

3M Research & Development

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Office



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 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 perfluoroalkyl 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

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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,



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 PPAR α - 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 ≥ 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

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 “*suggestive 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 3×10^{-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:

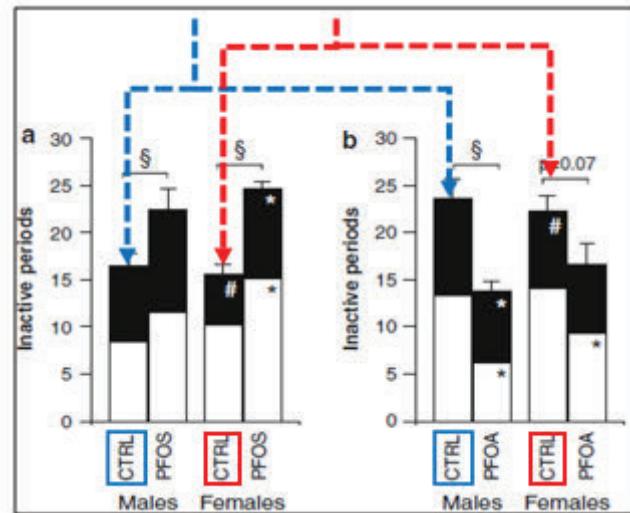
1. They are the same study. Albeit published five years apart, these two publications actually originated from one single study. From the same pregnant dams treated with dietary PFOA during gestation, the pups evaluated by Onishchenko et al. (2011) were litter-mates of the pups evaluated by Koskela et 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. The study design was flawed and insufficient to support a NOAEL or LOAEL. 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. Limited sample size. 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-

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. There were no serum PFOA data reported in these studies. 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 precluded 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. Timing of behavior assessments in pups were not appropriate. 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 mm²), 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. 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. 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. PFOA does not affect the reproductive system in laboratory animals. 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 did not 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, PPAR α knockout, and humanized PPAR α (Abbott et al. 2007; Albrecht et al. 2013; Lau et al. 2006; White et al. 2007). At inhalation dose up to 25 mg/m³/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. The developmental effects reported in laboratory animals for PFOA were primarily mediated by maternal effects. 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 only 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,

(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 PPAR α 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 than that 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	
6-12 months	103	
12-19 months	127	<u>Heilbron et al. 1991</u> <u>Pediatr Nephrol</u> . Jan; 5(1):5-11.
2-12 years	127	
20–29	116	
30–39	107	
40–49	99	
50–59	93	
60–69	85	https://www.kidney.org/sites/default/files/docs/11-10-1813_abe_patbro_gfr_b.pdf
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.

et al. 2003; Klaunig et al. 2012; Lake 2009; Ross et al. 2010), the qualitative differences bring us 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 PPAR α genes were knocked out (Abbott et al., 2007). This further supports 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 extrapolation when this uncertainty is already embedded in the large 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 2×10^{-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 PPAR α , 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 PPAR α 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 PPAR α 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 PFCs 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 PPAR α 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 (Goriner 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. The developmental effects reported in laboratory animals for PFOS were primarily mediated by maternal effects. 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 only 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 developmental effects were reported (e.g., decreased 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 PPAR α and CAR/PXR as a major factor in the rodent hepatic response 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 PPAR α /CAR/PXR activation as a mode of action. The transcriptional signature (mRNA) for PPAR α /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 PPAR α and PXR (Bijland et al. 2011). Rosen et al. (2009) observed the same transcriptional signature consistent with activation of PPAR α /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 PPAR α 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 PPAR α 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 PPAR α 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 PPAR α agonists differently than rodent hepatocytes, and activation of human PPAR α 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 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 PPAR α , Bjork et al. (2011) characterized the activation of several other hepatic nuclear receptors (PXR, CAR, the liver X receptor α (NR1H3 or LXRx), 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 PPAR α 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 μ M, with slight activation (much less than for positive control substances) of CAR3 and PXR occurring only at 100 μ M (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 PFOS 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 PFOS (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 (PPAR α) 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_{1,sd}) 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/chromodacryorhea; salivation; piloerection; appearance of fur; palpebral closure; respiratory rate/character; red, crusty deposits; mucous membranes/skin color; eye prominence; eye color; 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., F₁-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 \rightarrow 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 absence 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).

1. Inconclusive immune findings in human epidemiological data. 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. Serum PFOS concentrations in pups should be considered for POD because critical effects chosen by ATSDR were based on (developing) pups. 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 than that 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. HED for PFOS 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	
6-12 months	103	
12-19 months	127	Heilbron et al. 1991 <u>Pediatr Nephrol</u> Jan;5(1):5-11.
2-12 years	127	
20-29	116	
30-39	107	
40-49	99	https://www.kidney.org/sites/default/files/docs/11-10-1813_abe_patbro_gfr_b.pdf
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 PPAR α , 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 development 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	Minimal	0	10	10	10	10
	Mild	2	2	1	2	0
	Moderate	0	0	0	4	3
	Total	2	3	2	4	7
Incidence						

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 F₀ mice or in the F₁ 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	
6-12 months	103	
12-19 months	127	<u>Heilbron et al. 1991 <i>Pediatr Nephrol</i>. Jan;5(1):5-11.</u>
2-12 years	127	
20-29	116	
30-39	107	
40-49	99	https://www.kidney.org/sites/default/files/docs/11-10-1813_abe_patbro_gfr_b.pdf
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 GFRs from the population studied, 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 et 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” (*c.f.* 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 PPAR α , 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 development 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 modeled 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 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 where 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 al. 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 R² of these three models were 0.170, 0.174, and 0.265, respectively. However, the partial R² for PFOA (difference between R² 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 R² 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 R² or partial R² 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 ($\beta = 0.003$); Model 2 ($\beta = 0.012$); and Model 3 ($\beta = 0.011$) adjusted for the same number of covariates in addition to PFOA (2, 7, and 11). The R² 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 R² 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 R² 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 (0.65, 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 ($\beta=0.023$, $p=0.124$). Examining only those workers not taking cholesterol lowering medications (n = 840), the regression coefficient became $\beta=0.031$, $p=0.071$.

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 ($\beta=0.54$, 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 perfluorooctanesulfonyl 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 IU/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 μ M (\sim 360,000 – \sim 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, ATDSR 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 PPAR α 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 ramifications(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 perfluoroctanoate (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 clinical 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 PPAR α -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 underlying 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 PPARGα, 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 5th 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 - 1,530 μM (\sim 360,000 ng/mL - \sim 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 not 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 no 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; Kielson et al. 2016; Mogensen et al. 2015) followed by 4 diphtheria studies (Grandjean et al. 2012; Mogensen et al. 2015; Kielson 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

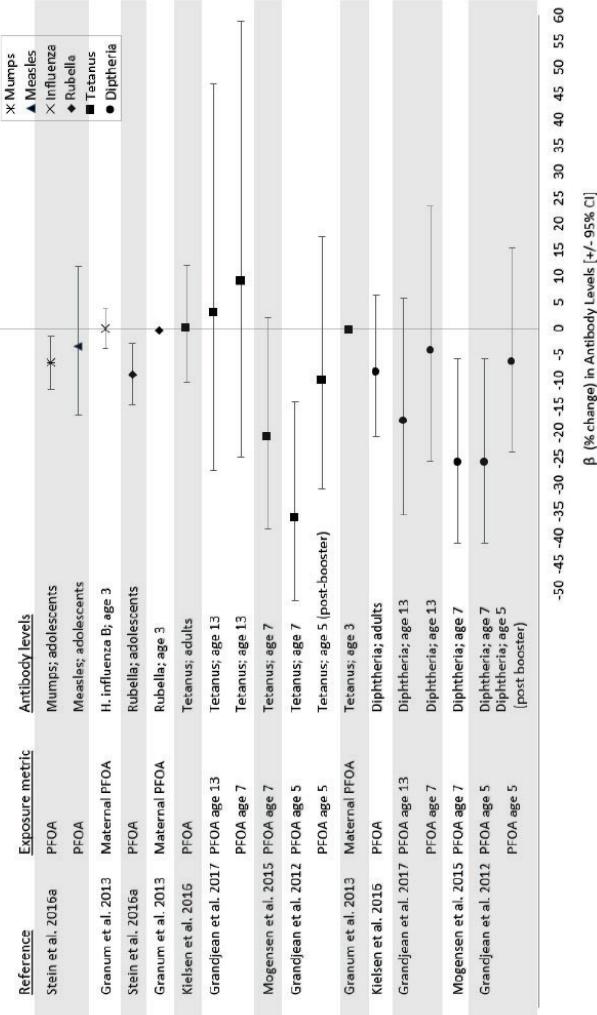
It is inappropriate for ATSDR to interpret antibody responses to these 8 distinct vaccines as a single health outcome (i.e., “decreased antibody responses to vaccines”). Commercially available vaccines differ depending on the nature of the vaccine antigen. Tetanus and diphtheria, for example, are toxoid 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 PFOS 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:

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, mothers 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/specifity 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 “*suggestive 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.

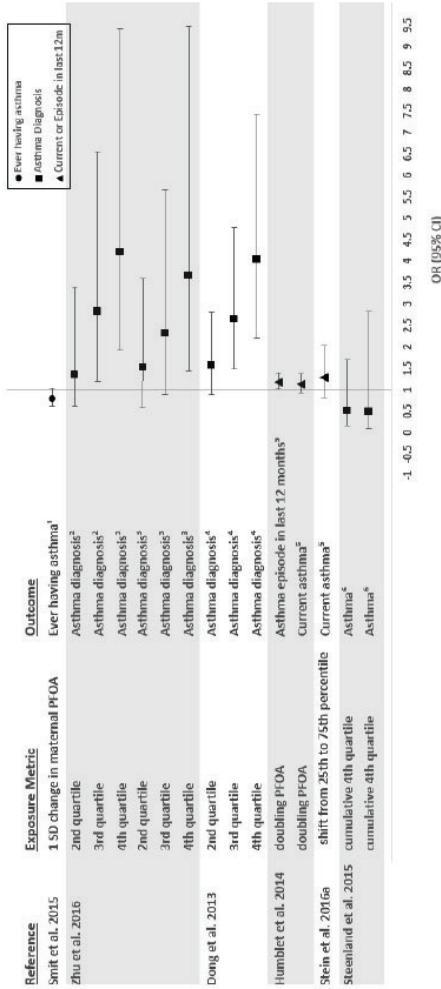
ATSDR provided no interpretation of the epidemiologic evidence or rationale for their conclusion of a “possible link”. In fact, the only conclusion ATSDR provided in the document is the following statement: “*In tests of hypersensitivity, there is some evidence of an association between serum PFOA and 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; Humbert 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) from 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.”

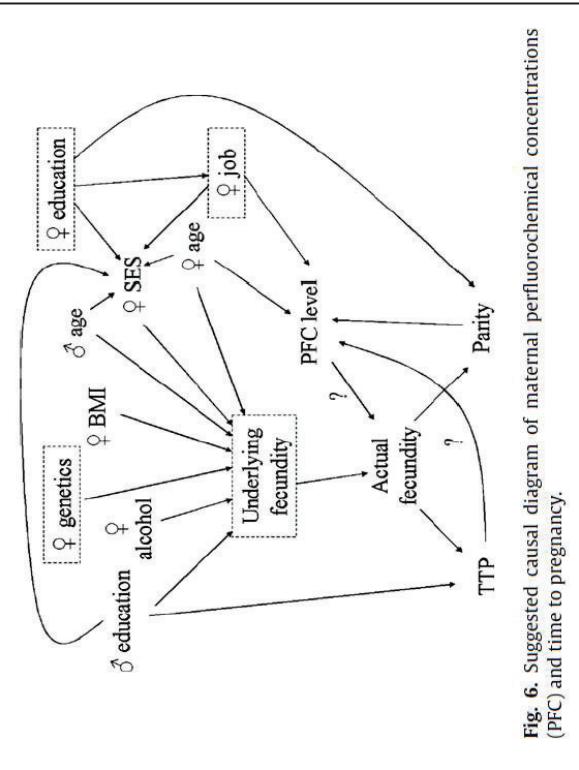


Fig. 6. Suggested causal diagram of maternal perfluorochemical concentrations (PFC) and time to pregnancy.

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 perfluoroctanoate (POFA) and perfluoroctane 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 acyclic 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 1.5-19 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 PFOS 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), Linters 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 online 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.

- [REDACTED] has served as a peer reviewer selected by ATSDR on the 2009, 2015, and now 2018 draft Toxicological Profiles for Perfluoroalkyls. [REDACTED] role as a peer reviewer on the draft 2009 Toxicological Profile should be acknowledged as well as ATSDR's request that [REDACTED] provide publicly available comments on the draft 2015 ATSDR Toxicological Profile. [REDACTED] has served as: 1) [REDACTED] of the 2005 U.S. Environmental Protection Agency Science Advisory Board Perfluoroctanoic 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. [REDACTED] 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.

- [REDACTED] 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. [REDACTED] 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 [REDACTED] 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-perfluoroctane sulfonamide) acetic acid (Me-PFOSA-AcOH)
 - 2-(N-Ethyl-perfluoroctane 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 Chemosphere 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, Sakre 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 PFOS 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. 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 “perfluoroalkyls” 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 t_{1/2} 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 al. 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 perfluropolyethers 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
Steenland et al. 2015	10
Steenland et al. 2015	14
Simpson et al. 2015	18
Winqvist et al. 2014	19
Steenland et al. 2015	31
Steenland et al 2015	42
Darrow et al. 2016	43
Darrow et al. 2016	44
Winqvist et al. 2014a	46
Steenland et al. 2015	71
Steenland et al. 2015	84
Winqvist and Steenland 2014b	86
Winqvist 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

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
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Bach et al. 2016	Should be cohort study	152
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