger than federal guidelines, they are still less protective than the state MCLs in Massachusetts and Vermont which limit the sum of 5 or 6 PFAS chemicals to no more than 20 parts per trillion.

With thousands of PFAS chemicals in commerce, these group standards are also a step closer to addressing the additive impacts of exposure to multiple PFAS chemicals via water. Although the Department of Health acknowledges this and calls the individual SALs for 5 chemicals a “reasonable initial approach” we urge the Department to consider people’s concurrent and lifelong exposure to a complex mixture of PFAS chemicals.

The field of PFAS toxicity and epidemiology is growing rapidly with new data on additional PFAS, and more sophisticated methods to measure the impacts of lower levels of exposure. We recommend that the Department of Health set up a mechanism to ensure that all SALs and MCLs for PFAS in drinking water be reviewed and updated regularly. These reviews should consider new data about the additive or synergistic effects of exposure to multiple PFAS chemicals.

Expanding to address more PFAS chemicals - Upon finalizing this guidance, the Department of Health should consider ways to require monitoring with analytical methods that capture a broader array of PFAS chemicals. The Total Oxidizable Precursor Assay (TOP) is a way to quantify PFAS chemicals that break down to form things like PFOS, PFOA, and PFHxS. Additionally, several analytical tests measure total organic fluorine (TOF) or total extractable organic fluorine (EOF), which would include all PFAS chemicals.

As soon as practical the Department of Health should require these tests be used to gauge the magnitude of human exposure to other unidentifiable PFAS chemicals and ensure selection of treatment technologies that are effective in reducing or eliminating exposure to multiple classes of PFAS compounds. We recommend that the Department of Health establish SALs for groups of PFAS chemicals detected by TOP, TOF or EOF, and require that all systems periodically test untreated drinking water with these methods. Systems that exceed the guideline should also test treated drinking water to ensure the final levels in drinking water are sufficiently low.

We support the Department’s new provisions to establish SALs and state MCLs for chemicals including in EPA’s periodic Unregulated Contaminant Monitoring Reporting (UCMR) program. PFAS are an example of a contaminant of emerging

concern that was detected through the UCMR program. While the UCMR 2013-2015 monitoring found widespread detections of PFAS in drinking water, EPA has been unable to set appropriate, timely and health-protective water quality standards for PFAS and any other chemicals, UCMR or otherwise. EPA's next round of UCMR-mandated monitoring for PFAS will include 29 specific chemicals. As a next step, Washington should consider setting SALs for these compounds.

Timeframe for testing and disclosure - Testing and data analysis should not be delayed until 2023. Too many people are drinking this water right now and will continue to do so. PFAS contamination is spreading to reach new waterways, and concentrations could be increasing due to the ongoing use of AFFF for fire fighting and poor control over discharges to waterways.

It is critical that the public is informed as soon as possible about where and what PFAS contamination exists in our communities. We advocate for the public availability of all such testing results of our water sources beyond simply what is served as drinking water. All "transient, non-community water systems" be monitored at least once to ensure they do not contain PFAS. We recommend that in addition to the current required public postings in the media and in the annual reports, notification with exact levels of PFAS in water samples exceeding the standards should be provided as soon as possible to each consumer by direct mail or a water bill insert.

Preserving water quality- Preventing further contamination of ground and surface waters is a crucial aspect of drinking water protection. A large number of measures are urgently needed to keep PFAS out of waters, ranging from setting protective Water Quality Standards for PFAS in surface waters, regulating discharges from point sources into the wastewater system, controlling the disposal of biosolids, landfill leachate, and cleaning up contaminated sites, among others. While many of these aspects are regulated by the Department of Ecology, they can collectively reduce the amount of PFAS in drinking water, and avert the need for costly technologies and permanent treatment regimens to remove PFAS from water at the point of human consumption.

In conclusion, PFAS chemicals pose clear threats to people and the environment. We thank you for your leadership in addressing these chemicals and we strongly urge you to finalize the proposed rules and continue to strengthen the regulation of PFAS in drinking water.

Thank you for your consideration.

Elaine Packard, Chair
Water & Salmon Committee
Washington State Chapter Sierra Club

From: colbyvoelker@everyactioncustom.com on behalf of Carol Voelker
To: DOH EPH DW PFAS
Subject: Strong PFAS drinking water standards
Date: Friday, August 20, 2021 1:02:16 PM

External Email

Dear WA Department of Health,

I appreciate you taking action to protect the health of our communities and ecosystems by setting State Action Levels (SALs) for five different PFAS chemicals in drinking water. As we have seen in communities like Coupeville, DuPont, Airway Heights, and Issaquah, the consequences of drinking water contaminated with PFAS are serious and we have a moral obligation to address PFAS pollution from the source.

As a person of faith, I care deeply about protecting children, elders, and other vulnerable populations from toxic PFAS chemicals. We cannot continue to allow these chemicals into a fluid we all need to survive.

Thank you for listening to faith communities and other stakeholders and editing the draft standard to require that important transient non-community (TNC) water systems near known PFAS contamination follow the same testing guidelines as other large Group A water systems.

In absence of a federal EPA standard, I'm grateful that the Department of Health is taking action on PFAS, but I would like to see more comprehensive action. I am concerned that the state SALs only cover five different PFAS chemicals but there are about 5,000 PFAS in the class. I urge the Department to require additional monitoring for total PFAS and implement a limit on all PFAS to protect drinking water and human health.

Under the current draft rule, if a water system exceeds the proposed SALs for PFAS, further testing/monitoring and public notification is mandated, but action to address the contamination, including clean-up, is not specified. I ask that the rule clearly articulate that if a PFAS SAL is exceeded, clean-up and/or other actions must be taken to return the drinking water supply to concentrations of PFAS below the SAL.

Thank you for your work to protect our communities from toxic PFAS.

Sincerely,
Carol Voelker
4059 31st Ave W Seattle, WA 98199-1701
colbyvoelker@gmail.com

September 3, 2021

Jocelyn W. Jones
Department of Health—Office of the Assistant Secretary
PO Box 47820-7820
Olympia, WA 98504-7820

Dear Ms. Jones:

Toxic-Free Future greatly appreciates the work of the Department of Health (DOH) to adopt drinking water rules that address per and polyfluoroalkyl substances (PFAS) in Washington State's drinking water.

DOH's proposed rule takes important steps to require testing for PFAS in drinking water in the state and establish State Action Levels (SALs). We thank the agency for these steps and request that the rule be strengthened before it is finalized.

Toxic-Free Future and a number of organizations petitioned DOH in 2017 to adopt drinking water standards and the urgency to finalize strong rules continues to grow:

- PFAS drinking water contamination has already had a serious impact on communities in Washington state, including Issaquah, Whidbey Island, Lakewood, and Airway Heights.
- Protecting communities from PFAS exposure is particularly important due to the ability of the chemicals to impact the immune system. PFAS can weaken the immune system and make people more likely to catch infectious diseases like colds, stomach bugs—and potentially Covid-19. This is suggested by several studies finding people with higher exposures to PFAS are at increased risk of communicable diseases. PFAS can also reduce vaccine effectiveness.
- Lawsuits are mounting. The Washington State Department of Corrections, the City of Airway Heights, the Lakewood Water District and the Kalispel Tribe have each filed lawsuits this year to help recoup the costs of clean-up and other impacts of inaction by the U.S. government and chemical companies.
- EPA data from 2016, not previously included in the PFAS Chemical Action Plan, shows significant PFAS groundwater contamination at a Moses Lake Superfund site.
- The Centers for Disease Control recently measured elevated levels of PFAS in the blood of Airway Heights residents. As stated in the supporting document for the draft rule, "a recent Center for Disease Control / Agency for Toxic Substances and Disease Registry study in the community of Airway Heights, Washington showed that study participants had mean serum levels of PFHxS that were 60 times higher than national norms even two years after PFAS contamination had been fully mitigated in their community drinking water."

- In 2020, new drinking water contamination was found by the Navy in an PFAS investigation of Kitsap Naval Base-Bangor.

We urge the Department of Health to strengthen the rule in the following ways:

1. **Address PFAS as a class**: There are more than 5000 PFAS in the class of PFAS and Dept of Health is proposing to address five. We urge the department to also obtain information on the presence of other PFAS by requiring testing for total fluorine or using the total oxidizable precursor assay. This approach is essential to our understanding of what chemicals are present in drinking water in our state, and will inform the department for development of future standards. DOH should also establish a limit for total PFAS detected.

2. **Do not delay testing or rule implementation until 2023**: We strongly urge immediate PFAS testing and implementation of the drinking water rule. Washington is far behind many states such as Michigan, New Jersey, and others. There is no reason for such a long delay and water systems have known this rule would be adopted since 2017. As mentioned above this is an urgent matter of protecting health, particularly the most vulnerable.

3. **Require PFAS testing for transient noncommunity systems once every three years.** For some systems, like those that serve churches and motels, the draft rule only requires testing if the department finds they are at risk. Transient noncommunity systems include several categories that often serve individuals for an extended period: motels, restaurants, churches, and farmworker housing. We urge the department to include transient noncommunity systems in the full monitoring requirement and require testing once every three years.

4. **When SALs are exceeded, it should be clear that mitigation is required to meet the SAL**. The draft rule requires water systems to notify consumers when SALs are exceeded as well as continued monitoring and investigation of the cause of contamination. It also requires action as directed by the department. The rule should more clearly require that systems ensure the SALs are not exceeded. What other actions would DOH require other than meeting the action level? It should be clear to water systems that these levels are to be met.

Thank you for the opportunity to provide these comments.

Sincerely,

Laurie Valeriano
Executive Director
Toxic-Free Future

STATE OF WASHINGTON
UTILITIES AND TRANSPORTATION COMMISSION
621 Woodland Square Loop S.E. ● Lacey, Washington 98503
P.O. Box 47250 ● Olympia, Washington 98504-7250
(360) 664-1160 ● TTY 1-800-833-6384 or 711

September 3, 2021

Jocelyn W. Jones
Department of Health
P.O. Box 47820
Olympia, WA 98504-7820

RE: Department of Health Per- and Polyfluoroalkyl Substances Draft Proposed Rulemaking

Dear Jocelyn Jones:

The Utilities and Transportation Commission (commission) appreciates the opportunity to comment on the Department of Health (DOH) Per- and Polyfluoroalkyl Substances (PFAS) Proposed Rule Making. The commission is limiting its comments solely on those sections of the CAP that potentially affect water companies under the commission's jurisdiction.

The commission has the following recommendations and comments:

1) In WAC 246-290-010(170), the definition of PFAS is defined by use. Would it be more useful to base it on the chemical composition characteristics similar to (263) "Trihalomethane (THM)"? Defining the composition structure is clearer for future integration of other chemicals in this family of products.

2) Consider breaking down rules into smaller sections, subsections, or use titles, subheadings, or use white space.
   a) WAC -300 is sixteen pages of text, covering several subjects in detail, making it a very long read. Many of the companies regulated by the commission are small operations with few staff. Simplifying the readability of rules is highly important to ensuring company personnel can quickly reference, understand, and comply with rules.

3) WAC -315(2)(a), what is the definition in the context for the word "fate"?

4) WAC -320:
   a) Is there a time frame, as in WAC 249-290-71006, that owners/operators must notify the public?
   b) Where will the additional "contaminant[s] not included in this chapter" mentioned in (11) be listed so the companies and public are aware of them?

5) WAC -480(1) would be easier to read as a bullet list.

6) WAC -71006(2)(b) language should be simplified: "as soon as practical, but no less than within 30 days" should be either "no longer than 30 days" or simply "within 30 days".

7) WAC -72004(5). If the section is denoting a line to be quoted in reports issued to the public, spelling out the acronyms would prevent confusion or frustration for consumers.

8) Will the notification also require specific information on the chemical found in the test, its half-life, and known potential effects? Will DOH assist the companies in relaying and explaining this information?

The additional information provided in the Significant Legislative Rule Analysis regarding the financial impacts on companies, and thereby customers, was helpful. However, there are a few questions:

1) Are the cost estimates only for the new PFAS testing, or do the estimates include all contaminant testing under current standards?

2) Does "Table 8: Estimated Mean Costs of Three Years of Monitoring on One Source" include only testing costs, or does it also include record keeping, reporting, notification, etc.?

3) Will operators need to send out notifications to customers for every contaminated test? E.g., if quarterly testing is required, will companies need to send quarterly notices to customers?

4) For consistency purposes, will the DOH provide documentation for companies to disseminate to customers, or aid in response to the public's questions regarding contaminants?

Thank you for the opportunity to comment on the proposed rulemaking. Please contact Benjamin Sharbono, Regulatory Analyst, at 360-664-1242 or at benjamin.sharbono@utc.wa.gov with any questions.

Sincerely,

Mark L. Johnson
Executive Director and Secretary

From: David Slight
To: DOH EPH DW PFAS; DOH EPH Lab Rule
Subject: PFAS Rulemaking – Formal Comments
Date: Friday, September 3, 2021 1:56:45 PM

External Email

Washington Water Advocates (WWA) are a group of water advocates sharing information to support the advocacy of cleaner, safer water in Washington State covering topics such as:

- forever-chemicals such as PFAS
- water recycling and reuse
- drinking water quality
- the quality and stewardship of the oceans and waterways
- and other environmental and tribal concerns such as fish and dams on the rivers.

Advocacy groups are critical to provide support for regulators but also to watch and monitor compliance.

We take an interest and are concerned for our future water supply. WWA is writing to urge the Department of Health to take strong and immediate action to protect state residents from toxic per- and polyfluoroalkyl substances (PFAS) chemicals in drinking water. Having reviewed the proposed approach by the Washington State Department of Health (reference PFAS: Per- and Polyfluoroalkyl Substances :: Washington State Department of Health) we are in favor of setting strict levels for PFAS and other chemicals in our water supply that will trigger and require action by local water suppliers.

We applaud the proposed water guidelines and approve and support this first step in establishing thresholds and monitoring regimes and standards for explicit PFAS reporting to protect public health as outlined in PFAS in Group A public drinking water systems encoded as State Action Levels for this first subset of forever-chemicals.

As others have suggested, the timeframe for testing and data analysis should not be delayed until 2023. Too many people are drinking this water right now and will continue to do so. It is critical that the public is informed as soon as possible about where and what PFAS contamination exists in our communities.

Our hope is that over time:

- the levels in various drinking water supplies will be monitored and openly published (supporting the change to within 30 calendar days)

- other specific substances can be added to this list (consider ways to require monitoring with analytical methods that capture a broader array of PFAS chemicals)
- acceptable levels are further reduced (supporting the current reductions to sulfate and chloride)
- the state will help local water districts with monitoring, remediation, and funding
- the state will also look at regulation and licensing around the production and usage of such chemicals in the first instance.

Please include us in future relevant communications and for further information please contact us at info@washingtonwateradvocates.org

Washington Water Advocates
http://www.washingtonwateradvocates.org
September 3rd, 2021