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July 19, 2019

Elizabeth Callahan
Massachusetts Department of Environmental Protection
One Winter Street
Boston, MA 02108

Re: **Proposed PFAS-Related revisions to the Massachusetts Contingency Plan -310 CMR 40.0000**

Dear Ms. Callahan:

The 3M Company (3M) appreciates this opportunity to provide the enclosed comments to the Massachusetts Department of Environmental Protection (MassDEP) proposed PFAS-Related revisions to the Massachusetts Contingency Plan (310 CMR 40.0000). As a science-based company, 3M encourages MassDEP to use the best available science when assessing these chemicals and developing groundwater and soil cleanup standards. As our comments reflect, 3M has substantial experience and expertise regarding PFOA, PFOS and other PFAS, informed in part by the fact that 3M scientists are authors or contributors to many of the studies cited in the references used by MassDEP. As a result of this expertise, 3M has significant concerns with PFAS-related proposals advances by MassDEP.

Please let us know if you have any questions.

Regards,

Oyebode A. Taiwo, MD, MPH

3M COMPANY's COMMENTS ON MASSACHUSETT MCP METHOD 1 STANDARDS

Summary

The 3M Company (3M) appreciates the opportunity to review and comment on the Massachusetts Contingency Plan (MCP) Method 1 Standards proposed by the Massachusetts Department of Environmental Protection (MassDEP) for six PFAS. Our comments focus primarily on the proposed Method 1 GW Standard for groundwater of 20 ng/L for PFAS. 3M has several significant concerns with this proposed value and conclude that it is unsupported by current science, nor adequately explained or justified by MassDEP. 3M has not specifically provided comments on the proposed soil standards. However, the soil standards rely on many of the assumptions and inputs as the groundwater standards, such as references dose, additivity, etc. Accordingly, 3M incorporates by reference its groundwater related reference to the proposed soil standards for PFAS.

First, MassDEP has not released any technical supporting documents detailing the derivation of the PFAS reference doses used and the associated Method 1 GW-1 Standards development. The scientific decision of lowering the PFAS reference doses appears to be based on MassDEP's review of published assessments done by ATSDR (2018), NTP (2016), and NJDQWI (2017, 2018), but MassDEP does not clearly nor thoroughly describe how it derived the reference dose used to set the proposed Method 1 Standards. MassDEP reduced the 2018 Office of Research and Standards Guideline (OSRG) value from 70 ng/L to 20 ng/L by applying a "data base uncertainty factor" but failed to specifically describe or document the need for this data base uncertainty factor or how it derived the specific uncertainty factor value. This lack of transparency and explanation hampers and limits the extent of comments that 3M or other interested parties can provide.

Second, the additivity grouping approach proposed by MassDEP to lump the six PFAS together is not scientifically supported. Further, other than some general statements on similarity across PFAS, MassDEP failed to provide any scientific support or explanation why or how it is valid to perform this grouping. 3M's comments provide a brief perspective on the why PFAS should not be grouped together as MassDEP has done.

Third, MassDEP needs to derive its own chemical specific RfDs and GW-1 standards for each of the six PFAS. The current approach used by MassDEP, lacks rigor and transparency and also shortcuts the risk assessment process typically followed EPA and other regulatory agencies, both within the US and internationally. The approach followed by MassDEP for this rulemaking is at odds with its own MCP requirements and guidance.

Fourth, there are problems with the water consumption assumptions embedded within the GW-1 Standards proposed by MassDEP. This includes a failure to recognize recent changes in lactating women water consumption values presented in EPA's Exposure Factors Handbook, inapplicability of applying lactating women rates to non-developmental endpoints and inconsistency with MCP required water consumption rates.

Fifth, the studies and conclusions by ATSDR, NJDEP and NTP relied upon by MassDEP contain critical flaws and cannot reasonably be used to justify support the proposed uncertainty factor. MassDEP should carefully and critically review this documents. 3M's comments outline concerns with these sources. Additionally, 3M has attached for MassDEP's reference copies of comments that 3M has previously filed with ATSDR, NJDEP and NTP regarding these documents.

Sixth, MassDEP should reconsider its use of relative source contribution. At a minimum, MassDEP should use a 50 percent value instead of the 20 percent currently proposed.

Seventh, epidemiological associations reported between human PFAS exposure and immune and developmental effects are often misstated or misinterpreted. 3M's comments address these critical issues that MassDEP need to understand when addressing these PFAS.

Therefore, 3M recommends that MassDEP perform a new evaluation of its proposed PFAS GW-1 standards taking 3M comments into consideration. 3M notes that MassDEP is concurrently considering a revised MassDEP PFAS ORSG (drinking water guideline) for use to evaluate public water supplies. Because MCP GW-1 Standards are typically set equal to any existing Massachusetts drinking water standard or guideline to promote regulatory consistency, MassDEP has stated "that any comments received apropos the proposed MCP GW-1 standard

will also be considered by the Department in the revision of the ORSG." Accordingly, 3M urges that MassDEP consider 3M's GW-1 Standards comments when preparing any PFAS ORSG.

3M Comments on the Method 1 GW Standards Rule Proposal

A. Summary of Method 1 GW Standards Rule Proposal

MassDEP proposes adding six perfluoroalkyl substances (PFAS) to the MCP Method 1 Standards list: perfluorodecanoic acid (PFDA), perfluoroheptanoic (PFHpA), perfluorohexanesulfonic acid (PFHxS), perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and perfluorononanoic Acid (PFNA). With respect to groundwater, MassDEP proposes Method GW-1 standards for these six substances to be set collectively at 20 ng/L (or parts per trillion – ppt).

These proposed GW-1 standards modify an Office of Research and Standards Guideline (ORSG) for PFOS, PFOA, PFNA, PFHxS and PFHpA issued by MassDEP on June 8, 2018. At that time, MassDEP collectively set the ORSG for these five substances at 70 ng/L. MassDEP based the ORSG on the PFOA and PFOS reference dose (RfD) and lifetime drinking water health advisory (DWHA) established by EPA in 2016. EPA established an RfD of 2.0 X 10-5 mg/kg-day and a DWHA of 70 ng/L.[1] In setting the 2018 ORSGs, MassDEP extended the PFOS and PFOA RfDs and DWHAs to PFNA, PFHxS and PFHpA, reasoning that because these substances are "structurally similar compounds" to PFOS and PFOA it was appropriate to do so.

The current rulemaking states that proposed GW-1 standards "*differ from the published US EPA Drinking Water Health Advisory and the June 2018 MassDEP ORSG in several ways, in consideration of toxicological studies and analyses that have been published subsequently*." Among these studies, MassDEP specifically identifies the 2018 Agency for Toxic Substances and Disease Registry (ATSDR) draft Toxicological Profile for Perfluoroalkyls, which included

[1] 3M notes that EPA derived the PFOS and PFOA RfDs and DWHAs independently of each other. The fact that the respective PFOS and PFOA values are identical is coincidental and not a reflection that the toxicity, mode of action or health based values for PFOA and PFOS are interchangeable.

Minimum Risk Level (MRL) values for PFOS, PFOA, PFNA and PFHxS.[2] MassDEP observed that ATSDR's draft MRLs for PFOS and PFOA were lower than USEPA's RfDs for these substances. MassDEP also notes that the MRLs are similar to RfDs proposed by New Jersey's Drinking Water Quality Institute (NJ DWQI).

MassDEP states that the differences between the EPA RfD and ATSDR MRL values prompted it to re-evaluate its approach to these compounds. MassDEP reports that its Office of Research and Standards (ORS) reviewed "*numerous published toxicological assessments and key primary literature publications including the USEPA Health Effects Support and Drinking Water Health Advisory documents for PFOA and PFOS; the ATSDR draft Toxicological Profile for Perfluoroalkyls; the National Toxicology Program (NTP) Monograph, Immunotoxicity Associated with Exposure to PFOA or PFOS; the NJ Drinking Water Quality Institute MCL recommendations for PFNA, PFOS and PFOA*."[3] [internal citations omitted] To the extent MassDEP reviewed and evaluated toxicological assessments and key primary literature publications other than the EPA, ATSDR, NTP and NJ DWQI documents, these publications are not cited by MassDEP.

Based on this review, MassDEP ORS decided to apply "*an additional data base uncertainty factor in the RfD derivations to account for evidence associating exposures to longer-chain PFAS (e.g. PFOS and PFOA) with several potentially adverse responses, including but not limited to effects on development and the immune system, in laboratory animals at dose levels below those used in the USEPA RfD calculations.*" This resulted in a four-fold downward adjustment of the EPA PFOS and PFOA RfD values, from 2 X 10-5 to 5 x 10-6 mg/kg-day.

ORS applied this "revised RfD" to PFNA, PFHxS, and PFHpA following the additivity grouping approach ORS followed in 2018. Additionally*, ORS extended this approach to include PFDA* "*based on structural similarity and data indicating it has a long serum half-life*." This

[2] MRL and RfD values are essentially equivalent and represent "estimates of a daily exposure or intake of a chemical expected to be without appreciable risk of adverse non-cancer effects."

[3] MassDEP did not explain the reasons why the ATSDR MRLs and the NJ DWQI RfDs differ from EPA's values. If MassDEP had made and explained this comparison, it would have noted that ATSDR and NJ DWQI each used different toxicology studies, critical endpoints, uncertainty factors and human equivalent dose calculation methods from EPA's, as well as each other. MassDEP did not critically review or assess, at least in a documented manner, the strengths and weaknesses of the work by ATSDR or NJ DWQI.

resulted in an updated ORSG for each of these six PFAS of 20 ng/L. Consistent with its established procedures for GW-1 standards, MassDEP set the proposed GW-1 standard for these PFAS at the revised OSRG of 20 ppt.

B. MassDEP Failed to Specifically Describe or Document the Need for a Data Base Uncertainty Factor and to Describe How It Derived the Uncertainty Factor Value

As described above, MassDEP determined that the findings of the ORS literature review warranted the application of a "data base uncertainty factor" be applied to the RfDs derived by USEPA for PFOS and PFOA. This resulted in a four-fold downward adjustment of the RfD values for PFOS and PFOA. Although MassDEP did not specify the safety factor value, it can be calculated to equal four (dividing the USEPA RfD of 2 X 10-5 mg/kg-day by the "adjusted" RfD of 5 x 10-6 mg/kg-day).

MassDEP failed to explain why a data base uncertainty factor was needed. It also failed to explain how it determined that the factor should be four. MassDEP merely stated that it included an additional data base uncertainty factor to "*account for evidence associating exposures to longer-chain PFAS (e.g. PFOS and PFOA) with several potentially adverse responses, including but not limited to effects on development and the immune system, in laboratory animals at dose levels below those used in the USEPA RfD calculations*." This decision lacks a logical scientific basis and is inconsistent with EPA guidance on setting an uncertainty factor based on database uncertainty.

According to EPA's guidance on uncertainty factor allocation:

> *"The database UF is intended to account for the potential for deriving an under protective RfD/RfC* ***as a result of an incomplete characterization of the chemical's toxicity.*** *In addition to identifying toxicity information that is lacking, review of existing data may also suggest that* ***a lower reference value might result if additional data were available****. Consequently, in deciding to apply this factor to account for deficiencies in the available data set and in identifying its magnitude, the assessor should* ***consider both the data lacking and the data available for particular organ systems as well as life stages****. In many respects, the additional 10-fold factor for infants recommended by the*

National Research Council (NRC, 1993) and by Schilter et al. (1996) and called for in the 1996 FQPA is similar to the database UF.

If the RfD/RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproduction study is missing, or a factor of 10 may be applied if both are missing (Dourson et al., 1996). Dourson et al. (1992) examined the use of the database UF by analyzing ratios of NOAELs for chronic dog, rat, and mouse studies and reproductive and developmental toxicity studies in rats. They concluded that reproductive and developmental toxicity studies provide useful information for establishing the lowest NOAEL, and if one or more bioassays are missing, a factor should be used to address this scientific uncertainty in deriving a chronic RfD."

See (https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf).

MassDEP's very limited discussion of its reason for adjusting the EPA RfDs by this uncertainty factor falls short of meeting EPA's uncertainty factor guidance requirements. MassDEP failed to identify whether its decision was based on the lack of data charactering one or all of the six subject PFAS compound's toxicity. If data was deemed lacking, MassDEP provided no information to ascertain what specific data was lacking. Similarly, if MassDEP believes there is not information to address toxicity to particular organ systems or at particular life stages, the rule proposal is similarly silent.

MassDEP merely asserts that several potentially adverse responses, including but not limited to effects on development and the immune system, occur in laboratory animals at dose levels below those used in the EPA RfD calculations. This is not the same as saying that data is lacking. MassDEP seems to be using its version of a data base uncertainty factor as a shortcut and an avoidance of actually deriving, for each PFAS, its own chemical specific RfD.

MassDEP also leaves as a mystery why or how it landed on a database uncertainty factor of four? The most MassDEP has to say on the size of the uncertainty factor is *"[u]se of an additional uncertainty factor of 10 was considered, but not selected in light of the likely conservativeness of the Relative Source Contribution factor used with the RfD to derive a*

drinking water guidance value." MassDEP provides no insights into its selection of four as the data base uncertainty factor.

C. The Additivity Grouping Approach Proposed by MassDEP is Not Scientifically Supported Nor Adequately Explained by MassDEP

MassDEP proposes to lump six PFAS together such that the sum of these six substance will be subject to the proposed Gw-1 standard of 20 ng/L. This is not scientifically justified nor does MassDEP provide an adequate basis for doing so.

MassDEP reaches this position in two steps. First, in 2018, ORS observed that the EPA DWHAs for PFOA and PFOS were both calculated to be 70 ng/L and "*because the adverse effects following exposure to both PFOA and PFOS are similar and include effects on the developing fetus, as well as effects on the liver, immune system and changes in organ and body weights, they have similar chemical structures and their US EPA HAs are both [70 ng/L], the US EPA recommends that the concentrations of both compounds be summed and compared to [70 ng/L].*" [4] Further, ORS asserted that the "*available data for PFHxS, PFNA and PFHpA demonstrate that these PFAS compounds are very similar in molecular structure to PFOS and PFOA, have long biological half-lives like PFOS and PFOA and elicit similar types of effects at similar dose ranges as PFOA and PFOS*." As a result, it extended "*the additivity approach used by the US EPA for PFOS and PFOA to PFHxS, PFNA and PFHpA*." In this rule proposal, MassDEP included PFDA "*based on structural similarity and data indicating it has a long serum half-life*." ORS extended this approach to include PFDA for its current proposal.

In deciding to group the PFAS compounds, MassDEP presents conclusory statements that that these PFAS have similar adverse effects, chemical structure, structural additivity and serum half-life. Beyond these general statements, MassDEP provides no detailed analysis and assessment to prove its point. There is no reference to any published study or research to backup such conclusions.

[4] More accurately, EPA actually stated: "Because these two chemicals cause similar types of adverse health effects, EPA recommends that when both PFOA and PFOS are found in drinking water the combined concentrations of PFOA and PFOS be compared with the 0.07 part per billion HA level." 81 FR 33250, 33521 (May 25, 2016)"

The grouping proposal by MassDEP fails to recognize that in fact there are distinct differences in potencies and modes of action (MOAs) for PFAS. MassDEP should not apply additivity grouping for its assessment**.** 3M strongly disagrees with MassDEP's proposal to apply additivity grouping on PFAS, especially given the myriad of data available that provide compelling evidence illustrating the differences. For example, among the long-chain PFAS such as the ones currently considered by MassDEP, there are appreciable and well-documented differences in:

- Solubility (PFOA > PFOS)
- Human half-lives (PFHxS > PFOS)
- Different in toxicological responses at equal molar across PFAS
- Sex difference in serum elimination in rats (yes for PFOA; yes for PFHxS; no for PFOS)
- PPARα-mediated hepatocellular hypertrophy (PFOA > PFOS)
- CAR/PXR-mediated hepatocellular hypertrophy (PFOS > PFOA)
- Primarily PPARα-mediated developmental MOA (yes for PFOA; no for PFOS)

These differences preclude a scientifically defensible grouping approach for PFAS. MassDEP has not addressed any of these considerations.

Moreover, while some states which have adopted the EPA DWHAs for PFOS and PFOA as their PFOA and PFOS guidance levels (including presumed additivity assumption by EPA[5]), other than Vermont, no other state which has individually assessed and derived water guidance levels for individual PFAS have adopted additivity grouping advocated by MassDEP (see for example, Minnesota, Michigan, New Hampshire, New Jersey). ATSDR has not followed this approach, nor have the national environmental protection agencies in Canada, Australia,

[5] 3M also notes that it disagrees with EPA's recommendation that when both PFOA and PFOS are found in drinking water the combined concentrations of PFOA and PFOS be compared with 70 ng/L. There are differences between PFOA and PFOS that also preclude this simplistic approach.

Germany, the Netherlands or Great Britain. For example, Health Canada recently stated that when PFOS and PFOA are found together in drinking water, it is appropriate "to consider both chemicals together … by adding the ratio of the observed concentration for PFOS to its [guideline value] with the ratio of the observed concentration for PFOA to its [guideline value]. ***Science currently does <u>not</u> justify the use of this approach for other PFAS***." [emphasis added].

D. Post Hoc Application of An Uncertainty Factor on the Reference Dose Derived by EPA is Without Precedent – MassDEP Must Perform Its Own Chemical Specific GW-1 Standard Assessment for Each of the Six PFAS

MassDEP attempts to shortcut the risk assessment process by applying an after the fact data base uncertainty factor to the EPA derived RfDs for PFOA and PFOS to address a number of issues and questions raised by the publications by ATSDR, NTP and NJDEP. 3M is unaware of any such similar approach taken by any other agency.

The better course, and the one followed by ATSDR and many other states, including Minnesota, Michigan, New Hampshire, and New Jersey is perform its own chemical by chemical assessment. This would entail a detailed review of the toxicological and epidemiological literature. Based on this review, MassDEP should select a critical study and health endpoint. From this study, MassDEP would determine the point of departure value, calculate human equivalent dose and apply as needed appropriate uncertainty factor to produce its own reference dose. Then, based on the standard exposure assumption MassDEP has used or recommends under the MCP, it could calculate its own chemical specific GW-1 Standards.

E. MassDEP's Implicit Adoption of Exposure Assumptions Embedded in EPA's DWHA is Contrary to Its Own Guidance for Determining GW-1 and Ignores Recent Changes in EPA Exposure Guidance Directly Affecting EPA PFOA ad PFOS DWHA

EPA derived PFOA and PFOS HAs using the water ingestion rate and body weight of a lactating woman (e.g., 54 mL/kg-day representing the consumers-only estimate of combined direct and indirect community water ingestion at the 90th percentile for lactating women) provided by the 2011 edition of EPA's Exposure Factors Handbook.[6] The GW-1 Standards by MassDEP implicitly adopt these exposure assumptions. There are several problems with this.

[6] EPA concluded that basing exposure on lactating women is also be protective for pregnant women.

First, these exposure assumptions are not specifically identified in the rule proposal or its supporting materials. EPA's exposure assumptions are barely mentioned in the 2018 ORSG development. It is critical that MassDEP be explicit and transparent in its exposure assumptions.

Second, such exposure assumptions are inconsistent with the assumptions routinely used by MassDEP for the derivation of GW-1 standards for other MCP substances. MassDEP's standard exposure assumption is a water consumption rate of 2 L/day by a 70 kg individual. This is equivalent to 28.6 mL/kg-day or nearly half the exposure rate that MassDEP implicitly uses for the six subject PFAS. See 310 CM 40.0983: Derivation of Additional Method 1 Groundwater Standards for Use in Method 2; see also Guidance For Disposal Site Risk Characterization, In Support of the Massachusetts Contingency Plan, Interim Final Policy #WSC/ORS-95-1 Chapter 7; and MassDEP guidance on MCP Numerical Standards https://www.mass.gov/files/documents/2017/12/27/MCP%20Numerical%20Standards%20-%20Derivation.pdf. 3M observes that NJDEP has based its proposed MCLs for PFOA and PFOS, and its final MCL for PFNA on a water consumption rate of 2 L/day by a 70 kg individual.

Third, the exposure assumptions that EPA adopted for PFOS and PFOA, may not apply to the other four PFAS being addressed by MassDEP in the proposed rules. EPA selected lactating women as the exposed population to protect because the RfDs it derived for PFOA and PFOS concerned developmental effects. Hence, EPA felt to necessary to account for the higher water consumption rates expected for lactating, as compared to the general population. Applying such exposure assumptions for the four other PFAS would only be appropriate if the concern was protecting against developmental effects. As various water guidance values proposed by other states show, developmental effects do not serve as the only health effect upon which a water guidance value have been proposed.

Finally, the water consumption rate used by EPA in its DWHA calculations has been recently reduced by EPA. In 2016, EPA used a water ingestion rate and body weight of for a lactating woman of 54 mL/kg-day representing the consumers-only estimate of combined direct and indirect community water ingestion at the 90th percentile for lactating women

provided by the 2011 edition of EPA's Exposure Factors Handbook. In February 2019, EPA updated the water ingestion chapter (Chapter 3) of the Exposure Factors Handbook. This updated chapter includes a downward revision of this recommended water ingestion rate from 54 mL/kg-day to 47 mL/kg-day. This revision was the result of EPA looking at additional studies and data sets to produce a revised set of recommended values for water ingestion rates for lactating women (see Table 3-3 of the 2019 Exposure Factors Handbook). Replacing the water ingestion rate of 54 mL/kg-day with a water ingestion rate of 47 mL/kg-day would raise the proposed GW-1 by approximately 15 percent.

F. Studies and Conclusion by ATSDR, NJDEP and NTP Include Critical Flaws, Cannot be Used to Support the Proposed Uncertainty Factors and Should be Carefully and Critically Reviewed By MassDEP

1. MassDEP's reliance of on the MRLs set by ATSDR because the MRLs are critically flawed, unsupported by the science, and should not be used by MassDEP for the reference dose derivation.

ATSDR' s selection of the critical toxicological endpoints and its derivation process used in establishing their provisional MRLs lacked scientific rigor.[7] Moreover, the best available science was not applied by ATSDR. 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. Some key concerns with ATSDR's MRL development, which relied on rodent toxicological endpoints, are uncertainties encompassing:

1) Human relevance: among the rodent developmental toxicological endpoints chosen by ATSDR for MRL calculations for PFOA, PFOS, and PFHxS, they have not been observed in humans. ATSDR failed to explain the relevance of these effects, if any, to human health. 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.

2) Best available science not applied:

[7] Comments submitted by 3M to ATSDR regarding ATSDR's draft PFAS toxicity profile in August 2018 are attached hereto as Attachment A.

- For PFOA, the two studies selected by ATSDR (Onishchenko et al., 2011 and Koskela et al. 2016) 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.). These two studies and the corresponding results should not be used in any meaningful risk assessment for humans.
- For PFOS, ATSDR did not take maternal toxicity influence as well as human relevance into consideration.
- For PFHxS, ATSDR failed to recognize that there are distinct differences in thyroid hormone regulations between rodents and humans; and similar to other nuclear receptor-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: a combined adjustment factor of 300 for PFOA, PFOS, and PFHxS MRLs in addition to the (large) dosimetric toxicokinetic 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.

4) Inappropriate human TK adjustment: for PFOA, PFOS, and PFHxS, the corresponding MRLs had 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 failed to 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.

2. The NTP immunotoxicity monograph (2016) on PFOA and PFOS provided insufficient support for extrapolating effects in animals to humans**.**

The suppression of the T-cell dependent antibody response (TDAR) in mice, which evaluates suppression of the "primary" IgM response, is used by NTP to support evidence of suppression of antibody titers to vaccinations in humans. However, because vaccine antibody titers actually represent the secondary IgG response, the observation in human epidemiological data was in great discrepancy with animal data in that no suppression of the secondary IgG response was observed in mice. There also are great incongruences between humans and animal data to support the final hazard conclusions reached by the NTP in the areas of hypersensitivity for PFOA, infection disease resistance for PFOS, and NK cell activity for PFOS. Collectively, NTP had overstated the final hazard conclusions.[8]

3. There was a serious technical error by NJ DWQI in its BMD modelling which resulted an artificially low PFOS MCL; and increased liver weights in rodents with PFOA exposure is not appropriate nor scientifically justified for human risk assessments.

The NJ DWQI selected an immunotoxicity study by Dong et al. (2009) for deriving a PFOS MCL. However, NJ DWQI made a serious technical error in its benchmark dose (BMD) modeling.[9] It used the standard error of the mean (SEM), rather than the required standard deviation when it assessed BMD applicability. This error led the DWQI to erroneously reject the otherwise preferred BMD modeling approach. Correcting DWQI's error results in a PFOS BMDL that is **five** times higher than the reference dose used by the DWQI. Making this correction would also result in a PFOS MCL that is **five** times higher than the MCL proposed by DWQI.

4. NJ DWQI's use of increased liver weights in rodents with PFOA exposure is not appropriate nor scientifically justified for human risk assessments.

[8] Comments submitted by 3M to NTP in 2016 are attached hereto as Attachment B.

[9] Comments submitted by 3M to NJDEP in 2019 pointing out this error and providing comments in NJDEP's proposed MCLs for PFOS and PFOA are attached hereto as Attachment C.

3M also disagrees with DWQI's use of increased relative liver weight as the basis for its PFOA MCL derivation. The assertion made by DWQI is inconsistent with USEPA guidelines and published expert opinions on the distinction between liver hypertrophy as a non-adverse adaptive change in rodents, rather than liver toxicity. Furthermore, NJ DWQI attempts to suggest an aura of database uncertainty in its MCL derivation for PFOS and PFOA is without scientific merit. Given the comprehensive toxicological database that are available for both PFOS and FPOA, the allocation lacks a logical scientific basis and contrary to EPA guidance.

G Relative Source Contribution

In its proposed rulemaking, MassDEP asks "*How should the GW-1 standard consider Relative Source Contribution? The target Hazard Index used to develop the Method 1 Standards is 0.2 to account for multiple chemical- and multiple pathway- exposures at and from 21E sites. PFAS has been described as "ubiquitous" in the environment, including exposures from common household products and foods. Is the assumption that 20% of a person's exposure comes from drinking water sufficiently protective?*"

3M believes that MassDEP confuses and conflates its Hazard Index concept with Relative Source Contribution (RSC). They are distinct and different concepts. EPA has defined relative source contribution as "The ratio between exposure from drinking water and total exposure is called the relative source contribution (RSC).[10] In contrast, EPA defines a hazard index as "The sum of hazard quotients (HQs) for substances that affect the same target organ or organ system" where a hazard quotient is the "ratio of estimated to reference level at which no adverse health effects are expected."[11]

EPA describes the RSC as the portion of the RfD attributed to drinking water (directly or indirectly in beverages like coffee tea or soup). The remainder of the RfD is allocated to other potential sources. In the case of PFAS, other potential sources could potentially include

[10] See e.g., Fluoride: Exposure and Relative Source Contribution Analysis, EPA Office of Water (820-R-10-015). December 2010.

[11] See Overview of Human Health Risk Assessment, RISK ASSESSMENT AND TRAINING EXPERIENCE Basics of Human Health Risk Assessment National Institute of Environmental Health Science, Superfund Research Program (November 5, 2014).

(depending on the type and nature of PFAS) ambient air, foods, bottled water, incidental soil/dust ingestion, consumer products and others.

EPA's exposure decision tree framework describes various considerations for determination of RSC. See *Methodology for deriving ambient water quality criteria for the protection of human health*. Washington, DC: Office of Science and Technology, Office of Water. EPA-822-B–00-004. Both methods use a lower and an upper bound RSC value of 20 and 80%, respectively.

The available chemical-specific data from PFOA drinking water affected communities, as reported by Emmett et al. (2006) and Landsteiner et al. (2015), provided substantial and compelling evidence that elevated PFOA levels in the drinking water is likely to be the primary route of PFOA exposure. This is similarly true for PFOS. While EPA used the default 20% RSC assumptions for its 2016 DWHAs for PFOA and PFOS, states such as Minnesota and New Hampshire have recently used a 50% RSC. Accordingly, 3M recommends that MassDEP at least a 50% RSC.

H. Other Health Science Considerations Mass DEP Needs to Consider in Its Rulemaking

1. The developmental epidemiologic association reported between lower birthweight per measured PFOA or PFOS maternal or cord blood is likely not causal but consistent with confounding and/or reverse causation via glomerular filtration rate (GFR).

3M is uncertain to the definition of what MassDEP means by adverse development outcome based on epidemiology studies. Most epidemiologic studies have centered on the association between measured maternal (or cord blood) PFOA and PFOS concentrations and lower birthweight. The following brief comments focus on this reported association and why it is not causal.

- Johnson et al. (2014) originally concluded in their meta-analysis of a set of epidemiology studies that there was an association between measured maternal (or cord blood) PFOA concentrations and lower birthweight. This analysis, however, did not directly take into account the possibility of confounding by the maternal glomerular filtration rate. Several months later, Verner et al. (2015) reported findings from their

PBPK model/Monte Carlo simulation models and a meta-analyses of a similar collection of epidemiologic studies. The work by Verner et al. was based on a study by Morken et al. (2015) who observed an association between the glomerular filtration rate (GFR) and fetal growth which meant that GFR could potentially confound an association between fetal growth and measured PFOA or PFOS concentrations. Indeed, Verner et al. found such confounding by the GFR as it biased upwards, up to 50 percent, in their modeling efforts of the association between fetal growth and measured maternal PFOA or PFOS concentrations. Furthermore, Verner et al. reported this confounded association was observed only in the second and third trimesters, not the first trimester, likely because the effect of GFR would be subsequent to the well-known plasma volume expansion that occurs during the first trimester.

- Another meta-analysis was subsequently published by Negri et al. in 2017 which expanded to 16 epidemiologic studies. Based on their sensitivity analyses, there were stronger associations from studies conducted in Asia and significant heterogeneity was observed when the measurement of PFOS was done later in the pregnancy or using cord blood. The latter is consistent with the simulation PBPK modelling done by Verner et al. (2015) as it relates to the potential confounding influence of maternal GFR with the timing of when PFOS is measured during pregnancy. Negri et al. also examined the laboratory animal 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 PFAS 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.
- Steenland, Barry, and Savitz (2018) recognized the distinction in timing of when the PFOA maternal measurement was made based on the findings from the Verner et al. study. They also elaborated upon the Negri et al. study by conducting a meta-analysis of 24 epidemiologic studies. They stratified their results as to whether the maternal PFOA

concentration was measured in the first or the combined second and third trimesters. Steenland et al. reported with first trimester measurements of maternal PFOA that there was a -3.3 gram (95% CI -9.6, 3.0) reduction in birthweight per ng/mL PFOA. When PFOA was measured during second/third trimester, there was a -17.8 gram reduction (95 CI -25.0, -10.6) in birthweight per ng/mL PFOA. Steenland et al. (2018) concluded "restriction to studies with blood sampling conducted early in pregnancy or shortly before conception showed little or no association such that these results are consistent with confounding and /or reverse causation being responsible for the inverse association seen in studies with low background exposure levels and blood sampling conducted later in pregnancy, when confounding and/or reverse causality are likely to be more important."

2. Human Epidemiological Evidence Does Not Support an Association Between PFAS Exposure and Immune Effects.

In the "Summary of Proposed MCP Method 1 Standards Revisions" MassDEP states that the lower RfD ($5x10^{-6}$ mg/kg/day) is supported, in part, by epidemiology studies that have reported associations between human PFAS exposure and adverse immune effects (MassDEP, 2019). MassDEP does not specify which immune effects were considered in their decision to apply an additional database uncertainty factor, nor do they provide references for these epidemiology studies. Rather MassDEP cites both the ATSDR Draft Toxicological Profile for Perfluoroalkyls (ATSDR, 2018) and the National Toxicology Program (NTP) Monograph, Immunotoxicity Associated with Exposure to PFOA or PFOS (NTP, 2016) as support for evidence of adverse immune effects in humans.

It is important for MassDEP to recognize that the 2018 ATSDR Toxicological Profile for Perfluoroalkyls is a **DRAFT** document. In August 2018, the ATSDR received over 60 comments from various stakeholders (including 122 pages of comments from 3M) during the public comment period (see https://www.regulations.gov/docket?D=ATSDR-2015-0004). To date, ATSDR has not addressed any public comments nor issued a revised draft or final PFAS Toxicological Profile. Also, it must be noted that ATSDR acknowledged that for PFAS there is no cause and effect established between health effects and exposure to humans, when it stated:

"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***." (ATSDR 2018).

Regarding immune effects in humans, ATSDR (2018) identified a potential association between PFAS (specifically, PFOA, PFOS, PFHxS and PFDeA) and a decreased antibody response to vaccines, and a potential association between PFOA and increased risk of asthma diagnosis. 3M disagrees with ATSDR interpretation of the epidemiologic literature and their conclusions for the reasons summarized below:

There is a lack of support for decreased antibody response to vaccines. Among the epidemiologic studies cited by the draft ATSDR document, antibody responses to eight distinct vaccines were measured. Observed changes in antibody response to a particular vaccine type should not be interpreted as consistent with changes in the antibody response to another vaccine type. Accordingly, 3M recommended that ATSDR 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 epidemiologic studies have found no association between PFAS levels and increased incidence of infectious disease. 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 and expert health panels have reached similar conclusions (Australia Expert Health Panel, 2018; Food Standards Australia New Zealand, 2016; Health Canada, 2017; National Institute for Public Health and the Environment, 2016).

There is a lack of support for 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.

In conclusion, the epidemiological evidence does not support an association between PFAS exposure and decreased vaccine response or increased risk of asthma in humans. Further, any hypothesized vaccine response effects appear to have no clinical significance as the data does not support a causal association between PFAS exposures and an increased risk of infectious disease.

ATTACHMENT A
3M COMPANY'S COMMENTS ON ATSDR DRAFT TOXICOLOGICAL PROFILE OF PERFLUOROALKYLS

August 20, 2019

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August 20, 2018

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The Proposed MRLs Fail to Reflect the Best Available Science

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

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

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

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

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

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

Epidemiological Associations Claimed by ATSDR are Not Supported by the Science

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

Many Other Concerns and Deficiencies Require Revisions to the Draft

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

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

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

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

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

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

Sincerely,

John Banovetz

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

Executive Summary of 3M's Comments

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

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

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

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

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

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

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

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

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

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

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

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

1) Toxicological endpoints and human relevance

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

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

2) Best available science not applied

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

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

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

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

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

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

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

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

4) Toxicokinetics and half-lives in humans

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

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

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

6) Chronic toxicology studies are available for PFOA and PFOS

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

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

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

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

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

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

2) Epidemiological association: Hepatic enzymes

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

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

3) Epidemiological association: Increased serum total cholesterol and LDL

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

4) Epidemiological association: Increased risk of thyroid disease

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

5) Epidemiological association: Decreased antibody response to vaccines

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

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

6) Epidemiological association: Increased risk of asthma diagnosis

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

7) Epidemiological association: Increased risk of decreased fertility

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

8) Epidemiological association: Small decreases in birthweight

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

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

Detailed Comments on PFOA MRL

ATSDR position (page A-16)

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

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

3M Conclusion

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

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

3M Comments (Details):

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

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

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

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

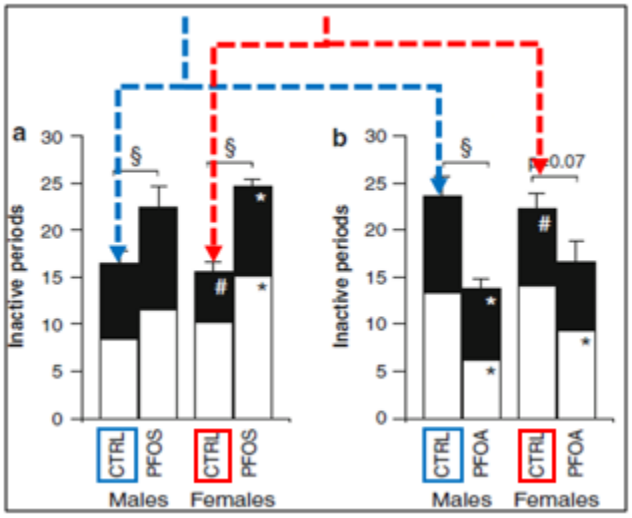
      Using a study that evaluated a single PFOA dose group was in absolute contradiction of what ATSDR stated in its MRL approach. On page A-6 of the draft profile, ATSDR explicitly stated that one of the MRL approach was to "*Identify laboratory animal studies that have evaluated dose-response relationship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

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

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

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

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



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

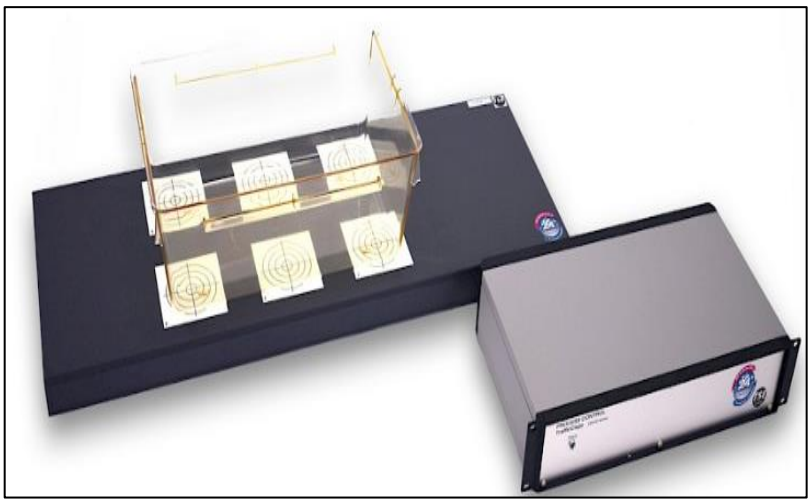


Illustration of TrafficCage™

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Detailed Comments on PFOS MRL

ATSDR Position (page A-36)

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

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

3M Conclusion

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

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

3M Comments (Details):

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Detailed Comments on PFHxS MRL

ATSDR Position (page A-49)

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

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

3M Conclusion

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

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

3M Comments (Details):

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ATSDR Position

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

3M Comments on Preeclampsia

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

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

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

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

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

3M Conclusion on preeclampsia

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

3M Comments on pregnancy-induced hypertension

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

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

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

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

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

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

3M Conclusion on Pregnancy-induced Hypertension

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

Detailed Comments on Hepatic Enzymes (alanine aminotransferase, ALT)

ATSDR position.

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

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

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

3M Comments

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

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

Several types of studies are discussed below.

Community studies (n = 2)

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

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

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

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

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

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

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

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

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

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

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

Occupational Studies (n = 4)

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

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

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

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

Experimental study (n = 1)

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

General Population (NHANES) studies

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

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

3M Conclusion

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

Detailed Comments on Cholesterol

ATSDR position on PFOA and cholesterol

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

3M Comments on PFOA and Cholesterol

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

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

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

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

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

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

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

3M Conclusion on PFOA and cholesterol

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

ATSDR position on PFOS and cholesterol

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

3M Comments on PFOS and Cholesterol

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

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

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

3M Conclusion on PFOS and cholesterol

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

Detailed Comments on Thyroid Disease

ATSDR position

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

3M Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

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

3M Conclusion on thyroid disease

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

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

ATSDR Position

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

3M Comments

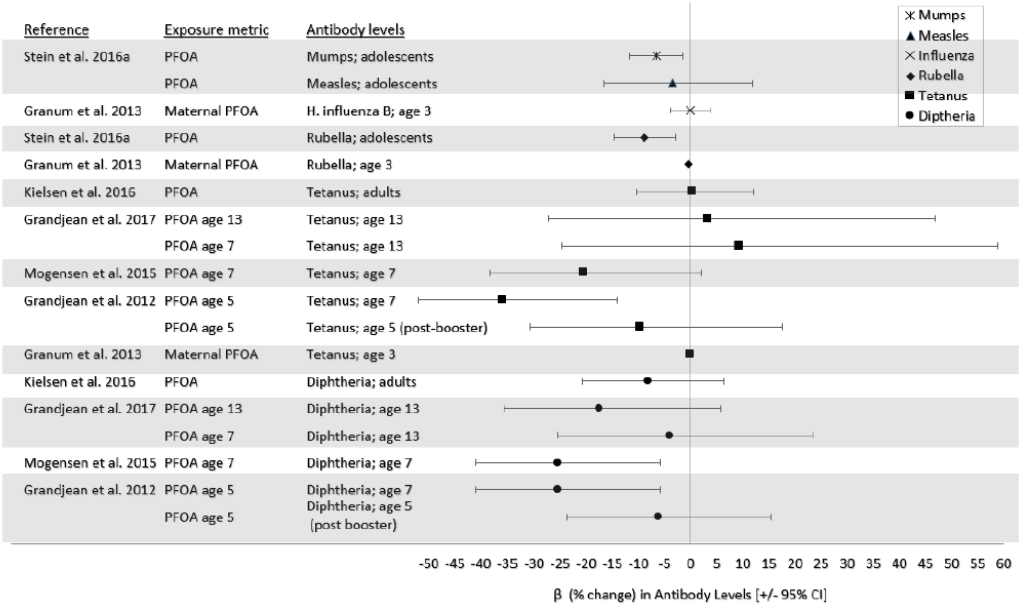
It is inappropriate for ATSDR to interpret antibody responses to these 8 distinct vaccines as a single health outcome (i.e., "decreased antibody responses to vaccines"). Commercially available vaccines differ depending on the nature of the vaccine antigen. Tetanus and diphtheria, for example, are toxoid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

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

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

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

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



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

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

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

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

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

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

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

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

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

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

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

3M Conclusion on decreased antibody responses to vaccines

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

Detailed Comments on Increased Risk of Asthma Diagnosis (PFOA)

ATSDR Position

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

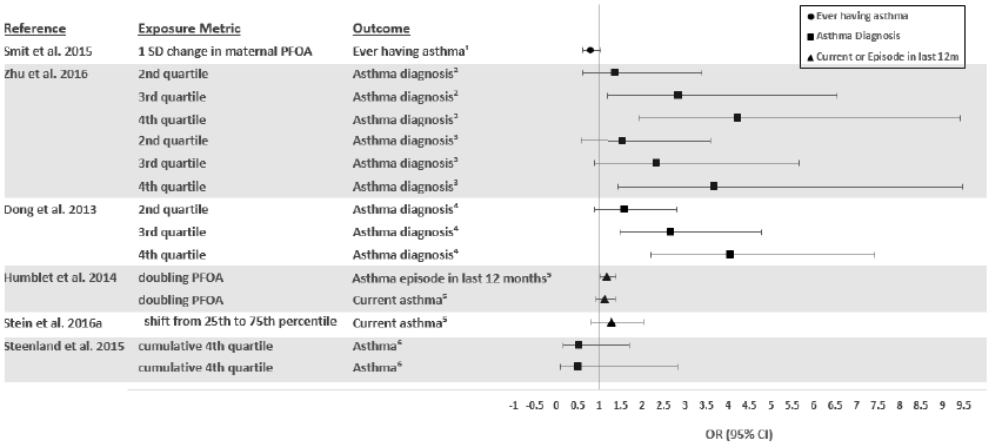
3M Comments

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

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

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

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



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

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

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

3M Conclusion on increased risk of asthma diagnosis

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

Detailed Comments on Increased Risk of Decreased Fertility

ATSDR position

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

3M Comments

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

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

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

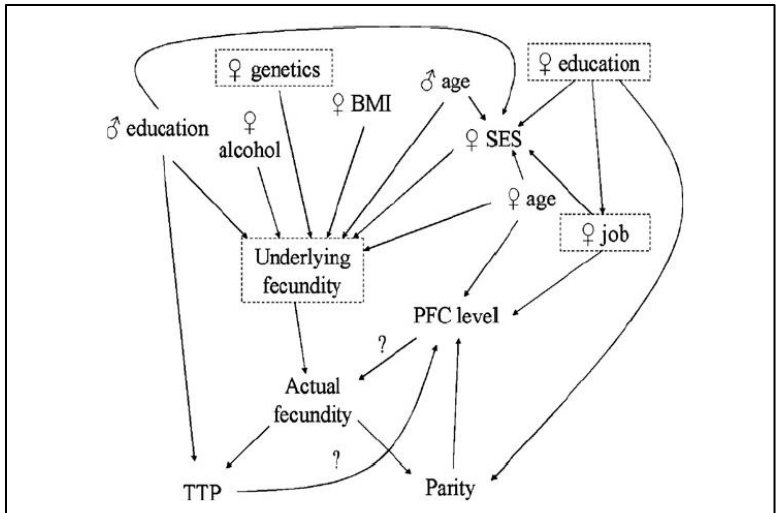


Fig. 6. Suggested causal diagram of maternal perfluorochemical concentrations (PFC) and time to pregnancy.

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

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

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

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

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

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

Furthermore, Fei et al. (2012) wrote,

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

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

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

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

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

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

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

3M Conclusion on increased risk of decreased fertility

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

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

Detailed Comments on Lower Birth Weight

ATSDR position

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

3M Comments

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

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

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

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

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

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

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

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

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

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

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

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

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

3M Conclusion on lower birth weight

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

Additional Comments

General note:

There is no authorship by chapters or sections within chapters.

Page v.

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

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

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

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

Page 1:

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

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

Page 2:

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

Page 3:

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

Page 4:

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

Page 5:

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

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

Pages 6 – 9:

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

Page 10:

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

Page 11:

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

Page 12:

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

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

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

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

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

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

Pages 13 and 14:

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

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

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

Page 21:

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

Page 22:

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

Page 23:

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

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

Page 24:

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

Page 25:

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

Page 108:

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

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

Pages 109 – 433:

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

Page 131:

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

Pages 244-300 (Section 2.14):

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

Pages 245-250, Table 2-16:

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

Pages 268 - 281:

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

Pages 270, Figure 2-19:

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

Pages 272, Figure 2-20:

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

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

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

Pages 277, Figure 2-21:

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

Pages 289-291 and Figure 2-27:

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

Pages 433 – 449:

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

Pages 434 – 438:

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

Pages 438 – 441:

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

Pages 441 - 443:

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

Pages 443-444:

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

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

Page 445:

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

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

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

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

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

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

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

Pages 447 – 449:

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

Page 450:

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

Page 450:

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

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

Page 514:

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

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

Page 518:

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

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

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

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

Page 539, Figure 5-2:

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

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

Page 541:

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

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

Page 581:

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

Page 596:

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

Page 633:

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

Page 636:

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

Additional comments:

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

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

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

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

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

Citations:

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Bach, C.C., Vested, A., Jørgensen, K.T., Bonde, J.P.E., Henriksen, T.B., Toft, G., 2016b. Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: a systematic review. Critical reviews in toxicology 46, 735-755.

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ATTACHMENT B

3M COMPANY'S COMMENTS SUBMITTED REGARDING THE DRAFT NATIONAL TOXICOLOGY PROGRAM MONOGRAPH ON "SYSTEMATIC REVIEW OF IMMUNOTOXICITY ASSOCIATED WITH EXPOSURE TO PERFLUOROOCTANOIC ACID (PFOA) OR PERFLUOROOCTANE SULFONATE (PFOS)

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Re: 3M Comments Submitted Regarding the Draft National Toxicology Program Monograph on "Systematic Review of Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS)."

Dear Dr. Xie,

The 3M Company (3M) appreciates the opportunity to review and comment on the NTP's draft monograph on the "Systematic Review of Immunotoxicity Associated with Exposure to Perflorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS)". The NTP monograph summarizes in great detail the objectives, specific aims and methods used to conduct this systematic review. The NTP should be commended for its transparency of the methodology it used and thoroughness of their study.

Given the myriad of scientific literature that has become available, we offer the following comments, which reflect in the spirit of assisting with that effort. Our prepared comments have also been peer-reviewed by Dr. Norbert Kaminski (Director, Institute for Integrative Toxicology, Michigan State University). There are several areas of the NTP systematic review on PFOA and PFOS in which insufficient animal data are used as supporting evidence for human findings and its final hazard conclusion. In particular, suppression of the TDAR in mice, which evaluates the "primary" IgM response, is used to support diminished antibody titers to vaccinations in humans. However, because vaccine antibody titers actually represent the secondary IgG response, the observation in human epidemiological data did not support the animal data because no suppression of the secondary IgG response was observed in mice. Similarly, there also are substantial inconsistencies between human and animal data to support the final hazard conclusions reached by the NTP in the areas of hypersensitivity for PFOA, infectious disease

OVERALL SUMMARY

i) Suppression by PFOA and PFOS of the IgM TDAR in mouse studies is deemed as supportive of human data from epidemiology studies showing an association between PFOA and PFOS exposure and a decrease in antibody titers to vaccines. The IgM TDAR is a primary antibody response, whereas, vaccine titers are mainly of the IgG antibody isotype. In addition, in the few animal studies where a bona fide memory response was evaluated, antigen-specific IgG was not suppressed by PFOA and PFOS. The final hazard conclusion for immunosuppression should be **downgraded**.

ii) The NTPs conclusion "there is **high confidence** that exposure to PFOA is associated with increased hypersensitivity responses based on the available animal data", should be downgraded. Two animal studies were primarily deemed as supportive of this conclusion, Fairley et al. and Ryu et al. (Fair et al., 2013; Ryu et al., 2014). By definition, hypersensitivity is an exaggerated immune response to an exogenous antigen. Importantly, Ryu et al. found that PFOA induced AHR in the absence of exposure to an allergen (Ova) and also PFOA did not potentiate the AHR response to Ova sensitization and challenge. Therefore the Ryu study does **not** support the conclusion that PFOA-induced AHR is due to a hypersensitivity response. By contrast, Fairley et al. showed an increase in AHR, which corresponded with an increase in serum anti-Ova IgE levels, which they concluded could be involved in enhanced AHR by PFOA. A common finding in both studies that deserves greater attention is the increase in airway associated inflammatory cells in PFOA treated mice, which could be involved in the underlying cause of AHR in a hypersensitivity-independent manner.

iii) The NTP concluded that "there is **moderate confidence** that exposure to PFOS is associated with suppression of NK cell activity in animals". The level of confidence should be downgrade to "**low confidence**", based on the fact that impairment of NK cell activity in the majority of studies cited occurred at doses well above those that are relevant to human exposure. Moreover, in several of the studies there were indications that doses producing a suppression of NK activity also induced overt toxicity as suggested by an elevation in corticosterone, decreased body and lymphoid organ weights and decreased lymphoid tissue cellularity (Dong et al., 2009; Zheng et al., 2009).

iv) The NTP final hazard conclusion based on the body of evidence for infection disease resistance is **"Suspected to be a Immune Hazard to Humans"**. Collectively, there does not appear to be sufficient supporting evidence in either humans or animals to support the NTP conclusion. The NTP should seriously consider down grading the final hazard conclusion for infectious disease resistance to something less than **"Suspected to be an Immune Hazard to Humans"**.

DETAILED COMMENTS

The NTP categorized the health effects of PFOA and PFOS on the immune system into three categories: (a) immune suppression; (b) hypersensitivity-related outcomes and (c) autoimmunity. For each of these categories, the NTP gave the greatest weight to primary outcomes (e.g., for immune suppression, suppression of antibody responses) and less weight to secondary endpoints (e.g., decrease in spleen weight, changes in cytokine production). Evidence related to secondary outcomes was used only as supportive evidence since the NTP felt that there was sufficient primary outcome data to draw conclusions. In addition, evidence for animal data was used to support human health outcomes in order to draw a final human hazard conclusion. This review of the NTP Systematic Review of Immunotoxicity Associated with Exposure to Perflorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS) will address each of the three health categories individually, for PFOA and PFOS, with a primary focus on whether the animal data supports the the NTP conclusions for human health outcomes.

PFOA Immune Evidence

A. **Immune Suppression:** Within the category, 'Immune Suppression", the NTP identified published studies in three subcategories antibody response, natural killer NK cell activity, and infection disease resistance based on the rationale that different cell types can be involved in each of these three responses.

1) Antibody Response: The NTP concluded that "there is **moderate confidence** that exposure to PFOA is associated with suppression of the antibody response in human based studies". Evidence for this conclusion comes from retrospective, cross-sectional and prospective epidemiological studies in which antibody titers to vaccinations were quantified in combination with measurements of serum PFOA levels coupled with supportive animal studies. The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016) and therefore will only be discussed within the context of animal data.

   Animal data supporting the NTP conclusion "there is moderate confidence that exposure to PFOA is associated with suppression of the antibody response in humans" is based on the observation that PFOA administration to mice suppressed the antigen specific (sRBC or hBRC) T cell-dependent IgM antibody response (TDAR) (DeWitt et al., 2009; Dewitt et al., 2008; Loveless et al., 2008; Yang et al., 2002). Results from the TDAR were viewed as especially important by the NTP for several reasons. The first being that the TDAR is viewed as one of the most sensitive immunotoxicological assays for identifying immune modulating agents. The rationale being that the TDAR requires the involvement of numerous immune cell types including B cells as effector cells (antibody secreting plasma cells), as well as T cells and macrophages as accessory cells for cytokine

secretion as well as antigen processing and presentation. The response also requires cell activation, proliferation and differentiation by B and T cells. Hence, the TDAR has many critical components and if one or more of these components is altered, it will affect the magnitude of the TDAR. Second, suppression of antibody titers to a number of different vaccines was observed in association with PFOA exposure in epidemiological studies. The NTP viewed suppression of humoral immune responses by PFOA in mice as being evidence of "high confidence" and supportive of human evidence deemed to be of "moderate confidence". The animal and human data collectively led the NTP to the final hazard conclusion for the antibody response: "**Presumed to be an Immune Hazard in Humans**". The critical humoral immune response data from animal studies is briefly summarized and discussed below.

Yang and coworkers administered PFOA in feed (0.02% w/w) for 10 consecutive days and a single sensitization with horse RBC followed by measurement of antigen specific IgM and IgG (IgG1, IgG2, and IgG3) using a plaque assay (enumerates the number of antibody secreting B cells) and also by ELISA. Suppression of both the IgM and IgG response was observed. Importantly, although antigen specific IgG was quantified, the measurements were to a single sensitization on day 6, which is not a secondary response to hRBC. Moreover, the actual PFOA serum concentrations were not determined as in other PFOA mouse immunotoxicology studies. Interestingly, Yang and coworkers demonstrated that removal of PFOA containing feed resulted in a rapid recovery from humoral immune suppression, which is difficult to explain based on the relatively long half-life of PFOA in mice. Yang et al. also suggested activation of the peroxisome proliferator activator receptor alpha (PPARα) as a putative mechanism for PFOA-induced suppression of the TDAR. Dewitt and coworkers showed similar sensitivity of the TDAR to suppression by PFOA in PPARα knockout and wild type mice, ruling out the involvement of PPARα. in suppression of the IgM TDAR.

Dewitt and coworkers (Dewitt et al., 2008) attempted to reproduce the Yang et al. studies. At high doses (15 and 30 mg/kg/day) suppression of the sRBC IgM TDAR was observed which coincided with a loss in body weight as well as spleen and thymus weight, suggesting PFOA at the doses used, induced overt toxicity. Using lower doses administered either by oral gavage or in drinking water, suppression of the sRBC IgM TDAR was observed at doses as low as 3.75 mg/kg/day, which occurred in the absence of decreased body weight or lymphoid organ weights. A second group of mice were also sensitized a second time with sRBC to assess the IgG response (memory response). In contrast to Yang et al., the IgG response was not suppressed by PFOA. At all doses with the exception of 30 mg/kg/day, the IgG response was enhanced by PFOA. Antigen specific IgM and IgG responses were determined ELISA.

Loveless and co-workers also evaluated the effects of PFOA on humoral immune responses in CD1 mice and CD(SD)IGS BR rats. Using the IgM TDAR, Loveless et al

observed suppression of the anti-sRBC response in mice at 10 and 30 mg/kg/day with a corresponding decrease in spleen and thymus weight as well as an increase in corticosterone levels. No suppression of the anti-sRBC IgM response was observed in the rat even at 30 mg/kg/day. In both the mouse and rat study, the anti-sRBC IgM TDAR was measured by ELISA. The authors speculated that suppression of the IgM TDAR in mice was putatively through release of corticosterone due to the high doses of PFOA used in the study. In a subsequent study, DeWitt and coworkers ruled out the involvement of corticosterone as the mechanism for PFOA-mediated IgM suppression using adrenalectomized mice, which exhibited similar sensitivity to PFOA as sham control mice in the IgM TDAR (DeWitt et al., 2009).

In spite of the importance placed on the evidence for suppression of humoral immune responses in mice ("high confidence"), which is viewed by the NTP as supportive evidence for suppression of humoral immune response in humans from epidemiology studies ("moderate confidence"), there exists a major incongruence in how the NTP reached its conclusions. The humoral immune response to vaccinations, as measured in the human epidemiology studies, is mainly a secondary IgG memory response. By contrast, the anti-sRBC/hRBC TDAR measured in mice is a primary, or IgM response. Virgin B cells (B cells never having been activated by an antigen) when activated by an antigen undergo clonal expansion and differentiate either in to short lived IgM secreting plasma cells or long-lived memory cells. Clearly, suppression of the IgM response by PFOA was demonstrated by at least three independent laboratories, albeit in several studies at doses that also induced signs of overt toxicity (i.e., reductions in body and lymphoid organ weight). Only in one mouse study by DeWitt and coworkers, was the IgG memory response correctly assessed such that mice received a second sensitization with antigen (sRBC) after induction of the primary IgM response (Dewitt et al., 2008). Yang and coworkers reported a decrease in the IgG response (IgG1, IgG2, and IgG3) but the response was not measured correctly, as mice only received a single antigenic sensitization, by i.v. injection. By contrast, when a bona fide secondary response was assessed in mice using two antigenic sensitizations with sRBC, PFOA treated mice demonstrated an enhanced IgG response (Dewitt et al., 2008).

It is difficult to interpret why the primary IgM response was suppressed in mice by PFOA and yet the secondary response was either not affected or enhanced. As discussed above virgin B cells after antigenic stimulation undergo numerous rounds of proliferation and then undergo commitment to become either an IgM secreting plasma cell or memory cell. Since the memory response in mice was either unaffected or enhanced, as determined by the IgG response, these data suggests that there is no impairment of memory B cell formation and in their capability to respond to antigenic stimulation to secrete IgG. This is in contrast to those epidemiologic studies suggesting suppression by PFOA of antibody titers to vaccinations, which is mainly an IgG response by memory B cells. The mouse

studies also suggest that, either: (1) PFOA suppresses B cell to IgM plasma cell differentiation; or (2) the same number of plasma cells are formed during the primary IgM response, in the absence and presence of PFOA, but the capacity of the plasma cells to secrete large quantities of IgM is partially impaired by PFOA. **Regardless of the mechanism responsible for suppression of the mouse IgM TDAR, it is mechanistically distinct from suppression by PFOA of antibody titers to vaccines reported in the human studies**.

It is also important to emphasize that with the exception of Yang and coworkers (Yang et al., 2002), the effect of PFOA on antibody responses in mice were quantified by ELISA. Although there are a number of methods to quantify humoral immune responses, either by enumerating antibody-secreting cells or quantifying secreted antibody, with both approaches being widely accepted and used, each provides different mechanistic information. As discussed above, suppression of antibody levels by a xenobiotic can be due to: (a) a decrease in the amount of antibody being secreted by each differentiated plasma cell with no affect on the total number of plasma cells; or (b) a decrease in the total number of B cells that have differentiate into plasma cells with no effect on the amount of IgM being secreted per plasma cell.

Finally, it is unclear mechanistically from either the animal or human studies, why PFOA decreased antibody titers to one vaccine in human subjects but not for another vaccine, even when the vaccinations were related (e.g., suppression to influenza type B but not type A/H1N1 or A/H3N3) (Looker et al., 2014).

Collectively, human and animal bodies of evidence for antibody response are divergent. Mouse studies show suppression of the IgM response with no impairment of the secondary antigen specific IgG response. By contrast, epidemiology studies suggest suppression by PFOA of antibody titers to vaccinations, which are mainly a memory IgG response.

2) Infectious Disease Resistance: The NTP concluded that "there is **low confidence** that exposure to PFOA is associated with suppression of infection disease resistance in human based studies". The basis for this conclusion is a lack of data due to few infectious disease endpoints having been measured in humans. The NTP also concluded that "there is **very low confidence** that exposure to PFOA is associated with a change in the ability of animals to respond to infectious disease because there are no experimental studies on disease resistance endpoints in mammals and wildlife studies have serious risk of bias". The conclusions by the NTP are appropriate for the effects of PFOA on infectious disease resistance.

3) Natural Killer Cell Activity: NTP identified no data on the effects of PFOA on human NK cell activity. The NTP also concluded that "there is **very low confidence** that exposure to PFOA is associated with suppression of NK cell activity in animals". Presently there is only one published study in mice in which the effects PFOA were evaluated on NK cell activity (Vetvicka and Vetvickova, 2013). Vetvicka and coworker used a single (20 mg/kg/day) dose of PFOA administered for 7 days, which suppressed NK cell activity. The study was viewed as having significant bias. In addition, there is one study in wildlife that showed no correlation between PFOA serum levels and NK cell activity in bottlenose dolphins (Fair et al., 2013). Based on the lack of data in combination with negative date, the NTP did not develop an evidence synthesis for PFOA and NK cell activity. Appropriately, NK cell activity was not considered by the NTP for hazard identification conclusions.

B. **Hypersensitivity-related Effects and Outcomes:** The NTP concluded "there is **low confidence** that exposure to PFOA during childhood is associated with increased hypersensitivity responses based on available human data". Evidence for this conclusion comes from retrospective, cross-sectional and prospective epidemiological studies of clinical measures and/or biomarkers of hypersensitive (e.g., asthma, rhinitis, skin disorders, serum IgE). The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016) and therefore will only be discussed within the context of animal data.

The NTPs conclusion "there is **high confidence** that exposure to PFOA is associated with increased hypersensitivity responses based on the available animal data", is based primarily on two studies both of which evaluated the effects of PFOA on airway hyperresponsiveness (AHR) in mice (Fair et al., 2013; Ryu et al., 2014). In addition, a study by Singh et al. (2012) is cited which showed that PFOA treatment in mice enhanced the IgE-dependent local allergic reaction in mice dosed dermally with 10 and 50 mg/kg/day PFOA for four days. In this same study i.p. injection of 1 and 5 mg/kg of PFOA increased histamine release (Singh et al., 2012).

In the Fairley study, PFOA was administered dermally in acetone for 4 consecutive days (0, 0.25, 2.5, 6.25, 12.5, 18.75, 25 and 50 mg/kg/day). Mice were then sensitized (i.p.) and challenged (pharyngeal aspiration) with ovalbumin (OVA) followed by measurement of airway hypersensitivity and AHR. At the 50 mg/kg/day dose a significant decrease in body weight, spleen weight, thymus weight, spleen cellularity and thymic cellularity were observed, all suggestive of overt toxicity. Mice treated with 25 mg/kg/day PFOA exhibited an increase in Ova-specific serum IgE and at doses of 18.75, 25 and 50 mg/kg/day an increase in total serum IgE. The increase in IgE serum antibodies was viewed as important since IgE is involved in type I hypersensitivity reactions by facilitating release of mast cell-derived mediators (e.g., histamine, prostaglandins, leukotrienes). As a measure of pulmonary function, penH values were determined in response to methacholine (MCH) challenge. Mice exhibited a trend toward increasing antigen-specific AHR with increasing concentration

of PFOA up to 50 mg/kg/day. Histopathology also showed a dose-dependent increase in airway associated inflammatory cells. Fairley et al. concluded that PFOA exposure increased IgE and AHR to Ova in mice that were concurrently exposed to Ova and PFOA. Overall the Fairley studies were of good technical quality and the study conclusions were consistent with results reported. It is also noteworthy that although serum PFOA levels were not determined, adverse PFOA related effects were observed primarily at high doses with the highest dose likely inducing overt toxicity.

Ryu coworkers (Ryu et al., 2014) also assessed the effects of PFOA on airway hypersensitivity and AHR but used a very different PFOA exposure paradigm compare to the Fairley study. Specifically, PFOA exposure was initiated in pregnant dams on gestation day 2 and continued through week 12 after birth by mixing 4 mg of PFOA/kg of diet with an estimated exposure level equivalent of 1 mg/kg oral gavage dose for 63 day (~105 mg/kg cumulative dose). Ryu et al also reported that PFOA exposure induced AHR but occurred in the absence of exposure to an allergen (i.e., Ova). Moreover, AHR induced by MCH challenge in mice sensitized and challenge with Ova was not enhanced by PFOA. These results suggest that PFOA does not appear to augment allergen-induces AHR. Interestingly, Ryu and co-workers also found that mice treated with PFOA only (i.e., no Ova treatment) exhibited an increase in inflammatory cells as assessed by bronchoalveolar lavage. The increase was primarily due to an increase in infiltrating macrophages. Serum level determinations showed that 12 week-old mice possessed 4,800 ± 1,100 ng/ml, which is significantly higher than what is observed in the general public (0.5 -12 ng/ml). The Ryu study was of good technical quality and the conclusions reached by the authors are supported by the study results.

Although both Fairley and Ryu reported that PFOA exposure induced AHR, only the Fairley study results support the NTP conclusion that PFOA AHR is mediated by a hypersensitivity response. By definition, hypersensitivity is an exaggerated immune response to an exogenous antigen. In the Ryu study, PFOA induced AHR in the absence of exposure to an allergen (Ova) and also did not potentiate the AHR response to Ova sensitization and challenge. It is noteworthy that although the PFOA daily dose in the Ryu study was significantly less than in the Fairley study, the overall cumulative dose in the Ryu study was at least an order of magnitude greater due to the duration of the exposure period. The mechanism for AHR by PFOA in the Ryu study is unclear but may be due, in part, to the marked increase in airway associated inflammatory cells, which was also identified by histopathology in the Fairley study. The NTP considered results by Singh and coworkers showing an enhanced IgE-dependent local allergic reaction in mice dosed dermally with 10 and 50 mg/kg/day PFOA and histamine release by i.p. injection of 1 and 5 mg/kg of PFOA as additional supportive evidence that PFOA induces hypersensitivity in mice. Importantly, in the Singh study it appears that histamine release by mast cells both *in vitro,* after direct addition of PFOA to cultured cells, and *in vivo*, after i.p. administration of PFOA, was due to

spontaneous release and not IgE mediated, as in a type 1 hypersensitivity response. In summary, the NTP considered both the both Fairley and Ryu studies as evidence for hypersensitivity related outcomes with "high confidence". **Both the Fairley and Ryu studies support the conclusion that PFOA at high doses can induce AHR in mice, but only the Fairley study supports hypersensitivity as a putative mode of action for AHR**.

Collectively, based on the human body of evidence, which was deemed by the NTP as "Low Confidence" and animal body of evidence as "High Confidence", the final NTP hazard conclusion based on hypersensitivity-related evidence was that PFOA is "Presumed to be an Immune Hazard in Humans".

C. **Autoimmunity:** The NTP concluded that "there is **low confidence** that exposure to PFOA is associated with ulcerative colitis and rheumatoid arthritis in humans based on epidemiological studies. The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016). No animal studies were identified by the NTP on potential associations between PFOA and autoimmunity.

PFOS Immune Evidence

A. **Immune Suppression:** Within the category 'Immune Suppression", the NTP identified published studies in four subcategories antibody response, natural killer NK cell activity, and infection disease resistance based on the rationale that different cell types can be involved in each of these three responses.

1) Antibody Response: The NTP concluded that "there is **moderate confidence** that exposure to PFOS is associated with suppression of the antibody response in human based studies". Evidence for this conclusion comes from epidemiological studies in which antibody titers to vaccinations were quantified in combination with measurements of serum PFOS levels coupled with supportive animal studies. The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016) and therefore will only be discussed within the context of animal data.

The NTP concluded that based on animal studies "there is a **high confidence** that exposure to PFOS is associated with suppression of the antibody response". The conclusion that PFOS suppresses antibody responses in mice is supported by a number of studies which show that exposure to PFOS at various life stages can suppress the IgM TDAR (Dong et al., 2011; Keil et al., 2008; Peden-Adams et al., 2008). Suppression of the IgM TDAR occurred at doses significantly lower with PFOS than PFOA. In several studies male mice exhibited greater sensitivity to suppression of the IgM TDAR than female mice (Keil et al., 2008) (Peden-Adams et al., 2008). In another study, Quazi and

coworkers showed that PFOS administered at 250 µg/kg/day over 28 days with a total administered dose of 7 mg/kg did not suppress the IgM TDAR (Qazi et al., 2010). Studies also show that PFOS does not suppress IgG after a single sensitization with antigen and, in fact, modestly enhanced the IgG response at a dose of 50 mg/kg/day (Dong et al., 2011).

As with PFOA, the NTP concluded that suppression of the IgM response in animal studies is supportive evidence of human data showing an association between PFOS exposure and decreased vaccine titers. As discussed above, antibody titers to vaccinations are primarily of the IgG antibody isotype and the animal studies demonstrating suppression of the primary antibody response, as measured in mice by the TDAR, is of the IgM isotype. It is also important to emphasize that the secondary IgG response was not appropriately induced to elicit a bona fide memory response as only a single antigen sensitizations was used in the mouse studies (Dong et al., 2011; Qazi et al., 2010). In addition, one study was identified in white longhorn chickens in which the secondary IgG (IgY) response was assessed after a secondary sRBC sensitization (Peden-Adams et al., 2009). These studies showed no suppression of the IgG response at PFOS doses of 1, 2.5 and 5 mg/kg egg weight, compared to control, although the IgM and combined IgM and IgY response was suppressed.

Based on the aforementioned studies the NTP concluded with respect to suppression of antibody responses, the human body of evidence being of "Moderate Confidence" and the animal body of evidence being of 'High Confidence" with the Final hazard conclusion "Presumed to be an Immune Hazard to Humans".

2) Infectious Disease Resistance: The NTP concluded that "there is **low confidence** that exposure to PFOS is associated with suppression of infection disease resistance in human based studies". The basis for this conclusion is limited data due to few infection disease endpoints having been measured in humans, weak or no association with PFOS exposure, and bias in experimental design. The NTP also concluded that "there is **moderate confidence** that exposure to PFOS is associated with reduced ability of animals to respond to infectious disease", which is based on one study in female mice (Guruge et al., 2009) and two wildlife studies (Kannan et al., 2006; Kannan et al., 2010).

Guruge et al. assessed the effect of PFOS on resistance to influenza virus A/PR/8/34 (H1N1) in B6C3F1 mice. In the Guruge and coworker study two doses of PFOS were employed, 5 or 25 µg/kg/day for 21 days yielding serum PFOS concentrations of 189 and 670 ng/ml, respectively. Mice exposed to PFOS at 25 µg/kg/day exhibited a significant decrease in survival (~15%) compared to control (~50%). The study appears to be of good technical quality.

In addition two wild life studies, one on sea otters found freshly dead on the California coast (Kannan et al., 2006) and a second in brown bats with white nose syndrome

(Kannan et al., 2010), were considered by the NTP. It is difficult to judge the conclusion from the wild life studies as there were many potential confounding factors. For example, in the sea otter study, the investigators categorized dead otters into one of three groups based on presumed cause of death, nondisease, emaciated, or diseased. It is not clear how there can be certainty on whether the cause of death was infectious disease-based. The investigators attempted to correlate PFOA/PFOS tissue levels to one of the three causes.

The NTP final hazard conclusion based on the body of evidence for infectious disease resistance is **"Suspected to be a Immune Hazard to Humans"**. There does not appear to be sufficient supporting evidence in either humans or animals to support the NTP conclusion. The NTP should seriously consider down grading the final hazard conclusion for infection disease resistance to something less than **"Suspected to be a Immune Hazard to Humans"**.

3) NK Cell Activity: The NTP identified no human data on the potential association between PFOS and NK cell activity. The NTP also concluded that "there is **moderate confidence** that exposure to PFOS is associated with suppression of NK cell activity in animals".

The NTP conclusion that "there is **moderate confidence** that exposure to PFOS is associated with suppression of NK cell activity in animals", is based on several studies in which NK cell activity was impaired in mice at dose from 0.833 to 40 mg/kg/day PFOS (Keil et al., 2008) (Dong et al., 2009; Vetvicka and Vetvickova, 2013; Zheng et al., 2009). Based on the studies cited, suppression NK cell activity by PFOS exposure appears to be a high dose phenomenon, which in at least one studies was also correlated with increased corticosterone serum levels (Dong et al., 2009), a biomarker of overt toxicity and known immunosuppressive factor. Specifically, Dong et al. showed increased NK cell activity at 5 mg/kg total administered dose (TAD) and suppression at 50 and 125 mg/kg (TAD), notably high PFOS doses. Peden-Adams showed increased NK cell activity at PFOS dose of 0.5, 1 and 5 mg/kg (TAD). Vetvika showed NK cell activity was decrease after 20 mg/kg/day administration for 7 days; a high PFOS dose. Final Keil et al., showed suppressed NK cell activity at 8 weeks post gestational exposure but not at 4 weeks, which the authors stated was an "unusual observation". The above studies suggest that PFOS impairs NK cell activity 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 (Dong et al., 2009; Zheng et al., 2009).

The animal studies do not support the NTP conclusion that there is a **"Moderate Level of Evidence"** that PFOS suppresses NK cell activity in the absence of overt toxicity.

B. **<u>Hypersensitivity-related Effects and Outcomes:</u>** The NTP concluded "there is **very low confidence** that exposure to PFOS is associated with increased hypersensitivity responses based on available human data". Evidence for this conclusion comes from epidemiological studies of clinical measures and/or biomarkers of hypersensitive (e.g., asthma, rhinitis, skin disorders, serum IgE). The strengths and weaknesses of the epidemiological studies have been extensively reviewed by the NTP and by Chang and co-workers (Chang et al., 2016) and therefore will only be discussed within the context of animal data.

   The NTP concluded "there is **low confidence** that exposure to PFOs is associated with increased hypersensitivity responses based on the available animal data". The conclusion is based primarily on limited data and inconsistencies within the relevant animals studies.

   Based on the above, the NTP did not develop an evidence profile or detailed discussions of the evidence for PFOS and hypersensitivity related outcomes.

C. **<u>Autoimmunity:</u>** The NTP appropriately concluded that "there is **very low confidence** that exposure to PFOS is associated with autoimmunity due to very limited data in this area. No animal studies were identified by the NTP on potential associations between PFOA and autoimmunity. The NTP concluded that there is an inadequate level of evidence to draw conclusions on whether exposure to PFOS is associated with autoimmunity.

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resistance for PFOS, and and NK cell activity for PFOS. Collectively, for these reasons NTP should consider downgrading the final hazard conclusions.

In addition to the comments provided herein (peer-reviewed by Dr. Kaminski), we encourage NTP to consider the insightful independent evaluations and comments by Drs. August, Beck, Chang, and Osterholm. We sincerely hope that these scientific emphases will be taken into consideration by NTP with the final assessment.

Sincerely,

[Signature Redacted]

Carol A. Ley, MD, MPH
Vice President & Corporate Medical Director

[Signature Redacted]

Sue Chang, Ph.D.
Senior Toxicology Specialist

ATTACHMENT C

3M COMPANY'S COMMENTS ON THE NEW JERSEY DEPARTMENT OF ENVIRONMENTAL PROTECTION'S PROPOSED RULES CONCERNING PFOS AND PFOA

May 31, 2019

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May 31, 2019

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Re: Ground Water Quality Standards and Maximum Contaminant Levels (MCLs) for Perfluorooctanoic Acid (PFOA) and Perfluorooctanesulfonic Acid (PFOS); Proposed Amendments: N.J.A.C. 7:1E Appendix A, 7:9C Appendix Table 1, 7:9E-2.1, 7:10-5.2, and 12.30, 7:14A-4 Appendix A and 7.9

Dear Mr. Knapick:

The 3M Company (3M) appreciates this opportunity to provide the enclosed comments to the New Jersey Department of Environmental Protection's (NJDEP) proposed groundwater quality standards and maximum contaminant level (MCL) drinking water standards for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). As a science-based company, 3M encourages NJDEP to use the best available science when assessing these chemicals and developing drinking water and groundwater standards. As our comments reflect, 3M has substantial experience and expertise regarding PFOA and PFOS, informed in part by the fact that 3M scientists are authors or contributors to many of the studies referenced by NJDEP. As a result of this expertise, 3M has significant concerns with the MCLs and groundwater quality standards being proposed.

Please let us know if you have any questions.

Regards,

Oyebode A. Taiwo, MD, MPH

3M COMPANY's COMMENTS ON THE NEW JERSEY DEPARTMENT OF ENVIRONMENTAL PROTECTION's PROPOSED RULES CONCERNING PFOS AND PFOA

On April 1, 2019, the New Jersey Department of Environmental Protection[1] (NJDEP) proposed to amend the New Jersey Safe Drinking Water Act (SDWA) rules to set maximum contaminant levels (MCL) for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). It also proposed amendments to the Ground Water Quality Standards (GWQS) to establish specific ground water quality standards for PFOA and PFOS. Further, NJDEP proposed adding PFOA and PFOS to the List of Hazardous Substances at N.J.A.C. 7:1E.

The following presents 3M Company's (3M) comments on these proposed rules.[2] Attachment A provides 3M's detailed comments on the proposed MCLs and GWQS. Attachment B provides 3M's comments on related recommended practical quantitation limits (PQL) for PFOS and PFOA. The following presents 3M's general comments and concerns regarding NJDEP's proposals, as well as specific comments relating to feasible treatment options for PFOS and PFOA and the proposed Hazardous Substance listing.

The Rule Proposal Ignored Best Available Science in Proposing Arbitrarily Low MCLs and Groundwater Quality Standards

As a science-based company, 3M has significant concerns with the MCLs and groundwater quality standards being proposed by NJDEP because they do not reflect the best and latest science regarding PFOS and PFOA. As 3M's detailed comments demonstrate, the proposed MCLs are overly conservative, technically flawed, do not reflect recently published studies and provide no additional protection compared to EPA's current drinking water health advisories. They are merely lower.

The MCL proposal ignores the best available scientific evidence and arbitrarily selects studies and toxicity endpoints to drive to lower MCLs. This was done without applying scientific rigor or assessing the reliability of testing for such low values. For example, NJDEP selected immunotoxicity as the critical effect for the PFOS MCL even though the body of human and animal studies is inconsistent on this endpoint. Further, NJDEP did not recognize methodological and technical flaws in its selected critical study for PFOS- the immunotoxicity study of mice by Dong et al. (2009). EPA and other agencies have elected not to use this study

[1] 3M recognizes the New Jersey Drinking Water Quality Institute (DWQI) made recommendations to the NJDEP regarding MCL levels, PQLs and treatment technologies. Because NJDEP has adopted the DWQI's recommendations in whole, with no revisions and because of the substantial overlap in the makeup of DWQI members and NJDEP staff, 3M's comments (unless otherwise stated) use DWQI and NJDEP interchangeably when addressing the above topics.

[2] NJDEP also proposed: (1) amending the Private Well Testing Act (PWTA) rules to require testing of private wells subject to sale or lease for PFOA, PFOS and perfluorononanoic acid (PFNA); (2) amending the SDWA rules to require testing of newly constructed wells for public noncommunity water systems and nonpublic water systems for PFOA, PFOS and PFNA; and (3) adding PFNA, PFOA, and PFOS to the Permit Application Testing Requirements/Pollutant Listings and the Requirements for Discharges to Ground Water in New Jersey Pollutant Discharge Elimination System (NJPDES) rules. 3M is not offering any specific comments to these proposed rules, other than to t to incorporate by reference its comments on the proposed MCLs to the extent the MCLs impact the foregoing proposed rule changes.

to set PFOS drinking water values. NJDEP appears to have selected Dong et al. 2009 as its critical study merely because it yielded the lowest possible MCL from its list of candidate MCLs.

The weight of scientific evidence does not support either MCL proposed by NJDEP. In addition to equating presence in the environment with harm, NJDEP treats PFOS and PFOA's long serum half-lives and tendency to accumulate in the blood at low exposures as synonymous with increased health risk and higher toxicity. The body of credible science does not support such a conclusion.

The Body of Scientific Evidence Shows Does Not Show Adverse Health Effects to Humans from PFOS or PFOA Exposure

The vast body of scientific evidence does not establish 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. *NJDEP dismisses careful and detailed evaluations of PFOS and PFOA made by EPA, Health Canada, Australia's PFAS Science Panel and other organizations. NJDEP focused on studies and findings reviewed and rejected by these entities.*

The Agency for Toxic Substances and Disease Registry[3] (ATSDR) recently acknowledged that for PFAS there is no cause and effect established between health effects and exposure to humans. It stated: "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***." ATSDR 2018; pages 635-636 (emphasis added).

A recently released review of studies involving perfluoroalkyls exposed populations commissioned by the Australian government also supports the lack of evidence of harm. In the May 2018 report by the Australian Expert Health Panel, "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."

This point is illustrated by the following table summarizing recent drinking water standards and guidance levels for PFOA and PFOS set by EPA, German, Dutch, Canadian, Swedish and Australian environmental authorities. As indicated by the chart, different national environmental protection authorities have arrived at different toxicity values and drinking water guidance levels for the same chemicals. Nonetheless, all of these drinking water guidance values are five times or more higher than the MCLs proposed by New Jersey.

[3] See ATSDR Draft Toxicological Profile for Perfluoroalkyls. June 2018

US and International Drinking Water Values ng/L							
	Germany (UBA) 2016	US (EPA) 2016	Netherlands (RIVM)	Sweden 2014	Canada (MOE) 2018	Australia (FSANZ) 2016	Proposed NJDEP 2019
PFOA	100	70	87.5 (2018)	-	200	560	13
PFOS	100	70	530 (2011)	90	600	70	14

Key Concerns with the Proposed PFOA and PFOS MCLs

As provided in 3M's detailed comments, there are numerous concerns with the proposed these MCLs. Key concerns include:

- The DWQI made a serious technical error in its evaluation of BMD modeling. This error led the DWQI to use a serum PFOS NOAEL of 674 ng/mL as the POD for calculating the PFOS MCL instead of a properly calculated serum PFOS BMDL. If DWQI's BMD modeling error is corrected, and NJDEP uses the preferred BMD modeling approach, a serum PFOS $BMDL_{1SD}$ at 3,400 ng/mL can be successfully determined as the POD for the PFOS MCL. This would raise the PFOS MCL to 0.064 µg/L, five times higher than the proposed MCL.
- NJDEP should not base the PFOS MCL on immunotoxicity. The Dong et al. 2009 study used by NJDEP for the PFOS MCL development fails to support PFOS-caused immune suppression in mice because the study had numerous technical deficiencies, such as incomplete antibody isotyping and partial assessments in some primary immune organs. Overall, this study fails to provide compelling scientific evidence to support the claim that PFOS causes immune suppression in mice. Moreover, the epidemiologic evidence is not sufficient to support an association between PFOS exposure and decreased vaccine response in humans.
- The PFOS serum half-life of 5.4 years used by NJDEP is not based on the best and most recent available science. NJDEP should instead use a serum elimination half-life of 3.4 years based on a 2018 study by Li et al.
- NJDEP use of the default relative source (RSC) value of 20% is not supportable. There is sufficient information for NJDEP to use an RSC of 50% or possibly higher.
- NJDEP's PFOA database uncertainty factor of 10 for PFOA lacks scientific merit and should be reduced to 3.
- NJDEP's selection of increased liver weight in rodents as the critical effect for a PFOA MCL is inconsistent with EPA guidelines and published expert opinions on the distinction between liver hypertrophy as a non-adverse adaptive change and other endpoints representing liver toxicity.

As a result, the PFOS and PFOA MCLs should be significantly higher.

NJDEP's Proposal Must be Assessed in Light of the Goals of Executive Order 63

Governor Murphy recent signed Executive Order 63, "Establishing new regulatory principles to foster economic growth and government efficiency". See N.J. Exec. Order No. 63 (2019) (EO 63). The following prefatory statements in EO 63 should guide any assessment of the rules proposed by NJDEP. They state:

> WHEREAS, **well-framed regulations** can fulfill statutory goals and mandates and carry out the government's ongoing mission of promoting the health, safety, and welfare of New Jersey, the protection of our land, air, and water, and the prosperity of our economy; and
>
> WHEREAS, **ill-considered or ineffective regulation can deter progress, unduly burden businesses**, hamper innovation and economic growth, and lead to stagnation, inefficiency, and inequity, while an informed and progressive approach to regulatory affairs can help avoid these shortcomings; and
>
> WHEREAS, as a general matter, an agency should not propose or adopt a regulation **without first making a reasoned determination that its benefits justify its costs**, with the recognition that some benefits and costs are difficult to quantify;

Compared to these goals, NJDEP's proposed rules are deficient. The exceedingly low proposed MCLs are ill-considered and not well-framed. They are not "informed" because the proposal ignores sound science in its MCL study selection and derivation. These values are no more protective in any real sense than EPA's current drinking water health advisories (DWHA) of 70 ng/L because both sets of values carry large margins of safety built into them. Neither are a bright line between safety and harm. They are, however, burdensome on business, local governments and water utilities. Nothing in NJDEP's rulemaking offers any reasoned determination that the benefits of the proposed MCLs justify its costs. In fact, other than limited cost considerations, such as monitoring costs by water utilities, NJDEP by and large ignore the larger costs to the whole of New Jersey's economy and citizens.

EO 63 also states "where federal regulation is inadequate to protect the environment, health, safety, and welfare of New Jersey's residents and communities, New Jersey should develop its own regulatory framework where it has the legal authority to do so", ***but*** "where federal regulation adequately protects the environment, health, safety, and welfare of New Jersey's residents and communities, New Jersey should operate under that framework in order to minimize confusion and complexity." EO 63, ¶ 2.a. On this count, New Jersey's efforts also fail. While there may be disagreement over the pace of EPA's regulation of PFOA and PFOS, EPA action is not non-existent. The current DWHA provide a national reference point, as EPA recent recommendations for groundwater remediation based on the DWHAs illustrate. Further, EPA's semi-annual regulatory agenda shows that EPA is on pace with meeting the requirements of the federal SWDA and will make an initial regulatory determination on setting MCLs for PFOA and PFOS by the end of the year.

While the proposed MCLs are nearly six times lower than the DWHA, they are not any safer given that the existing DWHA more than adequately protects public health. Taken together, the peer reviewed science does not show that exposures to PFOS or PFOA at or above either EPA's or NJDEP's value would pose any health risk. NJDEP has not demonstrated that

federal regulation of PFOA or PFOS is inadequate or that the DWHA are not protective. All NJDEP asserts is that its values are lower. The adoption of PFOS and PFOA values so dramatically different from EPA will, however add "confusion and complexity" in New Jersey, not only for business, but municipalities and the general population.

EO 63 also requires that "Governmental decisions should be **based on the best available data**, including scientific data if applicable. Where **scientific evidence is an important element** in developing or evaluating a rule, State entities should seek out and make productive use of scientific expertise available to them." EO 63, ¶ 2.c. As 3M's comments on the MCLs point out, NJDEP did not utilize the best or most recent science on PFOA or PFOS. For example, NJDEP selected as the critical study for calculating a PFOS MCL an immunotoxicity study of mice by Dong et al. (2009). This study has recognized methodological and technical flaws. Other than New Jersey, no other government or public health entity has used this study in any meaningful way or for setting regulatory or guidance levels for PFOS. Dong et al 2009 does not represent the "best available data. NJDEP selected it solely because it was "the most stringent potential Health-based MCL" of three potential MCLs calculated by NJDEP.[4]

Principles that Should Guide NJDEP's MCL Selection

3M urges NJDEP to consider the following as it reviews the comments of 3M and others:

- NJDEP should avoid the layering of very conservative parameter choices to derive an MCLs because this is NOT the same as being protective in a true and sound public health protection sense. A lower MCL value will not be any more protective than a higher MCL if the latter value already provides an adequate margin of safety. Unnecessarily low values do not provide more public health protection but will impose unwarranted costs on the public and instill unnecessary fear and anxiety in communities.
- Rely on the best available science in making parameter decisions.
- Consider the weight of the evidence and the totality of information available on toxicity, and exposure when selecting health effects end points and other input parameters.
- Many epidemiological studies regarding PFOS or PFOA are cross-sectional by design. This type of study design cannot address temporality (*i.e.*, time-dependent associations). This issue is important to acknowledge because confounding and reverse causation has now been shown to be the explanation for several different health outcomes initially reported in cross-sectional studies as indicating an association between PFOS or PFOA exposure and the outcome (e.g., chronic kidney disease, lower birth weight, early onset menopause).
- Primate toxicity data should not be minimized. When it comes to human relevance and risk assessment, given the many issues in extrapolating toxicology data from rodents (a lower order species) to humans (the highest order), primate data have always valued as

[4] If NJDEP had selected either of these two other studies otherwise approved by the DWQI, the resulting PFOS MCL would be five to ten times higher.

the most scientifically appropriate species for human risk assessment because it is the second-highest order species next to humans.

- In deriving their proposed guidance values, both ATSDR and EPA apply uncertainty factors on the assumption that humans are *more* sensitive than rodents to these effects. This has not been shown to be the case, however. Published data strongly support that rodents are likely to be much more sensitive to PFAS-induced effects than humans.[5]
- The body of scientific data for PFAS does not support the adverse human health effects that NJDEP associates with PFOS and PFOA and lists in its proposed Health Effects Language for Consumer Confidence Reports.
- As discussed in 3M's detailed comments herein, there is insufficient evidence to support using immunotoxicity as a basis for setting drinking water levels for PFOS or PFOA.

NJDEP's Reliance on ATSDR and EFSA for Support is Misguided

NJDEP attempts to bolster its MCL decisions by pointing to draft PFOS and PFOA maximum risk levels (MRLs) from the ATSDR, as well as recent preliminary tolerable intake levels proposed by the European Food Safety Authority (EFSA). While ATSDR and EFSA superficially support NJDEP's values, both the ATSDR and EFSA determinations are flawed in their own right, and add little, if any, value

NJDEP concludes that the ATSDR Intermediate MRLs for PFOS and PFOA provide "additional support for the Institute's PFOA and PFOS reference doses. There are significant problems, however with ATSDR's MRL development for PFOS and PFOA. 3M incorporates by reference and directs NJDEP to 3M's submitted to ATSDR (See 3M ATSDR Docket No. ATSDR– 2015–0004 submission). In our comments, 3M highlighted flaws in the draft ATSDR document, with particular focus on problems with both the PFOA and PFOS MRLs. For example, the two studies selected by ATSDR--Onishchenko et al. (2011) and Koskela et al. (2016)--lacked fundamental scientific rigor (*e.g.*, using a single dose study without any dose-response, small sample size with only six pregnant dams; no details on the reproductive nor the developmental hallmarks, litter bias, non-standard testing methods, no internal serum PFOA dosimetry data, etc.). Given these flaws, the proposed ATSDR MRLs were not derived using best available science and do not provide support for the NJDEP proposal.

With respect to EFSA, NJDEP said "the EFSA tolerable weekly intakes and associated daily intake values provide additional support for the Institute's reference doses for PFOA and PFOS" because the EFSA daily intake values are near or lower than the DWQI's PFOA and PFOS reference doses. EFSA values are based its tolerable levels on a novel approach of using human epidemiological studies concerning cholesterol to perform quantitative risk assessment to

[5] ATSDR has acknowledged the impact on these various differences on the reliability of its risk assessment, noting that "for the most part, adverse health effects in studies in animals have been associated with exposure concentrations or doses that resulted in blood levels of perfluoroalkyl compounds that were significantly higher than those reported in perfluoroalkyl workers or in the general population." This, along with "profound differences in toxicokinetics between humans and experimental animals," (such as the differences in half-lives between species) and issues related to PPARα, "make it somewhat difficult at this time to determine the true relevance of some effects reported in animal studies to human health." ATSDR 2018, page 10.

calculate the tolerable intakes. NJDEP's embrace of this approach contradicts a recent published position taken by DWQI members Gloria Post, Jessie Gleason and Keith Copper in December 2017. In this journal article, they stated "there is a **high bar for use of human epidemiology in quantitative risk assessment** due to its observational nature. … limitations in the current human database such as inability to determine the dose-response relationships for individual PFAAs due to cooccurrence of other PFAAs, **preclude the use of human data as the primary basis for PFAA drinking water guidelines**." See Post GB, Gleason JA, Cooper KR (2017) Key scientific issues in developing drinking water guidelines for perfluoroalkyl acids: Contaminants of emerging concern. PLoS Biol 15(12): e2002855. https://doi.org/10.1371/journal.pbio.2002855

Hazardous Substance Designation

NJDEP proposes to add PFOA and PFOS and hazardous substance under the Spill Act. NJDEP failed, however, to provide specific and adequate explanation and justification for this addition. NJDEP merely makes a conclusory statement that it "has determined that because PFOA and PFOS in the environment pose an unacceptable risk to public health, it is appropriate to include PFOA and PFOS on the DPHS Appendix A List of Hazardous Substances."

NJDEP merely offers a passing comment as to the persistence of PFOS and PFOA and a short recap of alleged health effects, for which no causation has ever been established in the scientific literature. NJDEP make no effort to explain why the presence of PFOA and PFOS in the environment pose an unacceptable risk to public health. NJDEP's rule proposal instead spends the bulk of its text discussing the outcome of adding PFOS and PFOA onto the list hazardous substances, such as cleanup liability under the Spill Act.

Recommended Treatment Options for PFOA and PFOS in Water

NJDEP's rule proposal references several Treatment Subcommittee reports that provide recommendations on perfluorinated compound treatment options for drinking water (dated June 2015; August 2016; November 2017). These reports summarize existing water treatment technology collected from various research organizations, as well as information from various operating Granular Activated Carbon (GAC) systems.

It appears that NJDEP has not fully assessed the economic impacts of the proposed standards as they relate to treatment costs. Several of the references state that "samples taken after GAC treatment" were either "non-detectable" or "have remained below the recommended [MCL]." The Treatment Subcommittee fails to recognize that several examples provided have raw water PFOS levels that are at or below the proposed MCL (NJAW – Logan System and NJAW – Penn's Grove). As such, while 3M agrees that GAC is effective for removal of PFOS and PFOA, a comparison of treatment effectiveness for these systems seems inappropriate and skews the economic impact analysis for the proposed PFOS MCL. The Subcommittee also fails to recognize that several of the treatment system operational costs are provided for treatment below higher drinking water guidance values (MDH – 0.3 ug/L; NJDEP 0.04 ug/L). There are no costs provided or calculated to meet the proposed MCL. Given the traditional GAC isotherm it would be expected that operating costs would be higher to operate a system at the proposed MCL.

Lastly, "the Subcommittee continues to advise that GAC and/or an equally efficient technology be considered for treatment…" The term "equally efficient" is vague and fails to consider the economic impacts of technology that may have equal treatment efficiency to GAC for PFOS and PFOA, but much higher capital and operating costs than GAC.

ATTACHMENT A

3M Comments on Proposed Groundwater Quality Standards and Maximum Contaminant Levels for Perfluorooctanoic Acid and Perfluorooctanesulfonic Acid[6]

EXECUTIVE SUMMARY

The New Jersey Department of Environmental Protection (NJDEP) is proposing to amend the New Jersey Safe Drinking Water Act (SDWA) rules to establish a maximum contaminant level (MCL) for perfluorooctanoic acid (PFOA) of 0.014 micrograms per liter (µg/L) and an MCL for perfluorooctanesulfonic acid (PFOS) of 0.013 µg/L. The MCLs proposed by NJDEP are based on recommendations made by the Health Effects Subcommittee of the New Jersey Drinking Water Quality Institute (DWQI) in 2017 (PFOA) and 2018 (PFOS). Upon reviewing the health effect documents prepared by DWQI Health Effects Subcommittee, 3M respectfully disagrees with the proposed MCLs, as well as its conclusions regarding the human health effects associated with exposure to PFOS and PFOA. 3M believes the following key scientific evidence was not fully considered by the DWQI Health Effects Subcommittee or NJDEP which led to incorrect scientific assumptions resulting in underestimations of the proposed MCLs.

Comments on the Proposed PFOS MCL

DWQI Made a Serious Technical Error in Its BMD Modeling Which Prevented Its Use for the PFOS MCL Calculation. The DWQI selected a study by Dong et al. (2009) as the point of departure (POD) study for deriving a PFOS MCL based on an immunotoxic endpoint. DWQI made a serious technical error in its benchmark dose (BMD) modeling by using the standard error of the mean (SEM) from the Dong et al. (2009) study, rather than the required standard deviation. This error led the DWQI to reject the otherwise preferred BMD modeling approach in this instance and to instead use a serum NOAEL of 674 ng/mL as the POD for calculating the PFOS MCL. If DWQI's BMD modeling error is corrected by using the standard deviation (rather than SEM), a serum BMD can be properly calculated and used as the POD for the PFOS MCL. Correcting DWQI's error results in a PFOS $BMDL_{1SD}$ at 3,400 ng/mL. Using this value as POD results in a PFOS MCL of 0.064 µg/L, five times higher than the proposed MCL.

The Study Selected by DWQI Fails to Support the Claim that PFOS Causes Immune Suppression in Mice. The Dong et al. (2009) study used by DWQI as the point of departure for the PFOS MCL was based on immunotoxicity. There were numerous technical deficiencies with the study that Dong et al. (2009) did not consider, such as incomplete antibody isotyping and partial assessments in some primary immune organs. Using a crude (non-specific) antigen SRBC, they only challenged the mice once without any follow up for a second challenge to elicit permanent antibody response (to antigens and/or vaccines). As a result, this study fails to provide compelling scientific evidence to support the claim that PFOS causes immune suppression in mice.

[6] Because NJDEP proposes use the same PFOA and PFOS values for both the proposed MCLs and the proposed GWQS, 3M incorporates by reference each of its comments regarding the proposed MCLs as comments on the proposed GWQS.

The Epidemiologic Evidence is Not Sufficient to Support an Association between PFOS Exposure and Decreased Vaccine Response in Humans. NJDEP asserts that human exposure to PFOS has been associated with decreased vaccine response. Contrary to this assertion considerable inconsistencies have been observed among the 9 epidemiological studies that have examined PFOS exposure to antibody responses to 10 distinct vaccine antigens. Because of these inconsistencies, these studies do not do not support an association between PFOS exposure and decreased vaccine response in humans. Further, any hypothesized vaccine response effects appear to have no clinical significance as the data does not support a causal association between PFOS exposures and an increased risk of infectious disease. As a result, they do not provide collaborative support to the immunotoxicity findings in laboratory studies in mice claimed by NJDEP.

Epidemiological Associations for Cholesterol and PFOS are Likely Non-causal. NJDEP also asserts that human exposure to PFOS has been associated with increased cholesterol. In experimental studies, PFOS has not been shown to cause an increase in cholesterol. The low dose response association based on certain observational epidemiologic data continues to remain only a hypothesis elusive of a foundational mode of action and not supported by experimental data.

Epidemiological Association for Birth Weight is Not Causal. NJDEP asserts that human exposure to PFOS has been associated with lower birth weight. The association with birth weight has been demonstrated to be the result of confounding or reverse causation.

The PFOS Serum Half-life Used by NJDEP is Not Based on the Best and Most Recent Available Science. NJDEP used a human serum elimination half-life estimate of 5.4 years from a retiree occupational population in the MCL derivation for PFOS which does not reflect overall general population demographics as well as age-dependent renal function. NJDEP should use 3.4 years as the serum elimination half-life estimate for PFOS for its MCL calculation. This estimate is based on a more recent study (Li et al. 2018) study of a Swedish population whose demographic characteristics are more similar to the community population used by DWQI for its selection of a PFOA serum half-life.

NJDEP Should Increase the RSC for PFOS MCL. DWQI chose a default relative source of contribution (RSC) of 20% for its PFOS MCL derivation stating, "there are insufficient data to develop a chemical-specific RSC for PFOS." The available chemical-specific data from PFOS drinking water affected communities provides substantial and compelling evidence that elevated PFOS levels in the drinking water has generally become the primary route of PFOS exposure in the general population. Other states such as Minnesota and New Hampshire have used 50%. NJDEP should use a higher RSC.

NJDEP's Proposed PFOS MCL Can Be Higher and Remain Protective. 3M's assessment of the proposed PFOS MCL identified three factors, each of which alone or in combination should result in a higher, but protective PFOS MCL. The PFOS MCL could be raised to 0.064 µg/L if NJDEP corrects the serious technical error that precluded the use of a BMD model and a $BMDL_{1SD}$ of 3,400 ng/mL derived from the study data by Dong et al. (2009) is used. If NJDEP adopts a serum half-life of 3.4 years the MCL would also increase. Finally, raising the RSC from

20% upwards to 50% would also proportionally increase the PFOS MCL. Table 1 illustrates the impact on the PFOS MCL of changing one or more of these factors.

Comments on the Proposed PFOA MCL

The Uncertainty Factor of 10 for Database Uncertainty is Inappropriate Because It Lacks Scientific Merit. DWQI allocated a database uncertainty factor of 10 to account for "sensitive effects that are not otherwise considered," specifically citing mammary gland development and hepatic toxicity not associated with liver weight. This decision lacks a logical scientific basis and contrary to EPA guidance.

- EPA guidance provides that the uncertainty factor for database uncertainty is intended to account for the potential for deriving an under-protective toxicity value when there is an incomplete characterization of the chemical's toxicity. In contrast, the toxicology database for PFOA is quite comprehensive. The convoluted action taken by DWQI for the allocation of an uncertainty factor of 10 is contrary to EPA guidance
- DWQI attempts to an aura of database uncertainty by focusing on mammary gland development concerns. In fact, DWQI derived a BMDL for PFOA and mammary gland development findings based on the study reported by Macon et al. (2011). It elected, however, not to proceed further for MCL derivation because this endpoint "*has not previously been used as the primary basis for health-based drinking water concentrations or other human health criteria"*. Therefore, it is improper for DWQI to include an uncertainty factor because there are "*more sensitive effects that are not otherwise considered.*" when it had considered mammary gland effects. Furthermore, the effect of PFOA exposure on mammary gland development in laboratory mice have not been consistently described in published literature. Contrary to the DWQI's assertion, it is not a robust endpoint. The study by Macon et al. (2011) used by DWQI had numerous technical deficits which preclude a meaningful interpretation in addition to its biological significance and relevance to human health.
- Accordingly, the UF should be reduced to 3. Changing this parameter would increase the proposed PFOA MCL to 0.042 μg/L.

Increased Liver Weight in Rodents Should Not be Used as a Critical Effect for a PFOA MCL. 3M disagrees with DWQI's use of increased relative liver weight as the basis for its PFOA MCL derivation.

- This decision is inconsistent with USEPA guidelines and published expert opinions on the distinction between liver hypertrophy as a non-adverse adaptive change and other endpoints representing liver toxicity (*vide infra*). If DWQI insists on using liver weight as a sensitive endpoint, DWQI should include additional available studies in mice and rats which capture sensitive life stages (i.e., gestation exposure) or with longer-term exposure duration (i.e., 13-week treatment).
- 3M disagrees also with DWQI's selection from the Loveless et al. (2006) study of only the data for mice that received linear/branched ammonium PFOA treatment for its MCL derivation. Only linear PFOA was detected in the general population in the latest

NHANES 2015-2016 cycle analyses. Branched PFOA was not detected. If DWQI continues to use the Loveless et al. (2006) study as the basis of its PFOA MCL, it should use a subgroup of the mice data that were treated with linear ammonium PFOA. This data results in a $BMDL_{10}$ of increased relative liver weight of 7,973 ng/mL (which is 1.8X higher than the current $BMDL_{10}$ used by DWQI). Using a $BMDL_{10}$ would result in a higher PFOA MCL to 0.026 µg/L by considering this parameter alone.

NJDEP Should Increase the RSC for PFOA. DWQI chose a default relative source of contribution (RSC) of 20% for its PFOA MCL derivation stating, "there are insufficient data to develop a chemical-specific RSC for PFOA." The available chemical-specific data from PFOA drinking water affected communities provides substantial and compelling evidence that elevated PFOA levels in the drinking water has generally become the primary route of PFOA exposure in the general population. Other states such as Minnesota and New Hampshire have used 50%. NJDEP should use a higher RSC.

Epidemiological Associations for Cholesterol and PFOA are Likely Non-causal. Epidemiological Associations for Birth Weight, Kidney Cancer, and Liver Enzyme ALT have been Demonstrated to be the Results of Confounding or Reverse Causation.

- **Cholesterol:** NJDEP asserts that human exposure to PFOA has been associated with increased cholesterol. In experimental studies, which include a phase 1 clinical trial in humans and a transgenic mouse model that mimics human lipoprotein metabolism, PFOA has been shown to cause a decrease in cholesterol at high concentrations. These observations are inconsistent with the observational epidemiologic associations showing higher cholesterol with markedly lower PFOA concentrations. Future research should address non-causal biologic explanations for this low dose response association for which an explanation(s) remains elusive of any foundational mode of action.
- **Birth weight:** NJDEP asserts that human exposure to PFOA has been associated with decreased birth weight. The association with birth weight in humans and maternal serum PFOA measured in the 2nd and 3rd trimesters has been shown to likely be the result of confounding and/or reverse causation due to maternal GFR.
- **Kidney cancer:** NJDEP states that human exposure to PFOA has been associated with kidney cancer. Although factually correct, it is highly misleading and improper to cite EPA's 2006 Science Advisory Board panel's (not unanimous) conclusion that PFOA is "likely" carcinogenic to humans. This long-outdated decision preceded the important studies subsequently published from the C8 Science Panel, 3M, and others which resulted in the "downgrading" of the classification to "suspected" by the EPA Office of Water which is comparable in hazard rating to the IARC "possibly carcinogenic to humans." DWQI should also acknowledge that the Raleigh et al. (2014) study did not show increased incidence of kidney cancer among the PFOA manufacturing workers who had been reported to have the highest serum concentrations of PFOA in occupational settings.
- **Liver enzyme ALT**: NJDEP asserts that human exposure to PFOA has been associated with increased liver enzymes as an indication of liver damage. There is no association between PFOA with liver disease including enlarged liver, fatty liver, or cirrhosis. Small percentage

changes in ALT, a liver enzyme, are reported inconsistently in epidemiologic studies but within normal physiological ranges. This small magnitude of change, if present, does not indicate liver "damage" by any standard clinical medicine of practice. Confounding cannot be ruled out as a possible explanation. Elevated ALT levels have been observed in some laboratory toxicological studies at very high doses.

The Epidemiologic Evidence Is Not Sufficient to Support an Association between PFOA Exposure and Decreased Vaccine Response in Humans. NJDEP asserts that human exposure to PFOA has been associated with decreased vaccine response. Contrary to this assertion considerable inconsistencies have been observed among the 9 epidemiological studies that have examined PFOA exposure to antibody responses to 10 distinct vaccine antigens. Because of these inconsistencies, these studies do not support an association between PFOA exposure and decreased vaccine response in humans.

NJDEP's Proposed PFOA MCL Can Be Higher and Remain Protective. 3M's assessment of the proposed PFOA MCL identified three factors, each of which alone or in combination should result in a higher, but protective PFOA MCL. The PFOA MCL could be raised to 0.026 µg/L based on the $BMDL_{10}$ of 7,973 ng/mL derived from mice treated with linear ammonium PFOA of the study data reported by Loveless et al. (2006). If NJDEP reduces the database uncertain UF of 10 to 3, the PFOA MCL would increase proportionally. Similarly, raising the RSC from 20% upwards to 50% would also proportionally increase the PFOA MCL. Table 2 illustrates the impact on the PFOA MCL of changing one or more of these factors.

Reported herein are 3M's detailed comments regarding each of these key points. In conclusion, 3M believes the current proposed MCLs for PFOS and PFOA were not based on the best-available science.

Table 1:

Possible PFOS MCL scenarios with proposed $BMDL_{1SD}$, Clearance Factor (CL), and/or RSC (proposed parameters highlighted in grey):

Critical Endpoint	MCL Scenarios	NOAEL or $BMDL_{1SD}$	Serum PFOS POD (ng/mL)	Total UF	Target human serum [PFOS] (ng/mL)	RfD = Target human [PFOS] x CL (ng/kg/day)	RSC	Water concentration (µg/L)
↓ Plaque forming cell response with PFOS (Dong et al. 2009)	**Current DWQI MCL**	**NOAEL**	**674**	**30**	$= \frac{674\ ng/mL}{30}$ **=22.47 ng/mL**	**=22.47 ng/mL x 8.1 x 10^{-5} L/kg/day = 1.8 ng/kg/day**	**0.2**	$= \frac{1.8\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.2}{2\ L/day}$ **= 13 ng/L = 0.013 µg/L**
	Possible Scenario #1:	NOAEL	674	30	$= \frac{674\ ng/mL}{30}$ =22.47 ng/mL	=22.47 ng/mL x 8.1 x 10^{-5} L/kg/day = 1.8 ng/kg/day	0.35	$= \frac{1.8\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.35}{2\ L/day}$ = 22 ng/L = 0.022 µg/L
	Possible Scenario #2:	NOAEL	674	30	$= \frac{674\ ng/mL}{30}$ =22.47 ng/mL	=22.47 ng/mL x 1.28 x 10^{-4} L/kg/day = 2.9 ng/kg/day	0.2	$= \frac{2.9\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.2}{2\ L/day}$ = 20 ng/L = 0.020 µg/L
	Possible Scenario #3:	NOAEL	674	30	$= \frac{674\ ng/mL}{30}$ =22.47 ng/mL	=22.47 ng/mL x 1.28 x 10^{-4} L/kg/day = 2.9 ng/kg/day	0.35	$= \frac{2.9\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.35}{2\ L/day}$ = 36 ng/L = 0.036 µg/L
	Possible Scenario #4:	NOAEL	674	30	$= \frac{674\ ng/mL}{30}$ =22.47 ng/mL	=22.47 ng/mL x 8.1 x 10^{-5} L/kg/day = 1.8 ng/kg/day	0.5	$= \frac{1.8\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.5}{2\ L/day}$ = 32 ng/L = 0.032 µg/L
	Possible Scenario #5:	NOAEL	674	30	$= \frac{674\ ng/mL}{30}$ =22.47 ng/mL	=22.47 ng/mL x 1.28 x 10^{-4} L/kg/day = 2.9 ng/kg/day	0.5	$= \frac{2.9\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.5}{2\ L/day}$ = 51 ng/L = 0.051 µg/L
	Possible Scenario #6:	NOAEL	674	30	$= \frac{674\ ng/mL}{30}$ =22.47 ng/mL	=22.47 ng/mL x 8.1 x 10^{-5} L/kg/day = 1.8 ng/kg/day	0.8	$= \frac{1.8\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.8}{2\ L/day}$ = 50 ng/L = 0.050 µg/L
	Possible Scenario #7:	NOAEL	674	30	$= \frac{674\ ng/mL}{30}$ =22.47 ng/mL	=22.47 ng/mL x 1.28 x 10^{-4} L/kg/day = 2.9 ng/kg/day	0.8	$= \frac{2.9\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.8}{2\ L/day}$ = 81 ng/L = 0.081 µg/L

Critical Endpoint	**MCL Scenarios**	**NOAEL or $BMDL_{1SD}$**	**Serum PFOS POD (ng/mL)**	**Total UF**	**Target human serum [PFOS] (ng/mL)**	**RfD = Target human [PFOS] x CL (ng/kg/day)**	**RSC**	**Water concentration (µg/L)**
↓ Plaque forming cell response with PFOS (Dong et al. 2009)	Possible Scenario #8:	$BMDL_{1SD}$	3,400	30	$= \frac{3400\ ng/mL}{30}$ =113.3 ng/mL	=113.33 ng/mL x 8.1 x 10^{-5} L/kg/day = 9.2 ng/kg/day	0.2	$= \frac{9.2\ (ng/kg/day) \times 70\ kg \times 0.2}{2\ L/day}$ = 64 ng/L = 0.064 µg/L
	Possible Scenario #9:	$BMDL_{1SD}$	3,400	30	$= \frac{3400\ ng/mL}{30}$ =113.3 ng/mL	=113.33 ng/mL x 8.1 x 10^{-5} L/kg/day = 9.2 ng/kg/day	0.35	$= \frac{9.2\ (ng/kg/day) \times 70\ kg \times 0.35}{2\ L/day}$ = 113 ng/L = 0.113 µg/L
	Possible Scenario #10:	$BMDL_{1SD}$	3,400	30	$= \frac{3400\ ng/mL}{30}$ =113.3 ng/mL	=113.33 ng/mL x 1.28 x 10^{-4} L/kg/day = 14.5 ng/kg/day	0.2	$= \frac{14.5\ (ng/kg/day) \times 70\ kg \times 0.2}{2\ L/day}$ = 102 ng/L = 0.102 µg/L
	Possible Scenario #11:	$BMDL_{1SD}$	3,400	30	$= \frac{3400\ ng/mL}{30}$ =113.3 ng/mL	=113.33 ng/mL x 1.28 x 10^{-4} L/kg/day = 14.5 ng/kg/day	0.35	$= \frac{14.5\ (ng/kg/day) \times 70\ kg \times 0.35}{2\ L/day}$ = 178 ng/L = 0.178 µg/L
	Possible Scenario #12:	$BMDL_{1SD}$	3,400	30	$= \frac{3400\ ng/mL}{30}$ =113.3 ng/mL	=113.33 ng/mL x 8.1 x 10^{-5} L/kg/day = 9.2 ng/kg/day	0.5	$= \frac{9.2\ (ng/kg/day) \times 70\ kg \times 0.5}{2\ L/day}$ = 161 ng/L = 0.161 µg/L
	Possible Scenario #13:	$BMDL_{1SD}$	3,400	30	$= \frac{3400\ ng/mL}{30}$ =113.3 ng/mL	=113.33 ng/mL x 1.28 x 10^{-4} L/kg/day = 14.5 ng/kg/day	0.5	$= \frac{14.5\ (ng/kg/day) \times 70\ kg \times 0.5}{2\ L/day}$ = 254 ng/L = 0.254 µg/L
	Possible Scenario #14:	$BMDL_{1SD}$	3,400	30	$= \frac{3400\ ng/mL}{30}$ =113.3 ng/mL	=113.33 ng/mL x 8.1 x 10^{-5} L/kg/day = 9.2 ng/kg/day	0.8	$= \frac{9.2\ (ng/kg/day) \times 70\ kg \times 0.8}{2\ L/day}$ = 258 ng/L = 0.258 µg/L
	Possible Scenario #15:	$BMDL_{1SD}$	3,400	30	$= \frac{3400\ ng/mL}{30}$ =113.3 ng/mL	=113.33 ng/mL x 1.28 x 10^{-4} L/kg/day = 14.5 ng/kg/day	0.8	$= \frac{14.5\ (ng/kg/day) \times 70\ kg \times 0.8}{2\ L/day}$ = 406 ng/L = 0.406 µg/L

Table 2:

Possible PFOA MCL scenarios with proposed UF, $BMDL_{10}$, an/or RSC (proposed parameters highlighted in grey):

Critical Endpoint	MCL Scenarios	BMR	Serum PFOA $BMDL_{10}$ (ng/mL)	Total UF	Target human serum [PFOA] ng/mL	RfD = Target human [PFOA] x CL, (ng/kg/day)	RSC	Water concentration (µg/L)
↑ RLW in mice treated with **linear/branched** PFOA (Loveless et al. 2006)	**Current DWQI MCL**	**10% ↑RLW**	**4,351**	**300**	**$= \frac{4351\ ng/mL}{300}$ =14.5 ng/mL**	**=14.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 2 ng/kg/day**	**0.2**	**$= \frac{2\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.2}{2\ L/day}$ = 14 ng/L = 0.014 µg/L**
	Possible Scenario #1:	10% ↑RLW	4,351	100	$= \frac{4351\ ng/mL}{100}$ =43.5 ng/mL	=43.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 6 ng/kg/day	0.2	$= \frac{6\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.2}{2\ L/day}$ = 42 ng/L = 0.042 µg/L
	Possible Scenario #2:	10% ↑RLW	4,351	300	$= \frac{4351\ ng/mL}{300}$ =14.5 ng/mL	=14.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 2 ng/kg/day	0.35	$= \frac{2\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.35}{2\ L/day}$ = 25 ng/L = 0.025 µg/L
	Possible Scenario #3:	10% ↑RLW	4,351	100	$= \frac{4351\ ng/mL}{100}$ =43.5 ng/mL	=43.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 6 ng/kg/day	0.35	$= \frac{6\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.35}{2\ L/day}$ = 74 ng/L = 0.074 µg/L
	Possible Scenario #4:	10% ↑RLW	4,351	300	$= \frac{4351\ ng/mL}{300}$ =14.5 ng/mL	=14.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 2 ng/kg/day	0.5	$= \frac{2\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.5}{2\ L/day}$ = 35 ng/L = 0.035 µg/L
	Possible Scenario #5:	10% ↑RLW	4,351	100	$= \frac{4351\ ng/mL}{100}$ =43.5 ng/mL	=43.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 6 ng/kg/day	0.5	$= \frac{6\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.5}{2\ L/day}$ = 105 ng/L = 0.105 µg/L
	Possible Scenario #6:	10% ↑RLW	4,351	300	$= \frac{4351\ ng/mL}{300}$ =14.5 ng/mL	=14.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 2 ng/kg/day	0.8	$= \frac{2\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.8}{2\ L/day}$ = 56 ng/L = 0.056 µg/L
	Possible Scenario #7:	10% ↑RLW	4,351	100	$= \frac{4351\ ng/mL}{100}$ =43.5 ng/mL	=43.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 6 ng/kg/day	0.8	$= \frac{6\ (ng/kg/day)\ x\ 70\ kg\ x\ 0.8}{2\ L/day}$ = 168 ng/L = 0.168 µg/L

Critical Endpoint	**MCL Scenarios**	**BMR**	**Serum PFOA $BMDL_{10}$ (ng/mL)**	**Total UF**	**Target human serum [PFOA] ng/mL**	**RfD = Target human [PFOA] x CL, (ng/kg/day)**	**RSC**	**Water concentration (µg/L)**
↑ RLW in mice treated with **linear** PFOA (Loveless et al. 2006)	Possible Scenario #8:	10% ↑RLW	7,973	300	$= \frac{7973\ \text{ng/mL}}{300}$ =26.5 ng/mL	=26.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 3.7 ng/kg/day	0.2	$= \frac{3.7\ (\text{ng/kg/day}) \times 70\ \text{kg} \times 0.2}{2\ \text{L/day}}$ = 26 ng/L = 0.026 µg/L
	Possible Scenario #9:	10% ↑RLW	7,973	100	$= \frac{7973\ \text{ng/mL}}{100}$ =79.7 ng/mL	=79.7 ng/mL x 1.4 x 10^{-4} L/kg/day = 11.2 ng/kg/day	0.2	$= \frac{11.2\ (\text{ng/kg/day}) \times 70\ \text{kg} \times 0.2}{2\ \text{L/day}}$ = 78 ng/L = 0.078 µg/L
	Possible Scenario #10:	10% ↑RLW	7,973	300	$= \frac{7973\ \text{ng/mL}}{300}$ =26.5 ng/mL	=26.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 3.7 ng/kg/day	0.35	$= \frac{3.7\ (\text{ng/kg/day}) \times 70\ \text{kg} \times 0.35}{2\ \text{L/day}}$ = 45 ng/L = 0.045 µg/L
	Possible Scenario #11:	10% ↑RLW	7,973	100	$= \frac{7973\ \text{ng/mL}}{100}$ =79.7 ng/mL	=79.7 ng/mL x 1.4 x 10^{-4} L/kg/day = 11.2 ng/kg/day	0.35	$= \frac{11.2\ (\text{ng/kg/day}) \times 70\ \text{kg} \times 0.35}{2\ \text{L/day}}$ = 137 ng/L = 0.137 µg/L
	Possible Scenario #12:	10% ↑RLW	7,973	300	$= \frac{7973\ \text{ng/mL}}{300}$ =26.5 ng/mL	=26.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 3.7 ng/kg/day	0.5	$= \frac{3.7\ (\text{ng/kg/day}) \times 70\ \text{kg} \times 0.5}{2\ \text{L/day}}$ = 65 ng/L = 0.065 µg/L
	Possible Scenario #13:	10% ↑RLW	7,973	100	$= \frac{7973\ \text{ng/mL}}{100}$ =79.7 ng/mL	=79.7 ng/mL x 1.4 x 10^{-4} L/kg/day = 11.2 ng/kg/day	0.5	$= \frac{11.2\ (\text{ng/kg/day}) \times 70\ \text{kg} \times 0.5}{2\ \text{L/day}}$ = 196 ng/L = 0.196 µg/L
	Possible Scenario #14:	10% ↑RLW	7,973	300	$= \frac{7973\ \text{ng/mL}}{300}$ =26.5 ng/mL	=26.5 ng/mL x 1.4 x 10^{-4} L/kg/day = 3.7 ng/kg/day	0.8	$= \frac{3.7\ (\text{ng/kg/day}) \times 70\ \text{kg} \times 0.8}{2\ \text{L/day}}$ = 104 ng/L = 0.104 µg/L
	Possible Scenario #15:	10% ↑RLW	7,973	100	$= \frac{7973\ \text{ng/mL}}{100}$ =79.7 ng/mL	=79.7 ng/mL x 1.4 x 10^{-4} L/kg/day = 11.2 ng/kg/day	0.8	$= \frac{11.2\ (\text{ng/kg/day}) \times 70\ \text{kg} \times 0.8}{2\ \text{L/day}}$ = 314 ng/L = 0.314 µg/L

3M's DETAILED COMMENTS ON THE PROPOSED PFOS MCL

A. There is a Serious Technical Error with DWQI's BMD Modeling for PFOS with Dong et al. (2009) data

The DWQI states that "The first step in dose-response analysis is identification of a Point of Departure (POD), which is the dose within or close to the dose range used in the study from which extrapolation begins." DWQI also recognized that "if a Benchmark Dose can be developed, it is **preferred** for use as the POD." Additionally, DWQI recognized that "Benchmark dose modeling is identified by the USEPA as **the preferred** approach for dose-response modeling when the available data are sufficient to support it."

DWQI reported that it was unsuccessful in its attempts to compute a BMD or BMDL based on the PFOS-included plaque forming cell response (PFCR) reported by Dong et al. (2009). As a result, it subsequently used the serum NOAEL of 674 ng/mL from the study as the POD for its MCL derivation.

3M's review of DWQI's BMD modeling discovered a major technical error in DWQI's BMD modeling (see details below). If corrected, an acceptable serum PFOS BMDL can be derived; specifically, a $BMDL_{1SD}$ of 3,400 ng/mL.

As NJDEP has recognized, a BMD and/or BMDL is the recommended and "preferred" approach for deriving a POD value. Accordingly, NJDEP should adopt the serum $BMDL_{1SD}$ and revise its POD value for PFOS. Because the serum $BMDL_{1SD}$ (3,400 ng/mL) is five times higher than the serum NOAEL (674 ng/mL), the PFOS MCL should be raised by a factor of five to 0.065 µg/L (0.013 µg/L x 5 = 0.065 µg/L).

1) <u>DWQI erroneously used standard error and not the required standard deviation in its BMD modeling.</u>

   Doses, number of animals, mean responses, and standard deviation are required to model summarized continuous response data using USEPA's Benchmark Dose Software (BMDS). According to DWQI's BMD modeling results for Dong et. al. (2009) PFCR data (*cf*. pages 236, 891 – 972, Appendix A - Health-Based Maximum Contaminant Level Support Document Perfluorooctane Sulfonate (PFOS)), the values in the standard deviation column are instead the standard error of mean values (SEM) provided by the study authors. This was a major modeling mistake by the DWQI. DWQI should have converted standard error to standard deviation by multiplying the standard error values by $\sqrt{N}$ ($\sqrt{10} \approx 3.16$). Therefore, its conclusion that the BMD modeling of the Dong et al. (2009) data did not give an acceptable fit to the data was based on faulty information.

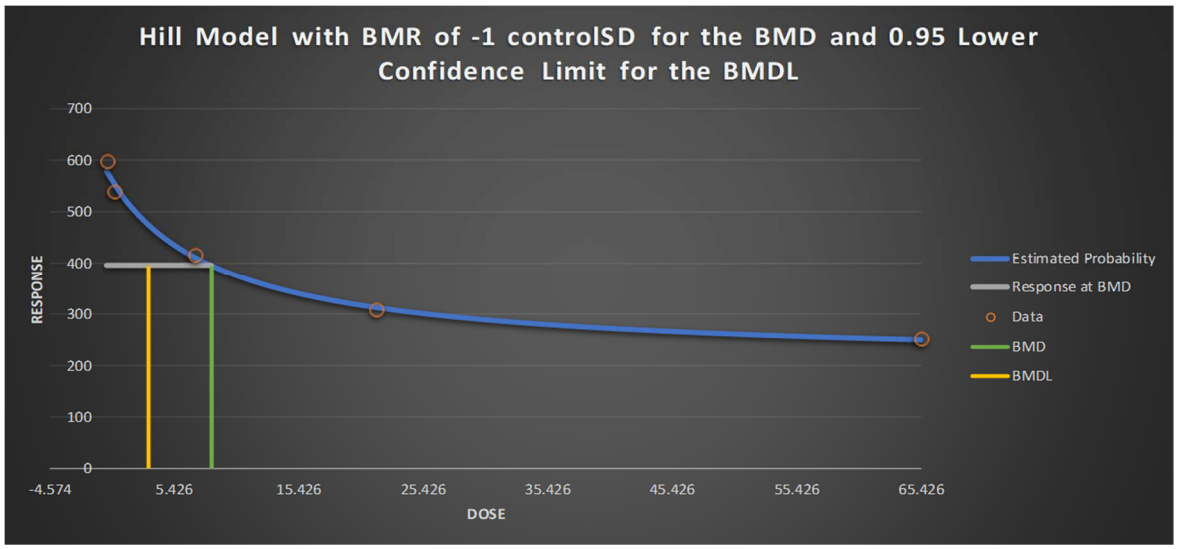
2) <u>$BMDL_{1SD}$ 3,400 ng/mL should be the POD for Dong et al. (2009) PFCR data</u>

   The "correct" standard deviation can be derived by taking SEM x $\sqrt{10}$. With this corrected value, the dataset from Dong et. al. (2009) was modeled using USEPA Benchmark Dose Software (BMDS) version 3.1., a lowest $BMDL_{1SD}$ (3,400 ng/mL serum PFOS) and lowest AIC and was deemed to be the "best" fit for the dataset. Specifically, the serum PFOS concentration vs. PFCR response dataset (minus the high dose group) was modeled using Exponential, Hill, Linear, Polynomial, and Power models, both with and without parameter

restrictions. All models were run using 3 user-defined options sets which assumed 1.) responses are normally distributed and variance is constant across dose groups; 2.) responses are log-normally distributed and variance is constant across dose groups; and 3.) responses are normally distributed and variance is non-constant (i.e. varies as a power function of the mean response. For all model runs, the benchmark response (BMR) was set to one control standard deviation and a BMDL equal to the 95% lower confidence limit on the BMD was calculated. Model viability was assessed on the basis of goodness-of-fit P-value, AIC, and visual inspection of graphs in accordance with BMDS technical guidance. The restricted Hill model assuming normally-distributed responses and non-constant variance had the lowest BMDL (3,400 ng/L serum PFOS) and lowest AIC and was deemed to be the "best" fit for the dataset (see Table 3).

Table 3: Benchmark Dose analysis (V3.1) for a 1 control standard deviation change in plaque forming cell response from PFOS administration in mice (Dong et al. 2009) – excluding highest dose group

Model	Serum PFOS (µg/mL)			Test 4 P-Value	AIC	BMDS Recommendation	
	BMD	BMDL	BMDU			Viable?	Notes
Exponential 4 (NCV)	10.03	5.10	24.02	0.74	626.74	Viable - Alternate	
Exponential 5 (NCV)	9.98	5.09	24.02	0.74	626.74	Viable - Alternate	
Hill (NCV)	***8.43***	***3.40***	***25.59***	***0.78***	***626.65***	***Viable - Recommended***	***Lowest AIC***



3) DWQI's rationale for concluding that the Dong et al. (2009) PFCR data is not amenable to benchmark dose modeling was incorrect.

DWQI performed benchmark dose modeling after excluding the high dose group which yielded 4 models with acceptable fits to the dataset:

- Restricted Hill Model, constant variance
- Restricted Hill Model, non-constant variance

- Unrestricted Hill Model, constant variance
- Restricted Hill Model, non-constant variance

The models that assumed constant variance were rejected because the constant variance test failed (Test 2 P-value was < 0.05), and we agree that the BMDLs calculated for these models should be used with caution. However, the version of BMDS that DWQI used (ver. 2.6.0.1) was unable to calculate BMDLs for non-constant variance Hill models. This software-based limitation has since been resolved in the more recent release of BMDS version 3.1. In fact, when we repeated DWQI's analysis (dropping the top dose and incorrectly entering standard error into the standard deviation column) using the most up-to-date version of the software, there were 3 viable models with calculated BMDLs obtained under the assumption of non-constant variance: Restricted Exponential 4, Restricted Exponential 5, and Restricted Hill.

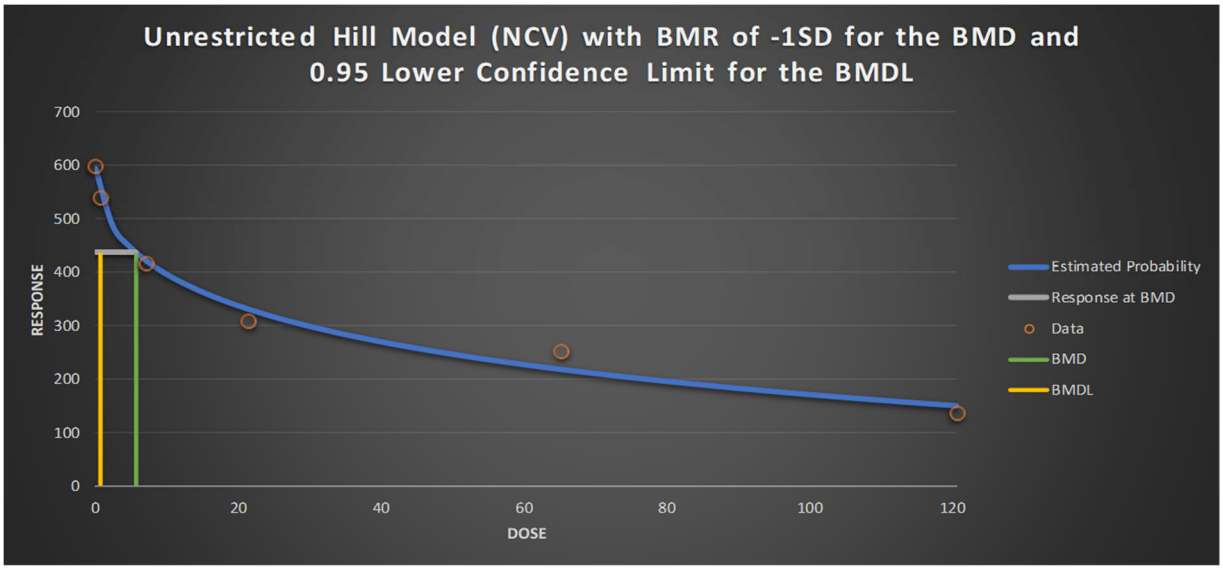
4) It should be noted that even if the highest dose group is included in the BMD modeling with the more recent release of BMDS version 3.1, there are no viable models that can be attained with the full dataset.
The complete dataset would yield 3 potential models for BMDL consideration (Table 4):

- Unrestricted Hill Model, non-constant variance
- Unrestricted Polynomial, Degree 4 Model, non-constant variance
- Unrestricted Polynomial Degree 3 Model, non-constant variance

Table 4: Benchmark Dose analysis for a 1 control standard deviation change in plaque forming cell response from PFOS in mice (Dong et al. 2009) – all dataset

Model	Restriction	Serum PFOS (μg/mL)			Test 4 P-Value	AIC	BMDS Recommendation	
		BMD	BMDL	BMDU			Viable?	Notes
Hill (NCV)	Unrestricted	5.6892	0.8301	22.0466	0.3025	736.7911	Viable - Recommended	Lowest BMDL WARNING: BMD/BMDL ratio > 5
Polynomial Degree 4 (NCV)	Unrestricted	11.9140	3.7914	13.3917	0.1881	738.8790	Viable - Alternate	Note: multiphasic curves
Polynomial Degree 3 (NCV)	Unrestricted	11.2946	7.8669	18.5970	0.4703	736.6554	Viable - Alternate	Note: multiphasic curves

However, in the unrestricted Hill Model, the ratio between BMD:BMDL > 5 reflects large uncertainty associated with the "true" shape of the dose-response curve in the low-dose region and caution should be used when selecting BMDLs from such models (Haber et. al., 2018).



The other 2 viable models (Poly 4 and Poly 3) have multiphasic curves with multiple inflection points which indicated non-monotonicity.

Taken together, these results suggest that all 3 unrestricted models should be excluded from consideration with BMDL selection which would mean no viable models were attained with the full dataset.

B. Evidence of Immune Suppression Was Not Supported by Dong et al. (2009) Data

There is insufficient evidence to support immunotoxicity with PFOS. Although NTP (2016) conducted a systemic review in 2016 and concluded that PFOS is presumed to be immune hazards to humans in connection with vaccine antibody response, there were several areas of the NTP systematic review where insufficient animal data were used as supporting evidence for human findings and its final hazard conclusion. In particular, suppression of the T cell-dependent antibody response (TDAR) in mice, which evaluates suppression of the "primary" IgM response, is used to support suppression of antibody titers to vaccinations in humans. However, because vaccine antibody titers reflect the secondary IgG response, the observation in human epidemiological data was in great discrepancy with animal data in that no suppression of the secondary IgG response was observed in mice. Similarly, there were incongruences between humans and animal data to support the final hazard conclusions reached by the NTP in the areas of infection disease resistance and NK cell activity for PFOS. For example, among the immunotoxicity data that had been reported for PFOS, inconsistent and inconclusive findings are being reported. When Peden-Adams et al. (2008) reported the immune suppression with PFOS at such low serum level (~91 ng/mL) in mice, Qazi et al. (2009a; 2010; 2009b) carefully designed and performed a series of studies trying to see if the results reported by Peden-Adams et al. could be replicated. They were not able to replicate the results. The weight of evidence would suggest that immunotoxicity responses are not rigorous nor robust to support risk characterization.

Based on immunosuppression effects observed in mice from a study by Dong et al. (2009), NJDEP (via its Health Effects Subcommittee within DWQI) had developed an MCL for PFOS in drinking water at 0.013 µg/L. The study by Dong et al. (2009) reported the reduction of plaque-forming cell response (PFCR) in male adult C57BL/6 mice as indication of immune response inhibition to a foreign antigen after 60 days of repeated PFOS oral administration.

The total administered doses (TAD) achieved in this study were 0, 0.5, 5, 25, and 125 mg/kg in the mice. The study authors concluded that several immune parameters had been altered due to PFOS treatment; in particular, PFOS treatment caused a dose-dependent decrease in IgM PFCR in splenic cells and the LOAEL for splenic IgM PFC response was determined to be at 5 mg/kg TAD. The NOAEL for IgM PFC response was therefore inferred to be at the next lower dose (0.5 mg/kg TAD). At that dose, the study authors reported a serum PFOS concentration of 674 ng/mL at the end of 60-day dosing period.

3M respectfully disagrees that Dong et al. (2009) study present the most sensitive data in animals when exposed to PFOS. From a fundamental immunology perspective, there were several important technical aspects that Dong et al. (2009) failed to address. The study also lacked overall scientific validity to support the conclusion that PFOS causes immune suppression. Specifically:

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), based on simple ANOVA and Dunnett's t tests, there appeared to be a difference in mean body weight change between the control group (mean body weight = 3.10 ± 0.13 g) and the NOAEL dose group at 0.5 mg/kg/day (mean body weight = 2.58 ± 0.15 g). With 1-sided test, the final body weights in the 0.5 mg/kg/day dose group were significantly lower than the control group at $\alpha = 0.10$ ($0.05 < p < 0.10$). With 2-sided test, it was statistically significantly different at $\alpha = 0.20$ ($0.15 < p < 0.20$). Therefore, Dong et al. (2009) data may have been confounded by decreased body weight effect which hindered the overall interpretation.

2) The standard clinical marker for antibody titers to vaccinations are secondary IgG antibody isotype, not primary IgM. Dong et al. reported the PFOS dose-dependent reductions in sheep red blood cell (SRBC)-induced IgM plaque forming cell assay *in vitro*; they did not evaluate IgG or other potential antibody responses that can develop, including IgG or IgE. In addition, the use of the SRBC-induced antibody response to measure antigen-induced antibody response is very crude and non-specific to T cell activation. There are better T-cell dependent antigens available for use in the immunology research (i.e., ovalbumin) and Dong et al. did not acknowledge such fact.

3) Furthermore, the study by Dong et al. (2009) did not take the time-based progression of IgM → IgG antibody class switching into consideration. The normal progression of antibody development involves the IgM production by B cells first as primary immune response. The B cells will subsequently proliferate and become activated when further

challenged by antigen, which, ultimately leads to antibody class switching to produce IgG, which is the clinical measurement for the assessment of antibody titer.

4) It is also 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 (SRBC) was challenged only once in the study.

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. The authors should have also looked at thymus and serum for IgM levels to illustrate that the responses are consistent in other primary immune organs. By way of similar scientific rationale, Dong et al. should have looked at IgG in addition to IgM, as well as evaluated IgG levels in thymus and serum.

6) 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. Similarly, while NK cell activity was reported for the spleen, it was not done for the thymus. These were major technical omissions.

7) With regards to NK cell activity, the LDH assay used by Dong et al. is not a typical assay used to assess NK cell activity. The LDH measurement is associated with cell membrane integrity and it is a non-specific assay and the LDH values reported by Dong et al. should not be used *in lieu* of NK cell activity data. The standard method for NK cell activity is flow cytometry, which Dong et al. did not perform and therefore the conclusions that NK cell activity is changed cannot be reliably drawn from this study.

8) 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, two major technical flaws associated with the study design limit a scientific support for this conclusion:

   - Dong et al. reported Concanavalin A (ConA)-mediated responses as antigen specific T cell receptor-based proliferation *in vitro.* However, ConA stimulates T cells via a different set of pathways than through the T cell receptor. The more appropriate method would have been using anti-CCD3/CD28 antibodies to mimic antigen specific cell stimulation *in vitro*.

   - The second concern is the use of the MTT assay to determine T cell proliferation *in vitro*. The MTT assay determines metabolic activity, not cell numbers. It is simply an indicator of cells' mitochondrial respiration state and is not a reflection any proliferative response(s). The standard assay for cell proliferation would be BrDU assay or PCNA staining, neither of which was used by Dong et al. and the readers were misinformed.

9) It was perplexing as to why Dong et al. did not look at / report blood lymphocyte counts, which is part of the standard CBC panel parameters.

10) It was unclear why Dong et al. did not provide any histological evidence for thymus, spleen, or bone marrow.

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

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. As discussed in detail above, Dong et al. (2009) misinformed the readers in their data presentation with incomplete antibody isotyping and partial assessments in some, but not all, primary immune organs. Using a crude (non-specific) antigen SRBC, they only challenged the mice once without any follow up for a second challenge to elicit permanent antibody response (to antigens and/or vaccines). They did not use the correct methods to evaluate cell proliferation and NK cell activity responses and improperly reported their data.

C. The Epidemiologic Evidence is Inconsistent and Does Not Support an Association between PFOS Exposure and Decreased Vaccine Response in Humans

The MCL recommendation for PFOS was based on a decreased plaque forming cell response in adult mice (Dong et al., 2009). The DWQI argues that this effect is supported by epidemiological evidence for an "analogous effect" of decreased vaccine response in humans. DWQI's review of the epidemiology literature is outdated and fails to accurately reflect the inconsistencies and mostly null findings across studies. DWQI acknowledges only 5 epidemiology studies, however, there are 9 published studies that have examined PFOS exposure and antibody responses to vaccines in children, adolescents and adults (Grandjean et al. 2012; Grandjean et al. 2017; Granum et al. 2013; Kielsen et al. 2016; Looker et al., 2014; Mogensen et al. 2015, Stein et al., 2016a; Stein 2016b; Zeng et al., 2019). These studies have measured antibody responses to 10 distinct vaccines: tetanus, diphtheria, rubella, measles, mumps, influenza A (H1N1), influenza A (H3N2), influenza B, enterovirus and coxsackievirus. While tetanus and diphtheria are the most commonly studied vaccine types, other vaccines have only been reported in 1 or 2 studies as illustrated below (Table 5):

Table 5

Vaccine type	Number of studies	Reference(s)
tetanus	5	Grandjean et al. 2012; Grandjean et al. 2017; Granum et al. 2013; Kielsen et al. 2016; Morgenson et al. 2015
diphtheria	4	Grandjean et al. 2012; Grandjean et al. 2017; Kielsen et al. 2016; Morgenson et al. 2015
rubella	2	Granum et al. 2013; Stein et al., 2016a
measles	2	Granum et al. 2013; Stein et al., 2016a
influenza A (H1N1)	2	Looker et al., 2014; Stein et al., 2016b
influenza B	2	Granum et al. 2013; Looker et al., 2014
influenza A (H1N2)	1	Looker et al., 2014
mumps	1	Stein et al., 2016a

enterovirus (EV71)	1	Zeng et al., 2019
coxsackievirus (CA16)	1	Zeng et al., 2019

Antibody responses to these distinct vaccine types should not be interpreted as a single health outcome (i.e. decreased vaccine response). Rather, antibody responses to each vaccine type should be considered separately as 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, conjugate or live attenuated depending on the strain and method of administration. 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), 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 type should not be interpreted as consistent with changes in the antibody response to another vaccine.

Moreover, the existing epidemiologic studies do not provide consistent evidence of a significant association between PFOS exposure and decreased vaccine responses. Contrary to DWQIs assertion that "*study findings are consistent and support a potential for PFOS to reduce vaccine response*" (DWQI 2018), mostly null findings have been reported across all studies and the results are inconsistent by vaccine type. For example, among the 5 existing studies that have examined antibody responses to the tetanus vaccine (the most commonly studied vaccine type) relative to serum PFOS levels, only one study reported a significant decrease in antibody levels (Grandjean et al. 2012) (Table 6). 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). More specifically, of the 11 statistical measures of association reported across these 5 studies, only 1 was significant (Grandjean et al. 2012). Clearly, the evidence from epidemiology studies examining tetanus vaccine response does not support a potential for PFOS to reduce this toxoid response as DWQI infers.

Table 6. Summary of epidemiology studies examining the association between serum PFOS levels and tetanus vaccine response

Reference	Study Population	PFOS levels (ng/mL)	Antibody levels (IU/mL)	Main findings
Grandjean et al., 2012	587 children/ Faroe Islands	27.3 (maternal) 16.7 (age 5)	0.22 (0.10-0.51) (age 5, pre-booster) 35.0 (16-96) (age 5, post-booster) 1.6 (0.65-4.60) (age 7)	Percent change in antibody concentration Maternal PFOS pre-booster, age 5: -10.1 (-31.9 to18.7) post-booster, age 5: -2.3 (-28.6 to 33.6) age 7: 35.3 (-3.9 to 90.6) PFOS at age 5 pre-booster, age 5: -11.9 (-31.9 to 18.7) post-booster, age 5: **-28.5 (-45.5 to -6.1)** age 7: 35.3 (-3.9 to 90.6)
Granum et al., 2013	56 children/ Norway	5.6 (maternal)	0.20 (age 3)	β (95% CI) -0.002 (-0.03, 0.02), p= 0.87
Mogensen et al., 2015*	459 children/ Faroe Islands	17.3 (age 5) 15.5 (age 7)	1.8 (0.6-1.6) (age 7)	Percent change in antibody concentration age 7: -9.1 (-32.8 to 23.0)
Kielsen et al., 2016	12 adults/ Denmark	9.5 (pre-vaccination)	4.0 (pre-vaccination)	Percent change in antibody concentration from day 4 to day 10 post-vaccination: -3.59 (5.51 to -11.91), p= 0.42
Grandjean et al., 2017	587 children/ Faroe Islands	15.3 (age 7) 6.7 (age 13)	Not reported	Percent change in antibody concentration age 7: 30.0 (-16.1 to 101.4), p=0.24 age 13: 22.2 (-12.4 to 70.3), p=0.24

* Same study population as Grandjean et al., 2012

It is also important to emphasize that small changes in antibody response, as observed in some studies, do not necessarily translate to an increased risk of infectious disease. Several epidemiologic studies (Dalsager et al. 2016; Fei et al. 2010; Impinen et al., 2018; Looker et al. 2014; Okada et al. 2012; Goudarzi et al., 2017; Granum et al. 2013) have examined PFOS levels and infectious disease outcomes (i.e., occurrence of common colds and otitis media, symptoms of infections, mortality from infectious and parasitic diseases and hospitalizations from infectious diseases). Across all reported measures, mostly inconsistent associations between PFOS levels and increased risk of infectious disease outcomes have been observed (Table 7).

Table 7. Summary of epidemiology studies examining the association between serum PFOS levels and infectious disease outcomes.

Reference	Study population	Study design	PFOS measure	Outcome
Dalsager et al., 2016	359 Odense children (age 1-4 years)	prospective	maternal	Symptoms of infection - fever: ↑ - cough: NS - nasal discharge: NS - diarrhea: NS - vomiting: NS
Fei et al., 2010	577 Danish children (average age = 8.2 years)	cross-sectional	maternal	Incidence of hospitalization for infectious diseases - all children: NS - age 0 - <1 years: NS - age 1 - <2 years: NS - age 2 - <4 years: NS - age ≥ 4 years: NS - girls: ↑ - boys: NS
Goudarzi et al., 2017	1558 Japanese children (0-4 years)	Prospective	maternal	Symptoms of all infectious diseases* - all children: ↑ trend - girls: ↑ trend - boys: NS trend
Granum et al., 2013	99 Norwegian children (age 0-3 years)	prospective	maternal	Symptoms of infection - common cold episodes: NS - common cold (y/n): NS - gastroenteritis episodes: NS - gastroenteritis (y/n): NS
Impinen et al., 2018	641 Norwegian children (age 0-10 years)	prospective	cord blood	Symptoms of infection - common cold episodes from 0-2 years of age: ↑ - lower respiratory tract infection episodes 0-10 years of age: NS
Looker et al., 2014	411 U.S. adults with drinking water exposure	cross-sectional	adult serum	Symptoms of infection - any "flu" infection in last 12 months: NS - any cold in last 12 months: NS - cold or flu in last 12 months: NS
Okada et al., 2012	343 Japanese infants (0-18 months)	prospective	maternal serum	Otitis media during the first 18 months of life - all infants: NS - males: NS - females: NS (< 5% reported chicken pox, bronchitis, RSV disease, rhinitis, pneumonia, skin infections, rotavirus, adenovirus and cytomegalovirus and were not included in the analyses)

* Infectious diseases included at least one case of self-reported otitis media, pneumonia, RS virus and varicella.

Note: ↑ = significant increase ($p < 0.05$); NS = not statistically significant

Relevant to this point, in the US, the annual number of cases of reported tetanus and diphtheria have not changed (where tetanus is a non-contagious disease, unlike the contagious diphtheria which can influenced by herd-immunity) (TABLE 8). From 2001-2015, 431 tetanus cases have been reported, but these cases occurred almost exclusively in unvaccinated or incomplete-scheduled vaccinated individuals (CDC 2018). The number of annual cases of diphtheria have been nearly 0 every year during this time frame while PFOS concentrations in the general population have continuously declined. During 1996-2016, a total of 15 diphtheria cases were reported in the entire United States with no cases of diphtheria that were reported in the state of New Jersey during this same period (NJDEH, 2019, see https://www.state.nj.us/health/cd/documents/chapters/diphtheria_ch.pdf).

These data show that the immunosuppression concerns expressed by Grandjean et al. (2012) are not well founded:

> *"If the associations are causal, the clinical importance of our findings is therefore that PFC exposure may increase a child's risk for not being protected against diphtheria and tetanus, despite a full schedule of vaccinations. Adequate formation of specific antibodies relies on several important immune functions, and serum antibody concentrations triggered by standardized antigen stimulations may therefore reflect the more general efficacy of the immune system in relation to infection. For this reason, PFC-associated decreases in antibody concentrations may indicate the potential existence of immune system deficits beyond the protection against the 2 specific bacteria examined in this study."*

Table 8 clearly illustrates that, relative to continuously declining PFOS concentrations in the general population, these data do not suggest a population whose immunity to tetanus or diphtheria might have been compromised by a decreased antibody response due to PFOS exposure. These data do not suggest there is an increased risk of infection to tetanus or diphtheria in a high-vaccinated population such as in the United States. Therefore, it is highly speculative to suggest there are immune system deficits beyond these "*2 specific bacteria*" when such risks do not exist for tetanus or diphtheria.

Table 8: Time series distribution of tetanus and diphtheria in the United States (WHO data)

See https://apps.who.int/immunization_monitoring/globalsummary/incidences?c=USA)

Diseases	2017*	2016*	2015	2014	2013	2012	2011	2010	2009	2008	2007	2006	2005	2004	2003	2002	2001	2000	1999	1998	1997	1996	1995
Diphtheria	_	_	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	2	1	1	5	2	0
Tetanus (neonatal)	_	_	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	_	_	_	_	_
Tetanus (total)	_	_	30	25	26	37	37	26	18	19	28	41	27	34	20	25	38	35	32	34	42	_	34

*Data not yet available

Further, the National Toxicology Program concluded that there is low confidence that exposure to PFOS is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease) (NTP, 2016). Other regulatory bodies and expert health panels

have made the following conclusions regarding immunotoxicity and exposure to PFOS and other perfluoroalkyls:

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):

"..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 (2017):

"Studies in environmentally-exposed populations have identified associations between PFOS levels and decreased antibodies against various illnesses, but the influence of PFOS exposure on clinical immunosuppression (i.e., incidence of illnesses) appears to be more tenuous." Health Canada further commented that "*a low level of consistency was observed across studies, with variations between genders, specific microbial immunoglobins, infections, mother vs. child exposure, and child years, amongst other characteristics. Moreover, the risk of residual confounding, bias, and chance cannot be discarded. These flaws impede concluding on a causative mechanism, and the nature of the association remains unclear*."

U.S. EPA (2016)

"*Another limitation of epidemiology studies that evaluate the immune response following PFOS exposure is that these studies have not demonstrated whether immune parameters measured in clinically normal individuals accurately reflect the risk of future immunological diseases. Given the immune system's capacity for repair and regeneration, apparent abnormalities that are detected at one point in time might resolve before producing any adverse clinical health effect. Thus, biomarkers that do not accurately diagnose or predict the presence or absence of a clinical health condition are not clinically useful*."

In conclusion, the inconsistent findings both within and across epidemiologic studies do not support an association between PFOS and decreased vaccine response in humans.

D. Epidemiological Associations for Cholesterol and PFOS Are Likely Non-Causal.

The European Food Safety Authority (EFSA) CONTAM Panel in March 2018 issued a tolerable weekly intake of 13 ng/kg body weight per week for PFOS. The EFSA CONTAM Panel considered an increase in serum total cholesterol with PFOS to be the critical effect which was based on three BMR models of cross-sectional epidemiologic studies (Steenland et al. 2009; Eriksen et al. 2013, and Nelson et al. 2010) where similar $BMDL_5$ values were calculated (25

ng/mL, 22 ng/mL, and 21 ng/mL, respectively). The BMDs were 27 ng/mL, 31 ng/mL, and 31 ng/mL, respectively. The much larger of the three cross-sectional studies, Steenland et al. (2009) appeared to have a plateauing of response at approximately 50 ng/mL. As noted on the report's first page, EFSA considers this CONTAM Panel report as provisional due to the scientific uncertainties described in this opinion as well as opinions expressed in minutes of a September 24, 2018 meeting. While DWQI Subcommittee on Health Effects acknowledged that most of these studies were cross-sectional by design, which means temporality between exposure and outcome cannot be determined, neither CONTAM Panel or DWQI considered a clinical chemistry study in monkeys with PFOS (Chang et al. 2017) in their assessments.

The study by Chang et al. (2017) evaluated the potential associations between serum PFOS and changes in serum clinical chemistry parameters in purpose-bred young adult cynomolgus monkeys (Macaca fascicularis). While the highest serum PFOS achieved was approximately 165,000 ng/mL, administration of PFOS to monkeys did not result in any toxicologically meaningful or clinically relevant changes in serum clinical measurements for coagulation, lipids, hepatic, renal, electrolytes, and thyroid-related hormones. A slight decrease (not increase) in serum cholesterol (primarily in HDL fraction), was observed. The corresponding lower-bound fifth percentile benchmark concentrations ($BMCL_{1sd}$) were 74,000 and 76,000 ng/ml for male and female monkeys, respectively.

These data corroborated the findings from a mechanistic study published by Bijland et al. (2011). Using the APOE*3-Leiden.CETP mouse model that expresses a human-like lipoprotein profile, high levels of PFOS lowered serum total cholesterol with enhanced lipoprotein lipase activity as well as decreased the rate of HDL particle maturation. At end of the three experimental studies where these mice were fed a Western higher fat composition diet, the PFOS concentrations ranged between 85,600 ng/mL and 124,700 ng/mL. Therefore, given the above toxicological data (Chang et al. 2017; Bijland et al. 2011), it is highly premature to suggest a low dose causal association between PFOS and cholesterol can be based on the current observational epidemiologic data without a well-defined mode of action.

3M Conclusion on Cholesterol and PFOS. As with the association between cholesterol and PFOA (*vide infra*), the toxicological evidence suggests a decrease in cholesterol with high concentrations of PFOS. The low dose associations between cholesterol and PFOS noted in certain observational epidemiologic studies, like that of PFOA, are likely due to yet-to-be-discovered mode of actions or confounding factors and are not causal. These include: 1) the possibility of decreased GFR with dyslipidemia that would confound an association between cholesterol and PFOS (or PFOA); 2) saturation of an underlying physiologic mechanism given the nonlinear association between PFOS (or PFOA) and cholesterol; 3) examination of shared organic anion transporters between lipids and PFOS (or PFOA) in the human in the small and large bowel, liver, and bile (as seen with URATE in the kidney proximal tubules; and 4) understanding the toxicokinetics of lipoprotein maturation with the possibility of incorporation of PFOS (or PFOA) into this maturation process of these lipoproteins. Until these and other hypotheses are thoroughly investigated, the low dose association based on observational epidemiologic data that has been suggested by some epidemiologists to be causal, continues to remain only a hypothesis elusive of a foundational mode of action and not supported by experimental data.

E. The Epidemiological Association for Birth Weight Has Been Demonstrated to Be the Result of Confounding or Reverse Causation

PBPK model/Monte Carlo simulation models by Verner et al. (2015) concluded that there was an association between GFR and fetal growth as well as confounding by GFR in the association between fetal growth and measured PFOA or PFOS concentrations. Verner et al. concluded such confounding could be upwards of 50 percent. More importantly, and what Post and Gleason did not recognize from the Verner et al. study, was this association between fetal growth and maternal measurement of PFOS was seen only in the second and third trimesters, not the first trimester, likely because the effect of GFR would be subsequent of plasma volume expansion that occurs in the first trimester. A similar situation occurred with PFOA in the Verner et al. study.

A meta-analysis was published by Negri et al. in 2017. They included 16 studies in their meta-analysis. The Negri et al. (2017) meta-analyses used both the untransformed and natural log transformations of PFOS. 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 PFOS was done later in the pregnancy or using cord blood. The latter is consistent with the simulation PBPK modelling done by Verner et al. (2015) as it relates to the potential confounding influence of maternal GFR with the timing of when PFOS 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 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. did not reference the Verner et al. (2015) PBPK simulation study who had demonstrated the potential confounding of maternal GFR, the timing of measurement of PFOS during and through pregnancy and reported birth weight.

An occupational study pertaining to fetal growth and PFOS was published more than 10 years ago by Grice al. (2007) of PFOS-related manufacturing workers at the 3M Decatur (Alabama) plant. Of 1895 past and present employees, 1400 (74%) responded including 263 female participants. Of these female participants employed at this manufacturing plant for one or more years, there were 421 singleton live births reported. The median birth weight was 3.5 kg for 32 singleton live births reported by women who were categorized as having had high cumulative PFOS exposure greater than 1 year compared prior to the birth compared to a median birthweight of 3.35 kg for the 312 singleton births reported among the women in the least exposed category (i.e., no direct occupational exposure) ($p = 0.15$). A non-statistically significant regression model estimated difference of 0.11 kg (95% CI -0.11, 0.33) was reported that adjusted for maternal age, gravidity and smoking status.

***3M Conclusion on birth weight*.** The association reported between fetal growth (few gram reduction) per ng/mL PFOS is likely not causal but rather consistent with confounding and/or reverse causation via GFR.

F. Use of a Human Serum Half-life Estimate of 5.4 Years in the MCL Derivation for PFOS is not Based on the Best Available Science:

Chemical-specific clearance factors (CL) used in risk assessment calculations are highly dependent on human half-life estimates. The human half-life estimates for PFOS have been reported in longitudinal studies across various age-groups and populations since 2007 ranging from 3.1 to 5.4 years for PFOS (Table 9).

Table 9. Serum elimination half-life estimates for PFOS from longitudinal studies

Reference	Study population	Sample size	Half-life (years)
Olsen et al., 2007	Retired production workers (aged 55-75 years) in Decatur, AL and Cottage Grove, MN	26 total (males = 24; females = 2)	4.8 (geometric mean) 5.4 (arithmetic mean)
Gomis et al., 2017	General population (US NHANES and Australia)	Modeled	4.9 (Australia males) 5.0 (Australia females) 3.8 (U.S. males) 3.3 (U.S. females)
Worley et al., 2017	Community in Alabama (mean age = 63 years) with drinking water exposure 2 samples collected without any water filtration)	45 total (males = 22; females = 23)	3.3 (males and females)
Li et al., 2018	Community in Sweden (aged 4-83 years) with drinking water exposure (samples collected after installation of GAC filter)	106 total males, 15-50 years = 20 females, 15-50 years = 30	3.3 (all ages) 4.6 (males, 15-50 years) 3.1 (females, 15-50 years)

For the PFOS MCL derivation, the DWQI selected an arithmetic mean serum elimination half-life estimate of 5.4 years (Olsen et al., 2007). This half-life estimate was based on a small study of 26 retired fluorochemical workers (24 males), whose mean age at the end of the study was 66 years. While the half-life estimate of 5.4 years is the most conservative estimate reported in the literature, it is not appropriate to use in the derivation of the PFOS MCL for the reasons discussed below.

Serum elimination half-lives are dependent on several factors including age, sex, and renal clearance of the study subjects. It is well-recognized that the glomerular filtration rate (GFR), an essential component of renal clearance of PFOS and other perfluoroalkyls, substantially declines with advancing age. The overall rate of decline in GFR in healthy persons is approximately 6.3 to 8.7 ml/min/1.73m^2 per decade (Berg, 2006; Linderman et al., 1985; Rule et al., 2010). Thus, the higher PFOS half-life estimate of 5.4 years, based on retired workers, is

likely explained by lower GFR and slower renal clearance of perfluoroalkyls in these older study subjects. This was not considered by the DWQI.

The DWQI also did not recognize that approximately 85% of the New Jersey general population is less than 65 years of age. Therefore, it is not justifiable to use a mean half-life estimate based solely on occupationally exposed retirees (who were primarily male) with markedly lower GFRs than most of the general population. The DWQI should consider alternative serum elimination half-life estimates that reflect overall general population demographics and GFRs. At a minimum, DWQI should present sensitivity analyses using these collective data.

Recently, a study by Li et al. (2018) reported PFOS half-life estimates from a community exposed to perfluoroalkyls through a contaminated water supply from a nearby military airfield. Upon the installation of GAC filters into the municipal water source, there was an abrupt mitigation of exposure to PFOS and other perfluoroalkyls in the drinking water. Study subjects (n=106), ages 4-83 years, were biomonitored a total of 7 times during a 26-month period following the installation of GAC filters. A serum elimination half-life of 3.4 years for PFOS was reported for these 106 subjects. Males (n=20) and females (n=30), ages 15-50 years, had half-lives of 4.6 and 3.1 years, respectively. It is well-known that various time-dependent physiological events (e.g., pregnancy, lactation, menstruation) affect clearance pathways that can result in lower concentrations in females.

Although the DWQI briefly discussed the Li et al. (2018) study, they failed to provide sufficient justification for not using the overall PFOS half-life estimate of 3.4 years reported in this study (DWQI 2019). Rather, the DWQI provided the following statement to justify their decision to use the half-life of 5.4 years from the Olsen et al. (2007) study:

> *"Although the men in Olsen et al. (2007) were all older than 50 years of age, the mean half-life of 4.6 years for men age 15-50 years from Li et al. (2018) is in reasonable agreement with the mean half-life of 5.4 years from Olsen et al. (2007). Additionally, the 95% CI of 3.9-6.9 years from Olsen et al. (2007) overlaps with the 95% CI of 3.7-6.1 years for men age 15-50 from Li et al. (2018)."*

DWQI did not address the fact that the PFOS half-life estimate of 5.4 years does not reflect overall general population demographics and age-related declines in GFR as discussed above. Furthermore, there is no scientific basis for stating that the mean half-life of 4.6 years from Li et al., (2018) is in "reasonable agreement" with the mean half-life of 5.4 years from Olsen et al. (2007). In fact, there is a difference of 292 days between these two half-life estimates which has a substantial impact on the derived MCL. If DWQI had used 4.6 years (1,679 days) for the PFOS half-life, based on men only, the MCL would be 15 ng/L. This MCL is 15% higher than the proposed MCL of 13 ng/L.

Regardless, the DWQI should have used the PFOS half-life of 3.4 years (for both males and females, ages 4-83 years) from the Li et al. (2018) study as this estimate is most representative of the general population. Furthermore, using this half-life would be consistent with the DWQI's decision to use a half-life for PFOA (2.3 years) based on another population that had contaminated drinking water mitigated following the installation of GAC filters (Bartell et al., 2010) rather than using the PFOA half-life based on the study of retired workers by Olsen at al.

(2007). Thus, the same DWQI decision-making process should be considered for PFOS as it was for PFOA. **If DWQI used 3.4 years (1,241 days) for the PFOS half-life while all the other parameters remain unchanged, the CL factor would become 1.28 x 10^{-4} L/kg/day and the MCL would be 20 ng/L. This MCL is 54% higher than the proposed MCL of 13 ng/L.**

Relevant to this point, it is noted that the Minnesota Department of Health used the mean half-life of 3.4 years from the Li et al. (2018) study in the derivation of their 2019 Health-Based Value for PFOS (see https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfos.pdf).

In conclusion, the DWQI used a half-life estimate of 5.4 years for PFOS that was not representative of general population demographics and GFRs, and not supported by the published literature. The DWQI should revise the MCL for PFOS to include a more appropriate and scientifically justifiable half-life estimate for PFOS consistent with their decision-making process for PFOA.

G. NJDEP Should Increase the RSC for PFOS

NJDEP chose a relative source of contribution (RSC) of 20% for its PFOS MCL derivation citing that:

> "*there are insufficient data to develop a chemical-specific RSC for PFOS*"

The available chemical-specific data from PFOS drinking water affected communities, as reported by Landsteiner et al. (2015) and Li et al. (2018), provided substantial evidence that elevated PFOS levels in the drinking water can be the primary route of PFOS exposure. Therefore, NJDEP could consider raising the RSC for PFOS. Other states such as Minnesota and New Hampshire have used 50%.

Also, it is incorrect for NJDEP to state that "There are no New Jersey-specific biomonitoring data for PFOS, and its more frequent occurrence in NJ PWS as compared to the U.S. as a whole suggests that New Jersey residents may also have higher exposure from non-drinking sources than the U.S. general population (e.g. NHANES)." Limited data reported by Graber et al. (2019) did not show that New Jersey residents have higher exposure (of PFOS). Study by Graber et al. was a cross-sectional biomonitoring study in Paulsboro area (New Jersey) where higher PFNA levels were detected in the community water supply system in 2009. Although PFOS concentration in the water was not reported, 13 PFAS serum concentrations were measured, including PFOS from 165 residents (> 12 years old). Compared to the representative data from NHANES, there was no difference in the PFOS serum levels from these community residents.

Table 3 Geometric mean and selected percentiles of selected per- and polyfluoroalkyl substances (PFAS) concentrations in serum (μg/L) for the population 12 years of age and older in Paulsboro New Jersey compared with U.S. NHANES 2013–2014 ($n = 165$)

PFAS		*n*	Geometric Mean (95% CI)	25th (95% CI)	50th (95% CI)	75th (95% CI)	95th (95% CI)
PFHxS	Paulsboro	124	2.03 (1.84, 2.25)	1.33 (1.20, 1.45)	2.02 (1.67, 2.38)	2.77 (2.49, 3.05)	4.70 (3.63, 5.76)
	NHANES	1432	2.18 (2.02, 2.36)	1.31 (1.24, 1.38)	1.93 (1.76, 2.10)	3.10 (2.74, 3.45)	6.46 (4.48, 8.44)
PFOA	Paulsboro	165	3.03 (2.70, 3.40)	1.94 (1.67, 2.22)	2.98 (2.43, 3.53)	4.69 (3.87, 5.51)	8.80 (6.91, 10.70)
	NHANES	2080	2.08 (1.91, 2.26)	1.35 (1.28, 1.43)	2.05 (1.89, 2.21)	3.06 (2.74, 3.37)	5.57 (4.63, 6.51)
PFOS	Paulsboro	164	5.37 (4.75, 6.06)	3.09 (2.57, 3.60)	5.66 (4.73, 6.59)	9.28 (7.93, 10.62)	14.76(11.62, 17.90)
	NHANES	2098	5.39 (4.98, 5.84)	3.20 (2.88, 3.51)	5.34 (4.95, 5.72)	8.76 (8.08, 9.43)	18.48 (15.26, 21.70)
PFNA	Paulsboro	165	3.50 (3.04, 4.04)	2.01 (1.60, 2.42)	3.89 (3.18, 4.60)	5.99 (4.47, 7.51)	12.41 (9.68, 15.13)
	NHANES	1599	0.91 (0.87, 0.96)	0.58 (0.57, 0.59)	0.78 (0.75, 0.81)	1.18 (1.09, 1.28)	2.22 (1.90, 2.54)

3M's DETAIL COMMENTS ON THE PROPOSED PFOA MCL

A. DWQI Allocated an Uncertainty Factor (UF) of 10 For PFOA MCL Derivation Without a Logical Scientific Basis; The UF Should Be Reduced To 3:

Uncertainty Factor Allocation

DWQI allocated a database uncertainty factor of 10 to account for "sensitive effects that are not otherwise considered," specifically citing mammary gland development and hepatic toxicity not associated with liver weight. This decision lacks a logical scientific basis and is inconsistent with EPA guidance on setting an uncertainty factor based on database uncertainty.

According to USEPA's guidance (https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf) in uncertainty factor allocation:

> *"The database UF is intended to account for the potential for deriving an underprotective RfD/RfC as a result of an incomplete characterization of the chemical's toxicity. In addition to identifying toxicity information that is lacking, review of existing data may also suggest that a lower reference value might result if additional data were available. Consequently, in deciding to apply this factor to account for deficiencies in the available data set and in identifying its magnitude, the assessor should consider both the data lacking and the data available for particular organ systems as well as life stages. In many respects, the additional 10-fold factor for infants recommended by the National Research Council (NRC, 1993) and by Schilter et al. (1996) and called for in the 1996 FQPA is similar to the database UF.*
>
> *If the RfD/RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproduction study is missing, or a factor of 10 may be applied if both are missing (Dourson et al., 1996). Dourson et al. (1992) examined the use of the database UF by analyzing ratios of NOAELs for chronic dog, rat, and mouse studies and reproductive and developmental toxicity studies in rats. They concluded that reproductive and developmental toxicity studies provide useful information for establishing the lowest NOAEL, and if one or more bioassays are missing, a factor should be used to address this scientific uncertainty in deriving a chronic RfD."*

Accordingly, it is misleading for DWQI to insert an uncertainty factor of 10 for incomplete database on the premise that "more sensitive effects that are not otherwise considered". The "more sensitive effects" that DWQI referred to are mouse mammary gland effects. While DWQI had selected relative liver weight for its final MCL derivation, it had also conducted a formal evaluation as well as benchmark analysis on the mammary gland data. In its Appendix A: Health-Based Maximum Contaminant Level Support Document" Perfluorooctanoic Acid (PFOA)", pages 206 – 210, DWQI went into great detail discussing this "sensitive" mammary gland effects based on the study by Macon et al. (2011). It is imperative for DWQI to acknowledge that its decision not to use the "more sensitive effects" for its MCL derivation is different than "more sensitive effects that are not otherwise considered". As a fact, on page 14 of Appendix A: Health-Based Maximum Contaminant Level Support Document" Perfluorooctanoic Acid (PFOA), DWQI explicitly stated that:

"Delayed mammary gland development is the most sensitive systemic endpoint with data appropriate for dose-response modeling, and a Reference Dose (RfD) was developed for this endpoint. It is believed that this endpoint has not previously been used as the primary basis for health-based drinking water concentrations or other human health criteria. Because the use of delayed mammary gland development as the basis for quantitative risk assessment is a currently developing topic, an ISGWQC with this RfD as its primary basis was not recommended."

Therefore, DWQI's decision to institute an uncertainty factor of 10 for PFOA MCL is unjustified. For PFOA, there is a rich dataset available on the key studies such as those mentioned by USEPA, and they include pre-natal toxicity, reproductive/developmental, two-generation, and even two bioassays (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; White et al. 2007; Biegel et al. 2001; Butenhoff et al. 2012a). Albeit the data are inclusive at best (let alone human relevance, if any), there are at least 8 studies that had evaluated potential mammary effects at various life stages (Albrecht et al. 2013, Macon et al. 2011, Tucker et al. 2014, White et al. 2007, White et al. 2009, White et al. 2011, Yang et al. 2009, Zhao et al. 2010). As we describe below in our critique of the key study (Macon et al. 2011) that DWQI characterized as demonstrating the most "sensitive" effects, there is no concordance on the mammary gland development findings among the published studies. Nevertheless, given the size of the available dataset, if the database uncertainty factor is not removed entirely, at a minimum NJDEP should revise its PFOA MCL by 3-fold to 0.042 μg/L.

PFOA and mammary gland development from Macon et al. (2011)

In its evaluation of toxicology studies, NJDEP (via its Health Effects Subcommittee within DWQI) focused on the effects of PFOA on the developing mammary glands in mice. NJDEP included a study by Macon et al. (2011) as a possible study for the PFOA RfD derivation. Macon et al. (2011) examined the effects of exposure to various ammonium PFOA (APFO) concentrations during gestation on mammary gland development in progeny born to CD-1 mice. A subset of females was dosed with APFO during almost the entire gestation when they received either 0 (vehicle control, DI water), 0.3, 1, or 3 mg/kg/day APFO from gestational day (GD) 1 – 17. In another subset of the study, other gestating females received APFO from GD10-17, at levels of 0 (vehicle control, DI water), 0.01, 0.1, or 1 mg/kg/day APFO. The study authors concluded there was "*significantly stunted mammary epithelial growth*" concomitant with fewer terminal end buds (TEBs) for all offspring and that a no observable adverse effect level for delayed mammary gland development could not be established.

3M disagrees with DWQI that mammary gland development is a robust endpoint for PFOA-related toxicity in laboratory animals and there were a number of specific concerns that warrant careful consideration before using data from Macon et al. (2011) for risk characterization as follows:

(1). Inadequate animal acclimation procedure

The pregnant mice used by Macon et al. were only acclimated to the new environment for *one* day between the time of arrival and the administration of APFO at the study facility. Given the fact that these mice were newly impregnated and had gone through various physical and environmental stresses in less than a week (*e.g.,* co-habituation with a male, mating, becoming pregnant, transportation-induced physiological changes, adaptation to a new vivarium with inherent differences in environmental conditions), it is hard to imagine that these mice did not experience undue stress between the day of arrival and the start of the study. For reasons such as these, many institutions require a minimum acclimation period for mice of 3 - 5 days prior to the initiation of any experimentation on animals (ILAR 1996).

A further reason for concern with maternal stress arises when the authors stated that 15% of females were not pregnant "*as expected*". The rationale for this so-called expectation was not explained or justified. Given that such rates of loss were stated to be unrelated to PFOA exposure, this outcome further suggested that dams were stressed. Macon et al. did not provide any indication of what treatment groups these losses occurred in or to what extent. They described that n=13 pregnant dams were assigned to each treatment group yet went on to say that 15% of dams were not pregnant; thus, group sizes of n=13 could not have been realized in the final study.

On a related note, dams in the full-gestation study were transported around day 0 of gestation, whereas dams in the latter study (late gestation exposure) were transported around day 8 of gestation. Thus, while all females experienced the same aforementioned short (1 day) acclimation period, this stressful experience was superimposed on different stages of fetal development, which may confound any extension of results.

(2). Maternal health

Guidance from various agencies such as the European Union (Section 3.7.2.4.1.) states:

> *"Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms."*

Therefore, it is important to be able to differentiate whether the developmental effects associated with APFO occurred in the presence or absence of marked maternal toxicity. The fact that Macon et al. did not provide any body weight data for the pregnant dams is unusual and disconcerting. Body weight data is an easy and objective clinical endpoint to measure and it is often the primary clinical index used to ascertain the well-being of an animal, especially those that are pregnant. In addition to lack of maternal body weight data, no data were provided for maternal liver weight or maternal PFOA concentrations.

(3). Litter handling / Sample selection bias

In their study, Macon et al. took newborn pups on postnatal day (PND) 1 and randomly distributed them with pups from other dams in the same treatment group. This allocation resulted in unequal numbers of pups per litter, with 7 – 9 pups per litter (4 – 7 females per litter). A well-planned developmental study would have attempted to cull and reach equal number of pups (per litter) with natural dams when possible. The authors stated they mixed pups and litters to be consistent with the approach used in their previous study (White et al. 2007). It was not clear why pups were randomly distributed to different dams because the instinctive and protective nature of a lactating dam (i.e., sensory recognition) can compromise the quality of the care for, and even the survival of, the foster pups. This oversight may be the reason why there were unequal numbers of pups per litter.

This oversight in experimental design may also be the reason why there were insufficient control female pups survived until PND 63 for sampling. Based on the experimental description, given that n = 13 dams (a seemingly sufficient number) was assigned to each treatment group, approximately n = 11 dams would have been expected to produce litters (with the expected 15% parturition loss). Given that the litter sizes were normalized to 4 - 7 females/litter, there should have been approximately 44 - 77 female pups available for necropsy across the 7 different postnatal ages (PND 7, 14, 21, 28, 42, 63 and 84) with a minimum of 6 female pups or more per postnatal time point for evaluation. It was not clear nor discussed by the authors as to why there were insufficient control female pups on PND 63. This not only raises the question as to the cause(s) and occurrence of postnatal death in the control group, it also reflected a poor study design and a lack of knowledge in animal handling.

(4). Mammary gland biology end points

a. Subjective scoring system for mammary gland development

The methods used by Macon et al. for assessing mammary gland development in offspring were performed *subjectively* on whole mounts using a categorical scale of 1 – 4 (1 = poor development and 4 = best development). In using this approach, the authors attempted to describe many different variables within the mammary glands as a single value rather than scoring or quantifying each variable. It is critical to recognize that the mammary glands undergo several developmental processes at once (i.e., ductal growth, branching, alveolar budding) and each of these landmark events must be quantified individually. Also, each of these processes is sensitive to different developmental and reproductive cues, and any comparisons of mammary gland development need to take the accompanying biology into account, such as age, metabolic bodyweight, stage of estrous cycle, and onset of ovarian function. It is worth noting that Macon et al. did not provide any information regarding stage of the estrous cycle, sex hormone concentrations, or histology of the reproductive organs. These baseline facts should have been adequately established to allow for a proper overall assessment.

What is most disconcerting is Macon et al. combined a subjective assessment of each variable within the mammary glands (i.e., ductal growth, branching, and alveolar budding) and integrated them into a single score that was not generated mathematically. The relative contribution or weighting of each variable in the final subjective score was never defined. The statement "*It should be noted that statistical differences found in a single quantitative endpoint did not necessarily determine aberrant development; rather, all quantitative and qualitative measurements were collectively utilized to determine overall developmental mammary gland scores*" reflected the fact that an undefined method was employed to generate their final scores for mammary gland development.

There are several significant limitations to this subjective scoring approach. A subjective scoring system precludes repetition by other laboratories, even those skilled in the art of mammary gland biology, given that the precise nature of the categorical scale is never documented. Moreover, even within the same laboratory there appears to be inconsistent definitions and use of this scoring system. In their study, Macon et al. used a scale where 1 = "*poor development*" and 4 = "*best development*" that they described as being similar to methods described by other laboratories (Hilakivi-Clarke et al. 1997a, Hilakivi-Clarke et al. 1997b, Welsch et al. 1988). It is interesting to note that 2 of the 3 referenced papers (Hilakivi-Clarke et al. 1997b, Welsch et al. 1988) used rats (not mice) as the test subjects. In addition, the development of the mammary glands in rats is considerably different from that in mice, thereby raising questions as to how the scale was developed or implemented. The remaining referenced study also used CD-1 mice (Hilakivi-Clarke et al. 1997a), but that paper did not provide sufficient information that would enable replication of their scoring method.

Macon et al. described that "*Scores were based on qualitative and quantitative histological characteristics of each developmental time point, including, but not limited to, lateral and longitudinal epithelial growth, change in epithelial growth, appearance of budding from the ductal tree, branching density, and number of differentiating duct ends (Hilakivi-Clarke et al. 1997a). Where applicable, at a given time point, mammary glands from both studies were compared on the microscope to ensure consistency in the scoring scale between studies*". It is unclear what other variables contributed to the subjective score given the results were not limited to those variables detailed above.

By contrast, in a similar study from the same research group, White et al. (2011) used a scale where 4 = "*excellent development/structure*" and 1 = "*poor development/structure*". The number of primary ducts and large secondary ducts, lateral side branching, appearance of budding from the ductal tree, and longitudinal outgrowth were assessed. Thus, in two studies from the same laboratory (Macon et al. and White et al.) published in the same year, there was variation between the scoring criteria and strategies used. Likewise, in both cases, it was not clear whether "*best development/structure*" and

"*excellent development/structure*" scores are synonymous, and whether a score of 4.0 represents that of an average control gland for a given age, which one might expect. In another instance, while Macon et al. reported the control mammary glands at PND21 with average scores of 3.3 (see Table 1, Macon et al. 2011) and 3.4 (see Supplemental Table 3, Macon et al. 2011), a recent study from the same laboratory, control glands from CD-1 mice at PND21 received a mean developmental score of 2.9 (Tucker et al, 2014). Even though the exact measures used to compute this score was not documented, it did appear that a score of 4.0 was realizable for control glands, as occurred at PND 84 (Macon et al. 2011).

Similarly, there appeared to be considerable variation among the population of CD-1 mice in this laboratory at PND21. The only data that were found to be statistically different at the 0.01 mg/kg dose at PND 21 was the value for this subjective developmental score (see Table 4, Macon et al. 2011). Even at 0.1 mg/kg, statistical differences were only detected for this subjective score and another quantitative measure of terminal end bud number. The ductal tree in the mammary glands of control females at PND 21 had only outgrown a few millimeters (see Figure 1A, Macon et al. 2011). By contrast, in comparable control CD-1 females at PND 21 in a recent paper from the same laboratory, the mammary ducts at PND21 have already reached the supramammary lymph node (see Figure 2A, Tucker et al. 2014), although again, no quantification of mammary growth was performed in that study. This difference is on the order of several millimeters, which relative to the size of the ductal tree at PND21, is substantial. *This dramatic difference in mammary gland development within the control population of CD-1 mice from this facility raises concern about how the mammary gland is being used as a toxicological end point*.

Another consideration that warrants further evaluation concerns the incorrect statistical inferences that have been made in analyzing the subjective mammary gland scores. By using a subjective scale, Macon et al. utilized a categorical method to generate their data. In performing their statistics by analysis of variance they assumed (incorrectly) that their mammary development scoring system increased linearly and/or with consistent increments. This assumption and statistical test is fundamentally invalid, further calling into question any conclusion about the low dose effects reported by Macon et al.

b. Lactation performance of dams as a critical variable

Another important consideration for this study, and any study of gestational exposure, concerns the consequences for the dam as she goes on to rear offspring. Specifically, the process of lactation is sensitive to a number of factors that can impact a dam's ability to provide milk to her offspring, thereby suppressing their development and that of their organs, including the

mammary glands. Two processes that are most susceptible to such exposures are: 1) functional development of the dam's mammary glands during pregnancy in readiness for lactation; and 2) dam's ability to metabolically adapt to the massive nutrient demands of milk synthesis and secretion.

Studies by the same research group suggested that exposure of pregnant mice to PFOA impaired the ability of the maternal mammary glands to undergo full growth and functional differentiation (White et al. 2007; White et al. 2009). In these studies, dams exposed to 5 mg/kg PFOA during gestation weaned pups at PND20 that were 33% lighter in bodyweight than controls (White et al. 2007), while White et al. (2009) also found reductions in weaned bodyweight following *in utero* plus lactational exposure to 3 mg/kg PFOA. It is unclear why Macon et al. did not find this same effect on progeny bodyweight at the 3 mg/kg dose.

Regardless, one must consider the potential for one or more aspects of pre-weaning development to be disrupted as a result of impacts on the lactational capacity of the exposed dams. A point that is relevant to the findings by Macon et al. is that growth of the mammary glands in female mice offspring before the onset of allometric growth at puberty is isometric – that is, mammary gland development is proportional to body size when it is expressed as a function of their "metabolic bodyweight" (typically considered to equal $BW^{0.66-0.75}$). Hence, any measure of mammary gland development should be expressed relative to metabolic bodyweight, not merely total bodyweight. This type of correction cannot be performed for subjective scores.

A parallel consideration that must be taken into account is the energy expenditure by dams when litter size varies as it did in this study. Each unit volume of milk secreted contains considerable energy derived from the dam's reserves and from her nutrient intake. This point is relevant when considering the work by Macon et al. given that litter size varied. For example, in full-gestation exposure study the authors state they balanced litters to 10 pups despite not being able to realize a 50/50 male/female target ratio. In late-gestation exposure study the authors declared they had litter sizes ranging from 7-9 pups. A difference in litter size such as this can dramatically affect maternal performance – a dam feeding 7 pups expends considerably less energy for milk production than a dam feeding 10 pups in a litter. In turn, these differences in metabolic state of the dam can have major ramifications for milk yield and quality that can then go on to affect many aspects of pup growth and development.

(5). Data presentation bias

Macon et al. (2011) provided a few quantitative measures in terms of mammary gland measurement for a subset of the study samples (from the late gestation study; see Table 1, Macon et al. 2011) although it is not clear (or explained) why no similar data were shown for the mice from full-gestation study (subjective scores only, supplemental data Table 3). Thus, one must consider that the data set, as presented, is incomplete. It

should be noted that Macon et al. disregarded significant outliers without explanation.

Regarding the mammary gland assessments for the full gestation study, Macon et al. stated that there were histological characteristics similar to previous findings; however, they did not show any histological data at all.

The representative mammary glands presented in Figure 1 from Macon et al. (2011) did not align well with the author's claims. Macon et al. stated that mammary glands from the 0.3 and 1.0 mg/kg treatment groups were less developed, however the variation was substantial and much of this could be explained by variables such as individual differences, stage of estrous cycles, or lack of, for that matter. In particular, it should be noted that being an outbred strain, CD-1 mice have more inherent variation within their phenotypes. Macon et al. also emphasized that in the mature mouse mammary gland "….*in the adult mouse at PND 84, there are no TEBs*". However, there did not appear to be any visual differences in the distribution of TEBs presented as the examples in histological sections of the mammary glands for PND 84 between control (Figure 1D) and female pups from 0.3 mg/kg (Figure 1E) and 1 mg/kg (Figure 1F) dose groups. This raises the question whether the qualitative scores used by Macon et al. have a strong foundation based on histological analyses. The histology data presented by Macon et al. should be carefully re-examined.

Regarding the late gestation study, the authors reported reduced elongation at PND 14 by 14.4 and 37% in the 0.1 and 1 mg/kg doses (see Table 1, Macon et al. 2011), whereas in Figure 4 the most pronounced reduction is at PND 21. This figure would have benefited from counting number of ductal branches. The authors show reduced TEB number at PND 21 in Table 1; it is unclear however where the other quantitative data for the rest of the experiment are.

The study by Macon et al. (2011) was flawed in several important aspects of study design and had numerous instances of inappropriate data interpretation. The authors failed to consider all aspects of biology and rather than scope out the best objective endpoints for the assessment, the study gave very few quantitative measures. The authors attributed various phenotypic consequences (i.e., reduction in mammary gland development) to the direct effects of PFOA. Alternative interpretations suggest that PFOA may be affecting mammary gland function in the lactating dams. Without any supporting evidence for maternal well-being, the data presented by Macon et al. are built on a great deal of speculation with a lack of definitive reproductive data combined with a lack of quantitative mammary gland analysis. The fact that the effects of PFOA on mammary gland development cannot be consistently described and quantified in all mouse models brings into question the biological significance of this phenotype as described, and its relevance to human health is unclear.

C. Increased Relative Liver Weights in Rodents Should Not Be Used as a Critical Effect for MCL and DWQI Should Consider Other Relevant Studies. DWQI Should Use the $BMDL_{10}$ Data from Mice That Had Been Treated with Linear PFOA.

Increased relative liver weights in rodents

Based on increased relative liver weight effects observed in mice by Loveless et al. (2006), NJDEP (via its Health Effects Subcommittee within DWQI) developed an MCL for PFOA in drinking water at 0.014 μg/L. In addition to increased absolute and relative liver weights, direct evidence of hepatic peroxisome proliferator-activated receptor alpha (PPARα) activation with PFOA exposure was also demonstrated by Loveless et al. (2006) shown as increased cyanide-insensitive hepatic peroxisomal β-oxidation activity from PFOA-treated animals.

3M respectfully disagrees with NJDEPs' use of rodent liver weight as a critical effect to establish a point-of-departure for derivation of a reference dose for PFOA. It is inconsistent with USEPA guidelines and published expert opinions on the distinction between liver hypertrophy as a non-adverse adaptive change and other endpoints representing liver toxicity (*vide infra*). Moreover, the observational human data as well as a significant body of mechanistic experimental data that relates to the liver response to exposure to PFOA strongly suggests that rodent liver weight as an endpoint for the human-health risk assessment of PFOA is inappropriate and needlessly conservative.

- The USEPA generally does not rely on liver enlargement as the sole critical endpoint for risk assessment. USEPA internal guidelines in place since 2002 provide a framework for evaluation of hepatocellular hypertrophy as indicative of an adaptive, non-toxic effect as opposed to an adverse, or toxic effect. As noted in the USEPA Office of Pesticide Programs HED [Health Effects Division] Guidance Document # G2002.01 on Hepatocellular Hypertrophy (USEPA, 2002), liver hypertrophy does not necessarily represent liver toxicity, nor is it necessarily a precursor to a particular manifestation of toxicity. Guidance Document # G2002.01 suggests a weight-of-evidence approach that includes evaluation of other findings, such as: 1) type and severity of observed effects; 2) onset, duration, and progression of effects; 3) study method and design; and, 4) other relevant effects and data. This guidance states that liver size or weight changes may be "indicative of adaptation which, by itself, is not necessarily adverse." In the absence of microscopic evidence of liver injury or change, at least two liver-related clinical chemistry parameters should be elevated with clinical significance ("at least 2-fold to 3-fold greater than control levels") before liver weight changes are ascribed to toxicity. The USEPA guidance specifically defines the NOAEL as "a dose which elicits either no response or only adaptive, non-adverse responses (e.g., hepatocellular hypertrophy [liver weight changes] alone)." The LOAEL is defined as a "dose which elicits adverse effects (e.g., hepatocellular hypertrophy in addition to other evidence of liver toxicity)."
- Additional guidance documents or articles similar to USEPA HED Guidance Document # G2002.01 have existed for many years. More recently, in 2012, the European Society of Toxicologic Pathology (ESTP) published the conclusions from the 3rd International ESTP Expert Workshop. This workshop was convened to "define more clearly when adaptive responses become adverse, and understand the long-term consequences of

hepatocellular hypertrophy in order to guide scientific opinion for risk assessment in man…" (Hall et al., 2012). Hall et al. provide an updated perspective on the consideration of liver hypertrophy as an adaptive versus adverse change which includes a thorough discussion of mechanistic, clinical, microscopic, and epidemiological evidence that allows for more certain interpretation of hepatic hypertrophic changes observed in experimental studies in the context of human health risk assessment.

- While PPARα-mediated increases in liver weight (absolute and/or relative) is a robust biological response in rodents when exposed to PFOA, non-PPARα mechanism, such as activation of other nuclear receptors CAR (NR1I3) and PXR (NR1I2) can also contribute to the hepatocellular hypertrophic observations in rodents. It is imperative for NJDEP to recognize these biological events are often adaptive in nature (i.e., reversible) and increase in liver weight alone (absolute or relative) should not be considered as an adverse effect unless it can be furthered supported collaboratively by microscopic evidence (i.e., necrotic lesions) and with liver-related clinical chemistry parameters (i.e., 2 – 3X or higher than the reference values). Furthermore, there are fundamental differences between the responses of human and rodent liver from exposure to agents that increase activation of these nuclear receptors and the key differences between rodent and human hepatocytes, especially the absence of a hyperplastic response in human hepatocytes exposed to PPARα and CAR activators, highlighted the less-likely and questionable human relevance (Elcombe et al., 1996; Goll et al., 1999; Hirose et al., 2009; Parzefall et al., 1991; Perrone et al., 1998; Elcombe et al. 2014; Corton et al., 2014)

DWQI should consider other relevant studies

NJDEP (via its Health Effects Subcommittee within DWQI) developed an MCL for PFOA in drinking water at 0.014 µg/L based on the increase in relative liver weight effects. While NJDEP concluded that PPARα-mediated increases in liver weight (absolute and/or relative) is a robust biological response in rodents when exposed to PFOA, it had also concluded that non-PPARα mechanism may also contribute to the hepatocellular hypertrophic observation.

While the observation of liver weight effects in laboratory animals and its relevance to human risk continues to be a scientific debate (Corton et al., 2014; Klaunig et al., 2003; Klaunig et al., 2012), based on these grounding premises established by NJDEP, it should consider a study by Abbott et al. (2007) which encompassed a more sensitive life stages (gestation and lactation) than the adult male mice from Loveless et al. (2006).

- Given that NJDEP emphasized that "the developmental period is a sensitive lifestage for PFOA's hepatic effects, and that increased relative liver weight is a relative and appropriate endpoint for PFOA's toxicity." (*cf*. page 210, Appendix A: Health-Based Maximum Contaminant Level Support Document" Perfluorooctanoic Acid (PFOA)), the increases in relative liver weights observed in lactating dams in mice should be considered by NJDEP, such as the study by Abbott et al. (2007). A key strength of using pregnant and lactating animal data is to reflect PFOA exposure in lactating women; another advantage of considering the abovementioned studies is that Abbott et al. (2007) not only administered PFOA during gestation to wild type mice, they also utilized

PPARα null (knockout) mice as well. The inclusion of PPARα null mice (in addition to wild type) is of particular importance because NJDEP has established its position that non-PPARα mechanism can cause hepatic hypertrophy with PFOA. Therefore, the data obtained from a non-PPARα responsive mouse model (e.g., PPARα null mice) should provide even more relevance than those obtained from wild type.

In their review of the studies, the Health Effects Subcommittee within DWQI had excluded the study by Abbott et al. (2007) for MCL consideration because serum PFOA concentration data (from lactating dams) were obtained at 3 weeks after last PFOA dosing (i.e., end of weaning / lactation). The difference in the timing of the tissue collection should not be the basis for data exclusion because the benchmark dose variables evaluated by DWQI were exposure (serum PFOA concentration) and effect (increased relative liver weight). Even though Abbott et al. (2007) did not measure serum PFOA concentration in dams until the end of lactation, there were still appreciable large amount of PFOA in the blood of these animals (mainly due to slow serum elimination half-life). Therefore, NJDEP should also consider evaluating the mouse dam data from Abbott et al. (2007) which encompassed more sensitive life stages (gestation and lactation) than the adult male mice from Loveless et al. (2006).

- In addition, on the sole premises of increased liver weight effects in non-pregnant rodents, another study that DWQI should consider is a 90-day dietary study in male Sprague Dawley rats by Perkins et al. (2004). Sprague Dawley rats were also included in the 14-day study by Loveless et al. (2006). The study by Perkins et al. was also excluded by the Health Effects Subcommittee within DWQI, citing a lack of time-dependent responses in increased liver weight over the study period. This is incorrect. In this study, serum PFOA appeared to have reached steady state by 4 weeks into the study and the attainment of steady state is a common observation in laboratory animals when perfluoroalkyls were administered at high doses or for extended exposure durations. This corresponded to a "saturation" status where (latter) additional PFOA administered was not absorbed efficiently. This natural occurrence does not invalidate the study data given that at every single time point of the study (4, 7, or 13 weeks post-dose), there were dose-dependent increases in serum PFOA concentrations as well as increases in relative liver weight. In addition, based on the data reported by Loveless et al. (2006), male Sprague Dawley rats appeared to be more sensitive than male CD-1 mice in terms of higher body burden when similar PFOA doses were administered. A key strength of including Perkins et al. (2004) study data reflects on the dietary PFOA exposure route used in the study design, which is a major pathway considered by NJDEP as potential PFOA source of exposure. Therefore, NJDEP should also consider evaluating the longer-term rat data from Perkins et al. (2004) for its PFOA assessment.

In conclusion, Table 10 below summarizes these differences between the three studies (Loveless et al. 2006; Abbott et al. 2007; and Perkins et al. 2004).

Table 10:

	Current study chosen by NJDEP	Other Relevant Studies that NJDEP Could Consider	
Reference study	Loveless et al. 2006	Abbott et al. 2007	Perkins et al. 2004
Study type	14-day oral gavage study	Gestation exposure study	13-week (~90 days) dietary study
Doses	0, 0.3, 1, 3, 10, and 30 mg/kg/day	0, 0.1, 0.3, 0.6, 1, 3 (PPARα null only), 5, 10, and 20 mg/kg/day	Equivalent of 0, 0.06, 0.64, 1.94, and 6.5 mg/kg/day
Species	Male CD-1 mice • Wild-type	Female Sv/129 mice • Wild-type • PPARα null	Male SD rats • Wild-type
Effect	↑ Relative liver weight	↑ Relative liver weight	↑ Relative liver weight
Why should NJDEP consider this?		• Sensitive life stage (emphasize by NJDEP) • Liver weight increases were observed • Included both PPARα and non-PPARα mouse models	• Extended exposure duration • Consistent concentration-dependent responses between serum PFOA and liver weight increases • Rat showed higher body burden than mice at the same dose

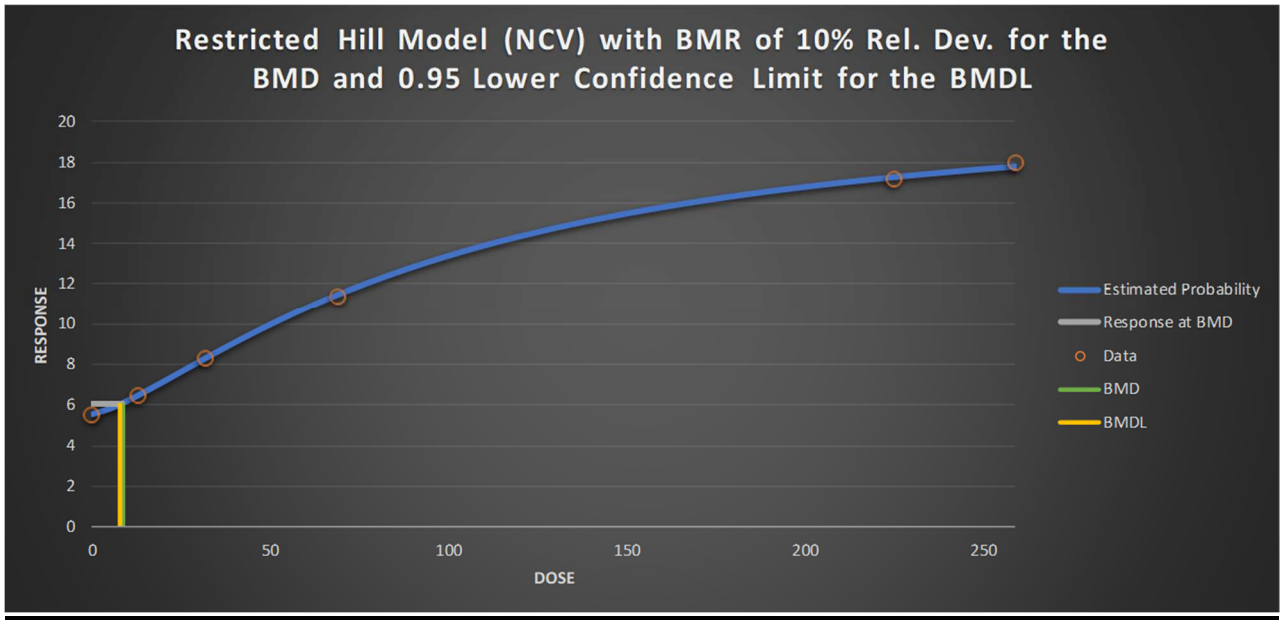
DWQI should use the $BMDL_{10}$ data from mice that had been treated with linear PFOA

As stated above, while the observation of liver weight effects in laboratory animals and its relevance to human risk continues to be a scientific debate, if DWQI is to continue using Loveless et al. (2006) study for its PFOA MCL process, it should use the data from mice that were exposed to linear ammonium PFOA. This is because NHANES data cannot detect the branched PFOA isomers in its latest 2015-2016 cycle analyses - only linear PFOA was detected in the general population. In the only other cycle year (2013 – 2014) that NHANES measured PFOA isomers, branched PFOA was only reported at or above 90th percentile in the population.

Based on a 10% shift in increased relative liver weight (identical to DWQI's modeling parameter) and by considering the data reported in Loveless et al. (2006) where mice were exposed to 14 daily treatments of linear ammonium PFOA; a serum PFOA $BMDL_{10}$ of 7,973 ng/mL can be obtained. This is 1.8X higher than the current $BMDL_{10}$ used by DWQI. If this $BMDL_{10}$ is considered by DWQI, it will result in a higher PFOA MCL (Table 11).

Table 11

Model	Restriction	Serum PFOA (µg/mL)			Test 4 P-Value	AIC	BMDS Recommendation	
		BMD	BMDL	BMDU			Viable?	Notes
Exponential 5 (NCV)	Restricted	8.138695	6.682145	9.696039	0.604588	109.4589	Viable - Alternate	
Hill (NCV)	**Restricted**	**8.638543**	**7.973335**	**10.28869**	**0.90215**	**108.6585**	**Viable - Recommended**	**Lowest AIC**
Hill (NCV)	Unrestricted	8.638544	7.973341	10.21833	0.90215	108.6585	Viable - Alternate	
Polynomial Degree 4 (NCV)	Unrestricted	7.135422	5.997349	8.55234	0.165892	112.3722	Viable - Alternate	



D. NJDEP Should Increase the RSC for PFOA

NJDEP chose a relative source of contribution (RSC) of 20% for its PFOA MCL derivation citing that:

> "*there are insufficient data to develop a chemical-specific RSC for PFOA*"

This is incorrect. The available chemical-specific data from PFOA drinking water affected communities, as reported by Emmett et al. (2006) and Landsteiner et al. (2015), provided substantial and compelling evidence that elevated PFOA levels in the drinking water will become the primary route of PFOA exposure. Therefore, NJDEP should raise the RSC for PFOA. States such as Minnesota and New Hampshire have used 50% RSC.

E. Epidemiological Associations for Cholesterol and PFOA are Likely Non-Causal; The Epidemiological Associations for Birth Weight, Kidney Cancer, and Liver Enzyme ALT Have Been Demonstrated Result from Confounding or Reverse Causation.

In March 2017, the DWQI recommended to NJDEP an MCL of 14 ng/L for PFOA and 13 ng/L for PFOS which was based on deliberations of its DWQI Health Effects Subcommittee. According to this Subcommittee, as stated by the NJDEP (page 7), exposure to PFOA has been associated with health effects including increased cholesterol, increased liver enzymes (an indication of liver damage), decreased vaccine response, decreased birth weight, and testicular and kidney cancer. Unfortunately, the NJDEP Subcommittee on Health Effects chose to not consider, discount, or not have available for review (due to not considering publications after March 2015), important information that suggests these reported associations are highly likely to be noncausal. 3M believes the following research that was not reviewed by the DWQI Health Effects Subcommittee due to either timing of publication or incorrect data interpretation, is germane to the conclusion of a misunderstanding of the biological associations reported with PFOA (or PFOS) and therefore impacts the proposed MCLs through the misguided attempt by the DWQI Health Effects Subcommittee to apply unjustified uncertainty factors for PFOS as well as incorrectly calculates the MCL for PFOA.

A brief discussion follows for each of the epidemiologic associations listed above.

Cholesterol

While the DWQI Health Effects Subcommittee was aware of a phase 1 dose-escalation clinical trial study that administered PFOA to cancer patients (see Appendix D, response found on page 41) due to the anti-tumorigenic properties of PFOA, it did not cite the existence of this study because it was only presented as an abstract (Macpherson et al. 2010) published in the journal Clinical Oncology. According to the DWQI Health Effects Subcommittee, the abstract was based on a clinical conference and was not peer-reviewed in the literature nor was there sufficient information in the abstract provided to even understand study design. Therefore, the Subcommittee chose to not consider the abstract. On the other hand, it should be noted that the draft 2018 ATSDR (https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf) report discussed this abstract. Regardless, such an explanation to cite or not cite an abstract is now moot as the findings from this phase 1 clinical trial study have been publicly available in the peer reviewed scientific literature since May 2018 (Convertino et al. 2018). To the best of 3M's knowledge,

this is the only experimental study of PFOA conducted in humans. All other epidemiologic research reported in the scientific literature is observational - whether it is from the general population, communities exposed to PFOA, or occupational. And, as noted by the DWQI Subcommittee on Health Effects, most of these studies were cross-sectionally designed studies which means temporality between exposure and outcome cannot be determined. Thus, the NJDEP needs to understand not only the phase 1 clinical trial study results because of its unique study design with direct exposure to humans. NJDEP also needs to consider an important toxicological study recently published in the same premier toxicological journal (Toxicological Sciences) where a genetically engineered mouse model designed to mimic human lipoprotein metabolism was used to assess administered PFOA (ammonium salt) dosages that resulted in environmental, occupational, and toxicological (similar to phase 1 clinical trial) concentrations of PFOA reported in humans (Pouwer et al. 2019). Summaries of both studies are presented below. Reduction of serum cholesterol in mice fed a Western diet was only observed at toxicological concentrations which were similar in magnitude to the phase 1 clinical trial in humans. Findings from these two studies indicate the epidemiologic observations are associative rather than causal.

1. Phase 1 clinical trial on PFOA (Convertino et al. 2018).

The sponsor of this phase 1 dose-escalation study was CXR Biosciences (Dundee, Scotland). The study was conducted by oncologists at the Institute of Cancer Sciences, Beatson Institute, University of Glasgow (Glasgow, UK) and the Aberdeen Royal Infirmary (Aberdeen, UK). At an invitation by CXR Biosciences that occurred several years after the study was completed and findings presented at international conferences (e.g., Macpherson et al. 2010), 3M licensed the non-identifiable clinical study data and the measured plasma PFOA analyses (via LC-MS/MS) and requested epidemiologists at the University of Minnesota School of Public Health to analyze these clinical trial data. They conducted different statistical metamodels of the clinical chemistry data with both PFOA dose groups and measured PFOA concentrations, including the use of generalized estimating equations, probability distribution functions, and a two-compartment PK/PD model.

The study included forty-nine primarily solid-tumor cancer patients who had not done well with standard therapy. These individuals received weekly PFOA (ammonium salt) doses (50 – 1200 mg) for 6 weeks. While the main limitation of the study was that it used as subjects late-stage cancer patients whose metabolic activity may differ considerably from healthy individuals, according to Convertino et al., there was no evidence that any of the cancers involved or treatments received prior to the study had systematic effects of the metabolic function studied. Baseline values prior to PFOA treatment were also recorded to determine measurable differences over the course of treatment. No more than one subject showed dose limiting toxicity at any dose so therefore the protocol defined maximum tolerated dose was not reached. Standard clinical chemistries were assessed including total cholesterol, LDL, HDL, ALT and other liver enzymes as well as liver function (e.g., prothrombin time), TSH and free thyroxine, creatine and uric acid. There was strong evidence that showed PFOA concentrations were associated with a reduction of total cholesterol as a there was a clear transition in shape and range of the probability distribution functions for a decrease in total cholesterol. The reduction of total cholesterol

was with the LDL cholesterol, not HDL fraction. This transition occurred at approximately between 175,000 and 230,00 ng/mL PFOA which are concentrations several orders of magnitude higher than reported in the general population, exposed communities through drinking water such as the mid-Ohio river valley community, or occupational workers. The findings at these high concentrations are, in fact, contrary to the positive association between PFOA and total cholesterol observed in cross-sectional studies that were modeled by EFSA in 2018.

2. Dose effect of PFOA (ammonium salt) on lipoprotein metabolism in a genetically engineered mouse model (Pouwer et al. 2019).

DWQI Health Effects Subcommittee mentioned two toxicological studies (Tan et al. 2013; Rebholz et al. 2016) which reported wild-type mice fed a Westernized (high fat) diet containing PFOA resulted in increased cholesterol. However, this Subcommittee appeared not to appreciate the fact that rodent lipoprotein metabolism is characterized by fast clearance of apoB-containing lipoproteins and the absence in the rodents of cholesteryl ester transfer protein (CETP) that results in a higher proportion of HDL-cholesterol relative to LDL cholesterol in the rodent. In contrary, humans have a much higher proportion of LDL-cholesterol relative to HDL-cholesterol due to the presence of CETP which results in transfer of cholesterol sterol from HDL-cholesterol to the much slower clearing apoB-containing lipoproteins in exchange for triglycerides. Therefore, wild-type mice are not the most suitable species to study human lipid metabolism in addition to the relevance and translatability of their findings to the human situation. It is premature for DWQI, inferring from the study conclusion by Tan et al. and Rebholz et al., to conclude that PFOA can cause hypercholesterolemia.

Instead, the APOE*3-Leiden.CETP mouse model has been commonly used to study the effect of pharmaceuticals on lipid metabolism and atherosclerosis for human evaluation. The APOE*3-Leiden.CETP mouse model was designed to mirror human lipoprotein metabolism with incorporation of cholesterol ester transfer protein expression and a delayed apoB clearance that occurs in humans.

Pouwer et al. (2019) used the APOE*3-Leiden.CETP mouse model to study the effect of PFOA on plasma cholesterol and triglyceride metabolism at concentrations relevant to humans relative to environmental, occupational, and toxicological (above phase 1 clinical trial) plasma PFOA concentrations. The other objective was to elucidate the mechanisms for the effects reported. Two experiments were conducted at either 4 weeks or 6 weeks of dietary intake. Dosages fed for 6 weeks were 10, 300, and 30,000 ng/g/d which corresponded at end of study to 65 ng/mL, 1500 ng/mL, and 144,000 ng/mL plasma PFOA, respectively. Serum concentrations were slightly less for 4 weeks dietary administration. At 30,000 ng/g/d, PFOA decreased plasma triglycerides, total cholesterol, and non-HDL-cholester0l whereas HDL was increased. The decrease in total cholesterol and non-HDL cholesterol was attributed to decreased very low density lipoprotein (VLDL) activity and increased VLDL clearance through enhanced lipoprotein lipase activity. The latter was likely due to decreased CETP activity and changes in lipoprotein metabolism. Gene expression and pathway analysis suggested that fatty acid oxidation and individual genes, all under

control of PPARα, were enhanced along with PXR regulation, but much less CAR mediated activity. These data are consistent with the findings from the phase 1 clinical trial in humans that demonstrated high serum or plasma PFOA concentrations result in lower cholesterol levels.

3. Another cholesterol-related paper worthy of consideration by NJDEP (Vanden Heuval 2013).

Vanden Heuval (2013) was a commentary to the premise offered by Fletcher et al. (2013), a member of the C8 Science Panel, who suggested that exposure to PFOA created a "hypercholesterolemic environment." As reviewed by Vanden Heuval (2013), a paper that was not cited by the DWQI Subcommittee on Health Effects, reverse cholesterol transport and cholesterol efflux involves HDL to stimulate the efflux of cholesterol from peripheral tissues, transport in plasma, uptake in the liver and then involve biliary excretion. Specific to the premise of anti-atherogenic properties of HDL, macrophage reverse cholesterol transport involves efflux of cholesterol from macrophage foam cells in the artery wall which involves many genes including the ABC transporters in the transport of free cholesterol from the cell. In their paper, Fletcher et al. (2013) reported inverse associations between serum PFOA levels and whole blood expression level of a small subset of genes involved in cholesterol transport among 290 mid-Ohio river valley subjects. Based on these data, Fletcher et al. suggested PFOA could be increasing circulating cholesterol and decreasing cholesterol efflux from macrophages in humans. However, according to Vanden Heuval, Fletcher et al. only examined 11 of 67 genes engaged in macrophage cholesterol efflux and reverse cholesterol transport and they did not take into account the redundancy and over-lapping functions in maintaining cholesterol homeostasis. Furthermore, most laboratory studies have examined the hepatic, not extrahepatic expression sites (e.g., peripheral lymphocytes and macrophages as was done by Fletcher et al.) that involve cholesterol metabolism. A mode of action was not formally studied by Fletcher et al. whereas the major target of the effects for PFOA (and PFOS) in laboratory studies involve PPARα, and likely other nuclear receptors such as PXR and CAR. Also, when Vanden Heuval compared cholesterol metabolism genes in mouse liver to those of peripheral lymphocytes, there was considerable inconsistency. Laboratory studies have often shown (e.g., see above study by Pouwer et al. 2019) decreases in cholesterol levels with exposure to PFOA, including the study by Loveless et al. (2006), which is being used by the DWQI Subcommittee on Health Effects for the point of departure for PFOA to set an MCL based on increased liver weight.

4. European Food Safety Authority (EFSA) benchmark response (BMR) model on cholesterol for PFOA.

The EFSA CONTAM Panel in March 2018 issued a tolerable weekly intake of 6 ng/kg body weight per week for PFOA. The EFSA CONTAM Panel considered an increase in serum total cholesterol with PFOA to be the critical effect which was based on two BMR models of cross-sectional epidemiologic studies (Steenland et al. 2009; Eriksen et al. 2013) where similar $BMDL_5$ values were calculated (9.2 – 9.4 ng/mL). The BMD ranged between 12 and 12.4 ng/mL. The much larger of the two studies, Steenland et al. (2009) reported a plateauing of response at approximately 25 ng/mL. In this mid-Ohio river valley population, while there was a non-monotonic increased risk for hypercholesterolemia, there was no

increased risk for coronary artery disease associated with increasing exposure to modeled PFOA exposure (Winquist et al. 2014). Nor has there been an increased risk for mortality from cardiovascular disease outcomes been reported in other highly exposed populations (Steenland and Woskie et al.2012; Raleigh et al. 2014). As noted on the report's first page, EFSA considers this CONTAM Panel report as provisional due to the scientific uncertainties described in this opinion as well as opinions expressed in minutes of a September 24, 2018 meeting. The CONTAM Panel report did not consider Convertino et al. (2018) or Pouwer et al. (2019) in their analyses.

3M Conclusion on cholesterol: It is highly premature to suggest a low dose causal association between PFOA and cholesterol can be based on the current observational epidemiologic data. Neither the DWQI Health Effects Subcommittee, EFSA (2018) or ATSDR (2018) reviewed any study, except Butenhoff et al. (2012b), that examined a hypothesized, peer-reviewed published research mode of action study for the low dose response association observed between PFOA and cholesterol, as evidenced in several (but not all) observational epidemiologic studies. On the other hand, there is considerable evidence to indicate a mode of action for the decreased cholesterol associated with PFOA seen in both human and animal high-dosed experimental studies. Several areas of investigation have been proposed to examine potential modes of action in low dose response studies including: 1) the possibility of decreased GFR with dyslipidemia that would confound an association between cholesterol and PFOA (or PFOS); 2) saturation of an underlying physiologic mechanism given the nonlinear association between PFOA (or PFOS) and cholesterol; 3) examination of shared organic anion transporters between lipids and PFOA (or PFOS) in the human in the small and large bowel, liver, and bile (as seen with URATE in the kidney proximal tubules; and 4) understanding the toxicokinetics of lipoprotein maturation with the possibility of incorporation of PFOA (or PFOS) into this maturation process of these lipoproteins. Until these and other hypotheses are thoroughly investigated, the low dose response association based on observational epidemiologic data, that has been suggested by some epidemiologists to be causal, continues to remain only a hypothesis elusive of a foundational mode of action and not supported by experimental data.

Birth Weight

Gleason and Post (2019) concluded, "Based on review of the relevant information, it was concluded that confounding by GFR does not account for the major portion of the decrease in fetal growth that is associated with PFOA." Post and Gleason (2019) limited their review to papers published by 2015. Subsequent research has shown the opinion of Post and Gleason (2019) is clearly not supported by the scientific evidence.

After a collection of studies conducted by Woodruff et al. (2014), Koustas et al. (2014), Johnson et al. (2014), and Lam et al. (2014) concluded, using a systematic review process, that "developmental exposures to PFOA adversely affects human health based on sufficient evidence of decreased fetal growth in both human and nonhuman mammalian species." A critical question arose as to whether the epidemiologic data presented on fetal growth was confounded by the maternal glomerular filtration rate (GFR). Vesterinen et al. (2015). did a systematic review the literature based on the hypothesis that reduction in fetal growth would lead to less plasma expansion resulting in less GFR and subsequently reduced filtration of

exogenous chemicals. This would result in higher concentrations in cord or maternal blood suggesting an association between lower fetal growth and higher cord or maternal blood concentrations of a chemical, including PFOA. Vesterinen et al. (2015) then asked the question whether there was an association established in the published literature between fetal growth and GFR (i.e., confounding or reverse causation). Based on their review, Vesterinen et al. (2015) concluded that there was insufficient evidence to support the plausibility of a reverse causation hypothesis between exposure to environmental chemicals during pregnancy and fetal growth; however further research would be needed to confirm or disprove this hypothesis. Post and Gleason (2019) mention the subsequent research by Morken et al. (2014) and the PBPK model/Monte Carlo simulation models by Verner et al. (2015) that definitely indicated there was an association between GFR and fetal growth as well as the confounding between GFR in the association between fetal growth and measured PFOA concentrations. Verner et al. concluded such confounding could be upwards of 50 percent. More importantly, and what Post and Gleason failed to recognize from the Verner et al. study, was this association between fetal growth and maternal measurement of PFOA was seen only in the second and third trimesters, not the first trimester, likely because the effect of GFR would be subsequent of plasma volume expansion that occurs in the first trimester.

A meta-analysis (not cited by Post and Gleason 2019) was published in 2017 by Negri et al. (2017). They included 16 studies in their meta-analysis. The meta-analyses by Negri et al. 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. 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 modelling 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 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. did not reference the Verner et al. (2015) PBPK simulation study who aptly demonstrated the potential confounding of maternal GFR, the timing of measurement of PFOS during and through pregnancy and reported birth weight.

Steenland, Barry, and Savitz (Steenland et al. 2018) did recognize this distinction from the Verner et al. study. They conducted a meta-analysis of 24 epidemiologic studies – 15 more than done by Johnson et al. (2014) and 8 more than Negri et al. (2017). They stratified their results as to whether the maternal PFOA concentration was measured in the first or the combined second and third trimesters. Steenland et al. reported with first trimester measurements of maternal PFOA, there was a -3.3 gram (95% CI -9.6, 3.0) reduction in birthweight per ng/mL

PFOA. When PFOA was measured second/third trimester, there was a -17.8 gram reduction (95 CI -25.0, -10.6) in birthweight per ng/mL PFOA. Steenland et al. (2018) concluded "restriction to studies with blood sampling conducted early in pregnancy or shortly before conception showed little or no association such that these results are consistent with confounding and /or reverse causation being responsible for the inverse association seen in studies with low background exposure levels and blood sampling conducted later in pregnancy, when confounding and/or reverse causality are likely to be more important." This statement clearly contradicts Post and Gleason's (2019) opinion that confounding by GFR does not account for the major portion of the decrease in fetal growth that is associated with PFOA.

Subsequent to the Steenland et al. (2018) meta-analysis, other studies have been, and will continue to be published regarding associations about fetal growth and the timing of measurements of PFAS, including those studies by Buck et al. (2018), Buck Louis et al. (2018), Manzano-Salgado et al. (2017), Marks et al. (2019), Meng et al. (2018), Shoaff et al. (20180, and Starling et al. (2017). The essential message from the meta-analyses conducted to date indicate physiological aspects of pregnancy, including plasma volume expansion, GFR, and when the maternal PFAS measurement was made during gestation, are critical important points to evaluate.

3M Conclusion on Birth Weight: The association reported between fetal growth (few gram reduction) per ng/mL PFOA is likely not causal but rather consistent with confounding and/or reverse causation via GFR.

Kidney Cancer

The DWQI Subcommittee on Health Effects (2017) chose not to specifically discuss the Raleigh et al. (2014) study under their discussion of kidney cancer. Nor did Post and Gleason (2019) mention the Raleigh et al. (2014) study, only that it was reviewed by IARC (2016). Post and Gleason chose only to present the epidemiological studies cited by IARC (2016) as the basis for IARC's conclusion that PFOA was a possible human carcinogen. This is a striking example of publication/reporting bias by Post and Gleason (2019). The Raleigh et al. findings are presented herein because this study was, indeed, very important in IARC's decision making process to consider the epidemiology data as "limited evidence" in their decision-making process by the Working Group in June 2014. The Raleigh et al. (2014) study was a cohort of Cottage Grove (MN) 3M APFO (ammonium salt of perfluorooctanoic acid for which PFOA is the dissociated anion measured in the blood) production workers (N = 4668) and the referent non-PFOA 3M production workers based in St. Paul (MN) (N = 4359) examined cancer mortality and incidence, including kidney cancer. This study was of comparable population size as the cohort mortality study of Steenland and Woskie (2012) and similar number of worker kidney cancer incidence cases as Barry et al. (2013). There was no evidence of increased risks for kidney cancer mortality or kidney cancer incidence in this population. There was a total of 35 kidney cancer incidence cases (16 in the Cottage Grove cohort; 19 in the referent cohort). Hazard ratios (HR) for kidney cancer incidence based on a quartile estimate of PFOA IH job and task-based exposure (cutpoints 2.9×10^{-5}, 1.5×10^{-4}, 7.9×10^{-4} $\mu g/mg^3$years were referent (nil exposure, HR = 1.00), Q1 HR = 1.07, Q2 HR = 1.07, Q3 HR = 0.98, and Q4 HR = 0.73). The exposures to APFO, and reported biomonitoring data of these 3M APFO manufacturing workers, were similar, if not

higher, than the DuPont workforce whose estimated cumulative exposure matrix (in µg/ml/year) was discussed by Steenland and Woskie (2012). (Note: the PFOA manufactured at the 3M plant was sold to DuPont for use at its Washington Works plant in West Virginia.)

An important difference between the 3M and DuPont cohorts was the near absence of exposure to tetrafluoroethylene (TFE) in the 3M workforce whereas such exposure was, in fact, present in the DuPont workforce because PFOA is a processing aid in the polymerization of TFE to make PTFE. This distinction is important because IARC declared TFE a "probable human carcinogen" in the same 2014 working group that labeled PFOA a 'possible human carcinogen", in part, because TFE causes kidney tumors in rats. Thus, TFE could be a confounding exposure when evaluating the relationship between PFOA and kidney cancer. Post and Gleason simply accepted the explanation offered by Steenland and Woskie (2012) that "appreciable exposures would have been unlikely (to TFE at the DuPont plant), since TFE exposure would have been well controlled due to its explosive and volatile nature." Yet Post and Gleason, cited in their review but did not discuss the detail in the paper by Olsen et al. (2014), who made the distinction that the lower explosion limit for TFE is 110,000 ppm! The 8 hour TWA is 2 ppm. Therefore, it is highly likely that low level TFE exposure could have occurred at the DuPont plant given the disparity between occupational exposure TWA level and the lower explosion limit." In fact, a multi-company cohort study of TFE/PTFE production workers could not "disentangle" the association between PFOA and TFE for cancer of the liver and kidney and leukemia mortality (Consonni et al. 2013). This study included the DuPont Washington Works plant. Sleeuwenhoek and Cherrie (2012), who constructed the exposure matrix for this study, reported it was possible based on their semi-quantitative exposure categorization of TFE that occasional occupational exposure to TFE was indeed possible in these PTFE production facilities. Production is primarily carried out in closed systems and the main exposure to TFE occurs from leaks, from opening autoclaves in the polymerization area or from decomposition of PTFE.

3M Conclusion on Kidney Cancer and PFOA: Although factually correct, it is highly misleading and prejudicial that NJDEP continues to cite the US EPA 2006 Science Advisory Board panel's (not unanimous) conclusion that PFOA is "likely" carcinogenic to humans. This long-outdated decision preceded the important studies subsequently published from the C8 Science Panel, 3M, and others which subsequently has resulted in the "downgrading" of the classification to "suspected" by the US EPA Office of Water which is comparable in hazard rating to the IARC "possibly carcinogenic to humans." More than 300 chemicals and physical agents have been categorized by IARC as "possibly carcinogenic to humans" including radiofrequency electromagnetic fields emitted by the cell phone. It was only recently that IARC removed coffee from its possible carcinogenic listing and replaced it with 'hot beverages.'

Liver Enzyme ALT

Post and Gleason overinterpret the epidemiological data as it relates to ALT and PFOA and the use of the phrase "liver damage" is misunderstood. ALT is a "leakage" enzyme and may be increased due to necrosis, injury or repair (Cattley RC, Cullen JM. Chapter 45. Liver and Gall Bladder. In (eds Haschek WM, Rousseaux CG, Wallig MA. Toxicologic Pathology. Third Edition. Elsevier:NewYork. 2013 Pages 1509 – 1566). 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 discussed below, those studies that have suggestion of an elevation of ALT remain well-within the expected physiologic range of measuring ALT. Using the term 'damage' in this context is therefore misleading. It is also possible to have quite modest but statistically significant increases in ALT that are not toxicologically relevant (Cattley and Cullen, 2013). Finally, it should be noted that the human half-life of ALT is approximately 47 hours (Hall et al. 2012). This is often not mentioned when cohort studies are conducted examining estimated (modeled) serum PFOA concentrations over time when there is only a single ALT value reported. Finally, it should also be noted that nonalcoholic fatty liver disease is the most common cause of mild elevations of liver enzymes (Gianni et al. 2005).

Several studies are worthy of careful evaluation as they relate to ALT and PFOA either because of the size of the population studied that was exposed to PFOA via the drinking water, they were occupational populations, or 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). Two studies were from the C8 Science Panel (one cross-sectional (Gallo et al. 2012), the other longitudinal based on an estimated cumulative serum (ng/mL-year) model (Darrow et al. 2016), four are occupational studies (2 cross-sectional (Olsen et al. 2007; Sakr et al. 2007a) and two longitudinal (Sakr et al. 2007b; Olsen et al. 2012), and 1 experimental phase 1 clinical trial (Convertino et al. 2018). Collectively, these studies do not suggest "liver damage" – see above - (2- to 4-fold increase) as measured by ALT associated with increasing serum concentrations of PFOA. Although some studies' regression coefficients for PFOA may be statistically significant, the percent variation explained of ALT by PFOA is minimal, at best, and the elevation of ALT very modest (generally an increase of 1 to 3 IU ALT). Nor is there any evidence of increased mortality from increased liver disease in epidemiologic analyses of community-based exposure to PFOA (Darrow et a. 2016) or in occupational cohort mortality studies (Steenland et al., 2012; Raleigh et al. 2014). A study of genetically engineered mice enabled to mimic human lipid metabolism observed an increase in ALT (U/l) only at the highest concentration which approximated a serum concentration 144,000 ng/mL PFOA (Pouwer et al. 2019). These studies are discussed below.

<u>Community studies (n = 2)</u>

1. Gallo et al. (2012).

   Gallo et al. reported on the C8 Health Project cross-sectional data collected in 2005-2006. Their conclusion was their finding of 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 ln-ALT was the independent variable. What is most important to note is that these models had an increasing number of covariates (2, 7, and 11) besides PFOA in each model. The R^2 of these three models were then 0.170, 0.174, and 0.265, respectively. However, the partial R^2 for PFOA (difference between R^2 including and excluding PFOA) remained 0.002, 0.001, and 0.002 for these three models, respectively. This clearly does not suggest that PFOA, although the coefficient was statistically significant

because of the study sample size (N = 47,092), 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. 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 (+1.3IU/L increase in ALT) but plateaued thereafter. The highest decile was 23 IU 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.

2. Darrow et al. (2016).

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

   In their analysis of estimated cumulative exposure of PFOA in the C8 Science Panel's community and worker study on liver function and disease (Darrow et al. 2016), Table S1 of Darrow et al. (see supplement) provided the linear regression coefficients for ln-transformed ALT per ln PFOA. These coefficients for PFOA for the 3 models were Model 1 (β = 0.03); Model 2 (β =0.012); and Model 3 (β = 0.011) adjusted for the same number of covariates in addition to PFOA (2, 7, and 11). The R^2 for these 3 models were 0.15, 0.232, and 0.235 respectively, similar in magnitude to Gallo et al. (see above paragraph) of 0.170, 0.174, and 0.265 for the same models adjusted for the covariates in their cross-sectional analysis, although PFOA in Darrow et al. was an estimated cumulative ng/mL-year metric versus measured (ng/mL). However, unlike Gallo et al., Darrow did not show the partial R^2 for PFOA. Because the coefficients of determination for the Darrow et al. models 1, 2, and 3 are very similar to Gallo et al. (despite a different metric for PFOA), it is highly likely the partial R^2 for PFOA in the Darrow et al. study also remained in the extremely low range of 0.001 to 0.002, thus ln PFOA (ng/ml-years) probably explained very little of the variance of ln ALT in the Darrow et al. paper, too, in Table S1.

   Darrow et al. also estimated, via modelling, 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 needs to be emphasized 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 the latter were Quintile 1 (reference); Quintile 2: 1.04 (0.82, 1.50); Quintile 3: 0.91 (0.64, 1.31); Quintile 4: 0.84 (0.59, 1.21); and quintile 5: 0.87 (0.61, 1.25). The hazard ratio for those prospectively followed since 2006 were Quintile 1 (reference); Quintile 2 (1.19 (0.75, 1.88); Quintile 3: 1.02 (065, 1.61), Quintile 4 (0.94 (0.60, 1.48), and Quintile 5: 0.92 (0.58, 1.47).

Thus, it would be inexcusable 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 draft 2018 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."

Occupational Studies (n = 4)

3. Sakr et al. (2007a)

   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 $R^2 = 0.276$), 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 $\beta = 0.031$, $p = 0.071$. Interpretation of this coefficient means ALT increased.

4. Sakr et al. (2007b)

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

5. Olsen and Zobel (2007)

   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.

6. Olsen et al. (2012)

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

Experimental studies (N = 3)

7. Phase 1 clinical trial

   A 6-week phase 1 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 probability distribution functions, ALT was invariant for any PFOA categorization with highest at 870 – 1530 µM (~360,000 – ~632,000 ng/mL) PFOA. Given the study conditions, authors concluded liver enzymes were not altered at PFOA concentrations that are 5 orders of magnitude greater than the general population measurements of PFOA.

8. General Population (NHANES) studies

It should be noted that several of the studies have 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, 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 relates to the analysis of liver enzyme data and its relationship with serum lipids. As shown by Deb et al. (Int J Hepatology 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 serum 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 2018 offered no insights into this issue between perfluoroalkyls, lipids, and liver enzymes. Neither has Post and Gleason (2019). What is certain, however, is that there has not been reports of 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. Thus, any possible weak association with ALT with perfluoroalkyls, if it is not confounded due to possible associations with obesity, alcohol, and lipids, does not reflect any clinical measures of reported liver disease in the general, community or occupational populations associated with measured PFOA (or PFOS) serum/plasma concentrations at orders of magnitude differences.

9. Pouwer et al. (2019)

In the aforementioned toxicological study by Pouwer et al. that used a genetically engineered APOE*3-Leiden.CETP mouse model to mimic human lipid metabolism in mice fed PFOA (ammonium salt) for 4 and 6 weeks, ALT values were also reported. There were no statistically significant increases in ALT (U/l) values with the control at the end of 6 weeks for the following dose groups: controls (ALT = 95 ± 27); 10 ng/g/d (ALT = 118 ± 70) and 300 ng/g/d (ALT = 123 ± 90) dose groups; but there was a statistically significant ($p < 0.001$) increase in ALT at the 30,000 ng/g/d (ALT = 740 ± 161) dose group. The mean plasma concentrations of PFOA for these 3 dose groups at end of study were 65 ng/mL ± 7, 1524 ng/mL ± 54, and 144,000 ng/mL ± 13,406, respectively. These serum concentrations correspond similarly to environmental, occupational, and toxicological levels as have been

reported in the epidemiological literature. ALT was not elevated in the above phase 1 clinical trial study of similar toxicological magnitude (Convertino et al. 2018).

3M Conclusion on ALT and PFOA: There is no association between PFOA with liver disease including enlarged liver, fatty liver, or cirrhosis. Small percentage changes in ALT, a liver enzyme, are reported inconsistently in epidemiologic studies but within normal physiological ranges. This small magnitude of change, if present, does not indicate liver "damage" by any standard clinical medicine of practice. Confounding cannot be ruled out as possible explanation. Elevated ALT levels have been observed in some laboratory toxicological studies at high doses.

F. Inconsistent Findings Both Within and Across Studies Do Not Support an Association Between PFOA Exposure and With Reduced Vaccine Response in Humans

The NJ Health Effects Subcommittee states that human exposure to PFOA has been associated with a decreased vaccine response (page 11). 3M respectfully disagrees with this conclusion for the following reasons:

- DWQI's review of the epidemiology literature is outdated and fails to accurately reflect the inconsistencies and mostly null findings across studies. DWQI reviewed 5 epidemiology studies. However, there are 9 published studies that have examined PFOA exposure and antibody responses to vaccines (Grandjean et al. 2012; Grandjean et al. 2017; Granum et al. 2013; Kielsen et al. 2016; Looker et al., 2014; Morgenson et al. 2015, Stein et al., 2016a; Stein 2016b; Zeng et al., 2019).
- Existing epidemiologic studies have measured antibody responses to 10 distinct vaccines (e.g., tetanus, diphtheria, rubella, measles, mumps, influenza A (H1N1), influenza A (H3N2), influenza B, enterovirus and coxsackievirus.) It is inappropriate to interpret antibody responses to distinct vaccines as a single health outcome (i.e. reduced vaccine response). Commercially available vaccines differ depending on the nature of the vaccine antigen. Consequently, each vaccine type elicits an immune response through various molecular and cellular mechanisms of the immune system. Further, 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), 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.
- Epidemiologic studies do not provide consistent evidence of a significant association between PFOA exposure and decreased vaccine responses. Mostly null findings have been reported across all studies and results are inconsistent by vaccine type. For example, among the 5 existing studies (Grandjean et al. 2012; Grandjean et al. 2017; Granum et al. 2013; Kielsen et al. 2016; Mogensen et al. 2015) that have examined antibody responses to the tetanus vaccine (the most commonly studied vaccine type)

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). Similar inconsistencies have been observed for PFOS.

- Small changes in antibody response do not necessarily translate to an increased risk of infectious disease. Several epidemiologic studies (Dalsager et al. 2016; Fei et al. 2010; Granum et al. 2013; Impinen et al., 2018; Looker et al. 2014; Okada et al. 2012; Goudarzi et al. 2017) have examined PFOA and PFOS levels and infectious disease outcomes (i.e., occurrence of common colds and otitis media, symptoms of infections, mortality from infectious and parasitic diseases and hospitalizations from infectious diseases). Across all reported measures, mostly null associations between PFOA levels and increased risk of infectious disease outcomes have been observed.
- Further, the National Toxicology Program concluded that there is <u>low confidence</u> that exposure to PFOA is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease) (NTP, 2016). Other regulatory bodies and expert health panels have made the following conclusions regarding immunotoxicity and exposure to PFOA and other perfluoroalkyls:

  <u>Australia Expert Health Panel (2018):</u>

  "*The strongest evidence for a link between PFAS and clinically important immunological effects is for impaired vaccine response. However, the human dose-response/threshold for potential immune effects is very poorly characterized, and the overall human evidence is weak.*"

  <u>Food Standards Australia New Zealand, FSANZ (2016):</u>

  A literature review commissioned by FSANZ concluded that *"there are both positive and negative studies showing associations for increasing PFOS and PFOA concentrations to compromise antibody production in humans. However, to date there is no convincing evidence for increased incidence of infective disease associated with PFOS or PFOA effects on human immune function".*

  <u>Health Canada (2017):</u>

  "Studies in environmentally-exposed populations have identified associations between PFOA levels and decreased antibodies against various illnesses, but the influence of PFOA exposure on clinical immunosuppression (i.e., incidence of illnesses) appears to be more tenuous. Health Canada further commented that "*Although all studies investigated the effects on the immune system, the outcomes were not specific (measured different effects), no clear dose-response was observed, and most associations were weak. Conflicting results were common in the dataset, with variations observed between genders, specific microbial immunoglobulins, PFAAs, infections, mother vs. child exposure, and child years, amongst other characteristics. These flaws impede concluding on a causative mechanism, and the nature of the association remains unclear."* (Health Canada, 2017).

National Institute for Public Health and the Environment (RIVM, 2016):

RIVM concluded that "*associations have been found between exposure to PFOA and a decreased vaccination response*", but the "*evidence is unclear*".

New Jersey Drinking Water Quality Institute (DWQI, 2017):

"Review of epidemiologic studies provides evidence of consistent findings among studies of decreased antibody concentrations following vaccination and PFOA. There is epidemiologic evidence of temporality. However, there are a limited number of comparisons across the same vaccination types, making consistency/specificity difficult to evaluate."

In conclusion, the inconsistent findings both within and across studies do not support an association between PFOA with reduced vaccine response in humans.

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ATTACHMENT B

3M Comments on Proposed Practical Quantification Limits for

PFOA and PFOS and Test Methods

Comments of Proposed PQL for PFOA

The DWQI Testing Subcommittee PQL recommendation is based on the Method Reporting Limit (MRL), using either the reporting limit or lowest calibration standard; whichever is lower. It decided not to recommend a PQL based on the MDL because the MDL is a statistical value while the others are actual concentrations verified within the analysis.

3M agrees that the Testing Subcommittee's decision to base the PQL on the reporting limit or lowest calibration standard is the more appropriate approach. However, the Testing Subcommittee's decision to select the lower of the two values is only valid if the laboratory has performed the required statistical analysis to determine both accuracy and precision at the quantitation level, by applying the QC criteria specified in Section 9.2.5 of EPA 537. The laboratory is required to perform this statistical evaluation when determining their MRL. EPA Method 537 indicates that background contamination is a significant problem for several of the method analytes and suggests that labs maintain a historical record of their laboratory reagent blank data.

The Testing Subcommittee also indicated that they based their decision on justifying the use a lower calibration standard value over the reporting limit value on the assumption that laboratories may be stating a reporting limit higher than what the laboratory is truly capable of achieving since performance data on emerging contaminants such as PFOA is largely client-driven. The Testing Subcommittee should have full knowledge on how testing laboratories are evaluating their lowest calibration standard before using these values in their decision-making process (i.e. what criteria does the laboratory use to demonstrate that all method analytes are below the lowest calibration standard and that all possible interferences from the extraction procedure do not prevent the quantitation of the method analytes at the lowest calibration level; what is the acceptance criteria for the recovery value of the lowest calibration standard).

If the reporting limit alone is not used as the primary source of performance data, and the acceptance criteria on the laboratory's acceptance of the lowest calibration standard is not known, the Testing Subcommittee should be conservative in its approach and use the Mean of RL value of 7.2 ng/L (Table 13). 3M notes that the analysis and recommendation of the Testing Subcommittee failed to consider that EPA Method 537.1 provides acceptance criteria for the lowest calibration level(s) that are ≤MRL in that the value must be within ±50% of the true value, and that the MRL acceptance criteria must be within ± 50% of the true value. The accuracy associated with the MRL or lowest calibration standard should be considered when setting the PQL. Taking the Mean of RL value 7.2 ng/L and adjusting for the 50% acceptance criteria (±3.6 ng/L), brings the PQL for PFOA to 10.8 ng/L, or 11 ng/L when rounded to two-significant values. This value is very close to the PFOA MCL and groundwater quality standard proposed.

It is recommended that laboratories, when reporting analytical test data, also include the analytical method uncertainty associated with the reported results. There are several references

in the literature for how to determine measurement uncertainly. This type of information is useful, especially when reviewing test data that is reported at or near the PQL.

Comments of Proposed PQL for PFOS

The Testing Subcommittee derived the PQLs for PFOS be using non-parametric statistical analyses of the mean and median from actual laboratory data. It derived the recommended PQL of 4.2 ng/L using the bootstrap upper confidence limit of the low calibration standards. The Testing Committee reported that its bootstrap analysis of the low calibration standards found that 15 of the 16 laboratories reviewed can meet the recommended PQL of 4.2 ng/L 95 % of the time.

3M believes that the Testing Subcommittee's decision to base the PQL on the lowest calibration standard is only valid if the laboratory has performed the required statistical analysis to determine both accuracy and precision at the quantitation level, by applying the QC criteria specified in Section 9.2.5 of EPA 537. The Testing Subcommittee should have full knowledge on how testing laboratories are evaluating their lowest calibration standard before using these values in their decision-making process (i.e. what criteria does the laboratory use to demonstrate that all method analytes are below the lowest calibration standard and that all possible interferences from the extraction procedure do not prevent the quantitation of the method analytes at the lowest calibration level; what is the acceptance criteria for the recovery value of the lowest calibration standard).

3M recommends that NJDEP should be conservative and use the Bootstrap Upper Confidence Limit of RLs (Table 12) value of 6.6 ng/L. 3M notes that the Testing Subcommittee's analysis and recommendation failed to consider that EPA Method 537.1 requires that the acceptance criteria of calibration levels that are ≤MRL must be within ±50% of the true value, and that the MRL acceptance criteria must be within ± 50% of the true value. Therefore, NJDEP must also consider this accuracy criteria when setting the PQL. Taking the Mean of RL value 6.6 ng/L and adjusting for the 50% acceptance criteria (±3.3 ng/L), brings the PQL for PFOS to 9.9 ng/L or 10 ng/L when rounded to one-significant value. Like PFOA, this value is very close to the MCL proposed by NJDEP.

3M further recommends that laboratories, when reporting analytical test data, also include the analytical method uncertainty associated with the reported results. There are several references in the literature for how to determine measurement uncertainly. This type of information is useful, especially when reviewing test data that is reported at or near the PQL.

PFOA and PFOS Test Method Considerations

As NJDEP notes, EPA Method 537.1 is likely to be the analytical method most often used to test for PFAS, including PFOS and PFOA. Based on the 3M Environmental, Health and Safety Laboratory's extensive experience using EPA Method 537.1, the drawbacks to EPA Method 537.1 include the following.

(1) EPA method 537.1 **is validated for drinking water only** but is often modified by contract labs and applied to other environmental matrices for which it is **not validated**. These modified methods **do not have** consistent sample collection, analytical operating

parameters, nor quality assurance parameters. We recommend other methods as they are validated for several matrices (i.e. groundwater, wastewater and soils, and sludges) and some have more efficient workflows.

a. 3M has developed a direct injection isotope dilution method which was **validated for drinking water, wastewater, and groundwater and was published in the peer reviewed literature 2013 (Wolf and Reagen 2013)**. The 3M method **greatly simplifies the sample preparation procedure** and was shown that for a 28-day sample holding times the method reporting limits were 10 ng/L – 20 ng/L.

b. As of 2019 EPA has a draft method SW846-8327 for the analysis of groundwater, surface water, and wastewater. The method uses direct injection and stable isotopes for determination of surrogate recovery and is similar to ASTM 7979.

c. As of 2019 EPA has a draft method SW846-8328 for the analysis of groundwater, surface water, wastewater, and solid samples. It a SPE-isotope dilution method.

(2) A second drawback to EPA 537.1 is that the laboratory is required to extract the entire sample as a single replicate, should a mistake or accident occur during sample preparation in the lab and/or an analytical equipment malfunction occur during analysis, the sample could be lost, and reanalysis of the samples would not be possible as the entire sample was already used**.**

(3) A third drawback to EPA 537.1 is the extraction procedure is lengthy and the opportunities for sample contamination can occur at any point during the multi-step procedure (i.e. contamination of the solid phase extraction (SPE) cartridge during the clean-up and conditioning of the SPE cartridge, extraction of the sample, sample bottle and cartridge rinse and elution, extract concentration blown down and addition of internal standard). Since contamination can occur at any point during the multi-step sample preparation procedure, the laboratory must prepare a sufficient number of method blanks to demonstrate that contamination has not occurred. If the method blanks show that contamination has occurred, the laboratory is unable to re-prepare the sample as the entire sample is consumed during the sample extraction procedure.

With the availability of isotopically labeled internal standards for the target analytes listed in EPA Method 537.1 (with the exception of perfluorotridecanoic acid for which the isotopically labeled internal standard for perfluorododecanoic acid is used as a surrogate internal standard), the 3M EHS Laboratory developed a direct injection isotope dilution method which was **validated and published in the peer reviewed literature 2013 (Wolf and Reagen 2013)**. The direct injection method **greatly simplifies the sample preparation procedure**. As with the 3M Laboratory SPE method, sample containers are spiked with a suite of isotopically-labeled internal standards and isotopically-labeled surrogate recovery standards prior to sample collection. The samples are prepared by diluting 1:1 with methanol. Again, the 3M EHS Laboratory has demonstrated that the direct injection method is appropriate through the use of target analyte field matrix spikes and target analyte travel blank spikes. At 28-day sample holding times, fortified laboratory reagent water samples analyzed using the 3M EHS Laboratory direct injection isotope dilution method had method reporting limits of 10 ng/L – 20 ng/L. **3M recommends the**

use of this direct injection isotope dilution method that was validated and published in the peer review literature in 2013.

The 3M EHS Laboratory performed a modified version of the EPA Method 537, validated and published the modified solid phase extraction (SPE) method in 2011 (Wolf and Reagen 2011). If a SPE method is used, we recommend the 3M SPE method as it is more robust and has improved quality assurance elements. The difference in the 3M SPE method as compared to EPA Method 537 I as follows

(1) The 3M SPE method uses internal standards for all target analytes, compared to Method 537 which uses just three IS compounds specified in the EPA method based on their functional group;

(2) The 3M SPE method adds the suite of isotopically-labeled internal standards and isotopically-labeled surrogate recovery standards to the sample container prior to sample collection, compared to Method 537 where surrogate recovery standards are added prior to sample extraction and internal standards are added after the SPE extraction, elution and blow down procedure. The addition of the internal standards to the sample container prior to sample collection eliminates the need extract the entire 250-mL sample and eliminates the need to solvent rinse the sample container after the entire sample has been passed through the SPE cartridge. The reason for solvent rinsing the sample container during the extraction procedure is due to the potential of several of the target analytes to adsorb to the surfaces of the sample container. Solvent rinsing the sample container is necessary when analyte specific internal standards are not used. However, if analyte specific internal standards are used and added to the sample container prior to sample collection, the potential for adsorption can be accounted for. The 3M EHS Laboratory has demonstrated that this method of adding internal standards and surrogate recovery standards to the sample container prior to sample collection is appropriate, through the use of target analyte field matrix spikes and target analyte travel blank spikes.

(3) The 3M EHS Laboratory SPE method allows for the preparation of a laboratory sample duplicate, laboratory matrix spike or re-analysis of the sample as needed, because unlike Method 537 the entire 250-mL sample is not consumed.

The 3M EHS Laboratory performed a modified version of the EPA Method 537, validated and published the modified SPE method in 2011. The difference in the 3M SPE method as compared to EPA Method 537 is

(4) Use of internal standards for all target analytes as opposed to just three IS compounds which were specified in the EPA method based on their functional group.

(5) The 3M method adds the suite of isotopically-labeled internal standards and isotopically-labeled surrogate recovery standards to the sample container prior to sample collection as opposed the EPA method where surrogate recovery standards are added prior to sample extraction and internal standards are added after the SPE extraction, elution and blow down procedure. The addition of the internal standards to the sample container prior to sample collection eliminates the need extract the entire 250-mL sample and eliminates the need to solvent rinse the sample container after the entire sample has been passed through the SPE cartridge. The reason for solvent rinsing the sample container during the extraction procedure is due to the potential of several of the target analytes to adsorb to the surfaces of the sample container. Solvent rinsing the sample container is necessary when analyte specific internal standards are not used. However, if analyte specific internal standards are used and added to the sample container prior to sample collection, the potential for adsorption can be accounted for. The 3M EHS Laboratory has demonstrated that this method of adding internal standards and surrogate recovery standards to the sample container prior to sample collection is appropriate, through the use of target analyte field matrix spikes and target analyte travel blank spikes.

(6) The 3M EHS Laboratory SPE method allows for the preparation of a laboratory sample duplicate, laboratory matrix spike or re-analysis of the sample as needed, since the entire 250-mL sample is not consumed.

References

1) S.T. Wolf, W.K. Reagen, "Method and Validation for the Analysis of Perfluorinated Compounds in Water by Per-sampling Isotope Dilution-Direct Injections-LC/MS/MS." *The Royal Society of Chemistry,* Anal. Methods, 5, 244-2454 (2013)

2) S.T. Wolf, W.K. Reagen, "Method for the Determination of Perfluorinated Compounds (PFCs) in Water by Solid-Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." *The Royal Society of Chemistry,* Anal. Methods, 3, 1485 (2011)