EXTERNAL SCIENTIFIC PEER REVIEW COMMENTS ON PROPOSED PUBLIC HEALTH GOALS FOR PERFLUOROOCTANOIC ACID (PFOA) AND PERFLUOROOCTANE SULFONIC ACID (PFOS) IN DRINKING WATER

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Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

This document contains the comments received from the external scientific peer review of the Office of Environmental Health Hazard Assessment's *Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water*. The draft document was released for public comment on July 21, 2021, and pursuant to Health and Safety Code section 116365(c)(3)(D), was submitted for scientific peer review following the closure of the comment period.

The peer review was coordinated by the CalEPA External Scientific Peer Review Program (the Program) via an agreement with the University of California, in accordance with Health and Safety Code section 57004. Reviewers identified by the University of California and approved by the Program were asked to review and comment on the draft document's scientific assumptions, findings, and conclusions as summarized in Attachment 2 of the peer review request (provided below).

Reviewers:
1. John Adgate, Ph.D., MSPH
Department of Environmental and Occupational Health
Colorado School of Public Health
University of Colorado Anschutz Public Health Campus
Aurora, Colorado

2. Hindrik (Henk) Bouwman, Ph.D.
School of Biological Sciences
North-West University
Potchefstroom, South Africa

3. Vaia Lida Chatzi, Ph.D.
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4. Jamie DeWitt, Ph.D., DABT
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5. Jennifer Schlezinger, Ph.D.
Department of Environmental Health
Boston University School of Public Health
Boston, Massachussetts

6. Robyn Tanguay, Ph.D.
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The Sinnhuber Aquatic Research Laboratory
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Attachment 2: Scientific Assumptions, Findings, and Conclusions to Review

Reviewers are asked to determine whether the scientific work product is "based upon sound scientific knowledge, methods, and practices."

OEHHA requests that you make this determination for the chemicals assessed in the draft document, "Public Health Goals – Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water." An explanatory statement is provided for each proposed PHG to focus the review.

Assumptions, Findings, and Conclusions

1. **Perfluorooctanoic Acid**

After reviewing the published literature on perfluorooctanoic acid (PFOA), OEHHA concludes that cancer is the primary adverse health effect associated with human exposure to this chemical. OEHHA is basing the PHG on this critical endpoint and its supporting studies. For the proposed PFOA PHG, OEHHA is using human epidemiological data for kidney cancer to derive the cancer slope factor (CSF). The underlying model used for deriving the CSF involves a linear regression between PFOA exposure and cancer relative risk. When a PHG is based on cancer, OEHHA also calculates a health-protective concentration for noncancer effects. The noncancer health-protective concentration for PFOA is based on the no-observed-adverse-effect concentration (NOAEC) for an increased risk of liver damage in humans.

PHG: US EPA reviewed the literature for PFOA and cancer published up to December 2015 and concluded that there was "suggestive evidence of carcinogenic potential" for PFOA and that, "Epidemiology studies demonstrate an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population" (US EPA, 2016b). In 2017, the International Agency for Research on Cancer (IARC) classified PFOA as possibly carcinogenic to humans (Group 2B) based on limited evidence in humans and in experimental animals (IARC, 2017a). With regards to the human evidence, IARC concluded that, "A positive association was observed for cancers of the testis and kidney." The National Toxicology Program (NTP) in its technical report on the carcinogenesis studies of PFOA in rats concluded there was "*clear evidence of carcinogenic activity* of PFOA" in male rats and "*some evidence of carcinogenic activity* of PFOA" in female rats (NTP, 2020).

Because several high quality human cancer studies were available for PFOA, dose-response analyses were performed using human data rather than animal data. The strongest and most consistent human evidence linking PFOA to cancer involves studies

of kidney cancer. Based on evaluations of statistical power, generalizability, potential bias and confounding, and other factors, OEHHA selected the human studies by Shearer et al. (2021) and Vieira et al. (2013) for cancer dose-response analyses. Two other epidemiologic studies identified associations between PFOA and kidney cancer (Steenland and Woskie, 2012; Barry et al., 2013). The high exposure occupational study by Steenland and Woskie (2012) was not used for dose-response analysis because information on a range of exposures more relevant to the general population was available from the Shearer et al. (2021) and Vieira et al. (2013). The study by Barry et al. (2013) was not used for dose-response analysis because it was performed in the same study area as the Vieira et al. (2013) study and these two studies likely involved a number of the same participants. Vieira et al. (2013) was selected over the Barry et al. (2013) because it presented dose-response data using a more appropriate exposure metric. An occupational study by Raleigh et al. (2014) did not report an association between PFOA and kidney cancer, but it used an inhalation exposure model that was not validated, and a detailed analysis of this study highlighted limitations that may have reduced the sensitivity of the study.

To derive the PHG, the CSF for each study was first calculated as the excess cancer risk associated with each ng/ml increase in serum PFOA (CSF_{serum}). This was then combined with the OEHHA-derived clearance rate (CL, see below) of 0.28 ml/kg-day to calculate a CSF_{intake}, which is the excess cancer risk associated with each ng/kg-day intake of PFOA. Because the studies selected for dose-response analysis were case-control studies, direct estimates of absolute risk were not available. Thus, the US lifetime kidney cancer risk and lifetime renal cell carcinoma risk in males were used as the baseline risk for the Vieira et al. (2013) and Shearer et al. (2021) studies, respectively. The dose-response slopes calculated from these two studies differed by six-fold, and the difference was not statistically significant. The geometric mean of the CSF_{intake} from each study was used to derive an overall CSF_{intake} of 0.0026 $(ng/kg\text{-}day)^{-1}$ for PFOA. Age sensitivity factors were not applied because the NTP (2020) animal bioassay showed no increased risks in combined adenomas and carcinomas from perinatal exposure compared to exposures later in life. Using a cancer risk of 10^{-6}, the CSF_{intake} of 0.0026 $(ng/kg\text{-}day)^{-1}$, and a 70-year lifetime weighted average drinking water intake rate of 0.053 L/kg-day, the proposed PHG is 0.007 parts per trillion (ppt).

Noncancer health-protective concentration: Because of the large number of high quality human studies of noncancer effects from PFOA exposure, dose-response analyses were performed using human data rather than animal data. OEHHA determined that the most sensitive noncancer endpoints for PFOA are immunotoxicity, liver toxicity, and alterations in lipid homeostasis. OEHHA selected the no-observed-adverse-effect concentration (NOAEC) of 9.8 ng/ml for an increased risk of liver toxicity, as indicated by elevated alanine aminotransferase (ALT) levels (Gallo et al., 2012) exceeding clinically based reference levels used by the International Federation of Clinical Chemistry and Laboratory Medicine, as the point of departure (POD). After applying the

CL of 0.28 ml/kg-day, a total uncertainty factor (UF) of √10, a relative source contribution (RSC) of 20%, and drinking water intake rate of 0.053 L/kg-day, the resulting health-protective concentration for noncancer effects is 3 ppt.

OEHHA-derived PFOA clearance rate (CL): The fact that there are dramatic differences in PFOA half-life between species necessitates incorporation of kinetic considerations in extrapolating dose from animal studies to humans. Even when evaluating human studies, which are often based on serum concentrations, kinetic considerations are important in conversion of the POD to a chronic intake dose per unit body weight. OEHHA determined the best method to address this is the application of a human clearance factor. Thus, a CL of 0.28 ml/kg-day was developed for PFOA based on human studies reporting exposure through drinking water, as well as other routes.

2. Perfluorooctane Sulfonic Acid

After reviewing the published literature on perfluorooctane sulfonic acid (PFOS), OEHHA concludes that cancer is the primary adverse health effect associated with human exposure to this chemical. OEHHA is basing the PHG on this critical endpoint and its supporting studies. For the proposed PFOS PHG, OEHHA is using the benchmark dose (BMD) approach for CSF derivation from animal toxicology studies. The noncancer health-protective concentration for PFOS is based on the lowest-observed-adverse-effect concentration (LOAEC) for increased total cholesterol in humans.

PHG: Although there are a few epidemiologic studies that show some association of PFOS with breast, liver, and bladder cancer, the results are mixed or the sample sizes are small. OEHHA did not identify any epidemiologic studies of PFOS that could be used for quantifying cancer risk in humans. Thus, the proposed PHG for PFOS is based on cancer data in laboratory animals. Butenhoff et al. (2012b) reported hepatocellular tumors in male and female Sprague Dawley rats and pancreatic islet cell tumors in male rats exposed through the diet for two years. OEHHA's cancer mode of action analysis and other mechanistic considerations support the relevance of these tumors to humans and the use of linear extrapolation in deriving the PFOS CSF. The CSF of 15.6 $(mg/kg\text{-}day)^{-1}$, derived from liver and pancreatic tumors in male rats, is used to derive the proposed PHG for PFOS. Due to the similarity of PFOS to PFOA in chemical structure and toxicological profile, early-in-life exposure to PFOS is not expected to result in increased cancer risk compared to exposures later in life, as was shown for PFOA. Thus, age sensitivity factors are not applied in calculating the PHG. Using a cancer risk of 10^{-6}, the CSF of 15.6 $(mg/kg\text{-}day)^{-1}$, and a lifetime weighted average drinking water intake rate of 0.053 L/kg-day, the proposed PHG is 1 ppt.

Noncancer health-protective concentration: Because of the large number of high quality human studies of noncancer effects from PFOS exposure, dose-response analyses

were performed using human data rather than animal data. OEHHA determined that the most sensitive noncancer endpoints for PFOS are immunotoxicity and alterations in lipid metabolism or production. OEHHA selected the lowest-observed-adverse-effect concentration (LOAEC) of 16.4 ng/ml for an increased risk of elevated total cholesterol (Steenland et al., 2009) above the clinical reference level published by the American Heart Association as the POD. After applying the CL of 0.39 ml/kg-day, a combined UF of 10, an RSC of 20%, and drinking water intake rate of 0.053 L/kg-day, the resulting health-protective concentration for noncancer effects is 2 ppt.

OEHHA-derived PFOS clearance rate (CL): The fact that there are dramatic differences in PFOS half-life between species necessitates incorporation of kinetic considerations in extrapolating dose from animal studies to humans. Even when evaluating human studies, which are often based on serum concentrations, kinetic considerations are important in conversion of the POD to a chronic intake dose per unit body weight. OEHHA determined the best method to address this is the application of a human clearance factor. Thus, a CL of 0.39 ml/kg-day was developed for PFOS based on more recent human studies reporting exposure through contaminated drinking water.

Additional Considerations

Reviewers are not limited to addressing only the specific topics presented above, and are asked to consider the following:

(a) For each proposed PHG, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in the derivation of PHGs based on cancer.

(b) For the proposed noncancer health-protective concentrations, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in the derivation of each health-protective concentration.

(c) For each chemical reviewed, please comment on whether a relevant study useful for assessing dose-response relationship or otherwise informing the PHG development was missed.

(d) PHGs must be protective of known sensitive populations. Please comment on whether each PHG is health protective of sensitive populations.

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February 13, 2022

Review of "Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water," Draft date: July 2021.

Per the Reviewer instructions, and based on my expertise and experience, I have reviewed the findings, assumptions, and conclusions I agreed I could review with confidence, which are: 1. Cancer Epidemiology (for PFOA); 2. Environmental Epidemiology (for PFOA and PFOS); 3. Exposure Assessment and Toxicokinetics (for PFOA and PFOS); and 4. Toxicology and Risk Assessment.

Requested Summary Statement: Based on my review I have determined that the CalEPA organization's assumptions, findings, and conclusions, including the scientific material from the peer reviewed scientific literature and other authoritative sources, rely on materials that are based on sound scientific knowledge, methods, and practices. The overall write up and the methods employed are based on sound science and the methods and approaches used are defensible. Thus their use in cancer and non-cancer health risk assessment also defensible.

Summary Comments: The overall draft is well written, with the major assumptions clearly identified and rationale clear in most places. There are instances where the presentation could be improved by more explicit promulgation and/or presentation of the range of uncertainties present in both the underlying data and modeling approaches applied to those data. Some of this is in the summary tables, for example, the ones presenting the range of PODs using different modeling data sets and approaches, but this general approach should be extended to show how these uncertainties address the final endpoints, e.g., PHGs and HPCs. My judgment is that relatively minor improvements in the presentation and interpretation of the human and animal studies will improve the clarity, transparency, and scientific rigor of the document.

A second issue of note is that much of the analysis of human and animal data seems over concerned with p-values, particularly in epidemiologic studies, an approach that the epidemiology research community is moving away from in favor of reporting all findings, uncertainty bands, and trends (see, for example, Savitz, DA, 2013 and related publications on this topic). Similarly, the analysis of animal studies in also focused on counting the number of studies with positive findings, which is only meaningful if the total number of studies with and without positive findings for each endpoint are also enumerated and then summarized while considering their overall findings in the larger scientific context. So while the approach taken is defensible, the document could be

improved by summarizing what is already a comprehensive accounting of the existing literature and more clearly summarizing how, for example, the endpoint analysis from animal studies does or does not contribute to overall weight of evidence findings of the cancer or noncancer endpoints for both PFOA and PFOS.

In my judgment the overall the approach to updating previous authoritative review documents from the USEPA and ATSDR is reasonable and, despite my concerns described in the last paragraph, the report authors do a good job indicating where additional information has been found and incorporated into their summary. I will note that sometimes the human and animal data tables do not stand alone as clearly interpretable units that are well integrated into the surrounding text. These should be examined for consistency of message, units and footnotes consistency, etc. A few examples are provided below, but also note soe formatting issues (e.g., tables break across Pages, headers are missing, etc.) that, if addressed, will improve readability.

Comments on the Major Areas of Review

The Summary pulls together the essential information from the various sections in accessible language. The comments below refer to both the summary and the presentation of these issues in the body of the document.

1. Cancer Epidemiology (for PFOA);
    - The proposed PHG and HPC are consistent with the underlying science and the accompanying analysis (see comments on study selection/quality below)
    - This scientific presentation could be improved by providing uncertainty bands around the cancer potency estimates. This is especially important for the PHG of 0.007 ppt, a level that is orders of magnitude lower than current best practice detection limits for PFOA in water and other environmental media, but could be done for the HPC (and PFOS slope factor) as well

2. Environmental Epidemiology (for PFOA and PFOS);
    - This section uses a systematic approach to identifying and critiquing studies, with a focus on the important potential confounders and other scientific issues (e.g., co-occurrence of other PFAS) and their potential effect on the overall analysis
    - The weight of evidence determination for the studies to use for the PFOA/ kidney cancer determination is reasonable and based on high quality studies with a large number of participants
    - Use of the geometric mean for cancer potency is reasonable, per my summary comment above, presenting the range of estimates is also important

to make clear the relationship between this finding and the uncertainty around the PHG

3. Exposure Assessment and Toxicokinetics (for PFOA and PFOS);
    - The use of California specific drinking water rates is a defensible choice that is clearly articulated
    - Continued use of the default RSC is justified based on the lack of any other compelling data
    - The toxicokinetic modeling approach is reasonable: further characterizing the impact of uncertainties (particularly relative to other sources of uncertainty) is important for transparency

4. Toxicology and Risk Assessment
    - The final risk characterization section is brief but has the essential elements
    - I concur that serum/plasma is the "least uncertain" approach to compare to toxicokinetic model outputs.
    - The comparison to other State standards/health guidance levels is important, incorporating uncertainty bands around the PHG for PFOA in particular, will improve transparency and provide context for what the State of CA is proposing, though it would be useful to repeat that the typical detection limits in water are in the range or, for the PFOA, well above the PHG
    - On page 228 a more detailed explanation of why OEHHA's clearance factors differ from the USEPA's should be provided here.

Additional Minor Comments on specific pages/issues

Chapter 3: PRODUCTION AND USE.

Page 27: Note that the description of the Beesoon et al 2012 study is confusing "indoor air concentrations in the dust samples" makes little sense—if it's PFAS adsorbed to particulate matter measured in indoor air say that; if it's PFAS concentrations measured in house dust that's a different environmental media.

Page 31: Human exposure paragraphs: the text does note that the major route of exposure in the general population is typically food, which is correct, but should also tell the reader that for many of the studies in highly exposed human populations are due to water contamination (e.g., the C8 studies, Ronneby, etc.) in which food, indoor air, dust, precursors are a relatively small contributors to overall exposure in those populations. While you do cite the Vestergren and Cousins (2009) paper on PFOA concentrations in water, the more salient point for this document is that the studies done in high drinking

water exposure human populations are the scientific basis for standards/health guidance in most states and California's PHGs for PFOA/PFOS, and there are uncertainties inherent in translating findings from this context populations with more typical exposure profiles.

Chapter 4: TOXICOKINETICS

Pages 35-40: suggest you avoid the use of "applied dose" as it is a vague concept. Wherever you can use either administered or internal dose.

Page 40: "Clearance factor" (CF is used later in the document) important in this document. It's important for conversions but it's not in the table of acronyms at the beginning. Furthermore, the units in Table 4.5.1 are unclear (and not consistent with the Summary) and there are unstated assumptions on the conversation of PFAS volume to mass. In general this issue is much more clearly explained in the section on ADIs and related calculations on pages 182 and 197.

Chapter 5: EVIDENCE OF TOXICOLOGICAL EFFECTS

Page 65: The write up here is too focused on statistical significance and does not reflect best practice in the interpretation of epidemiological studies. A more nuanced presentation will look at both direction and trend in all the endpoints is warranted here: see Savitz reference and related papers in the literature.

Page 66: Noting the sample size in the text here, i.e., the N for the vaccine response studies in this section, is important and places in context the important observation that the BMDL is 6-13 fold lower than the lowest "dose" in these studies.

Page 95: Table 5.4.2 is very important and would be it would be better if it did not break across pages so the reader can compare the human and animal data on PFOA and PFOS and liver toxicity.

Page 96: The text at the top of the page here probably belongs in the lipids section and not before it.

Page 104: Again, counting studies versus and a more nuanced presentation is not the most defensible scientific approach. The qualitative scoring needs to be explained more clearly in the main text as it refers to an appendix here.

Page 105: There is more recent data particularly in humans on half-lives particularly in highly exposed communities. While some of these studies are small, they show shorter half-lives (at least in highly exposed communities) though there's a considerable

variance between people. My point here is that the half live number cited from the Olsen study may not reflect what is observed in these high exposure communities; what that means for more typical serum levels found in the general population should be addressed somewhere here.

Chapter 6: DOSE RESPONSE ASSESSMENT

The general approach used here is scientifically justified, though having uncertainty bands would help in illustrate the uncertainties. Also, consider comparing various approaches for dose response and give the reader confidence that the various approaches provide evidence of consistency independent of the model chosen.

Page 182: the explanation/use of the PFOA clearance factor here is much clearer than earlier text

Page 184: The animal section here is not terribly helpful given the decision to use human data; there should at least be an explicit statement what, if anything, the animal data adds. In contrast, the PFOS human studies analysis is clear and justified.

Page 200: Section 6.2—the cancer potency derivation process is clearly described, though the significance of, for example, bolded text is sometimes confusing.

Page 228: See comment above under risk characterization.

References

Savitz DA. Commentary: reconciling theory and practice: what is to be done with p values? Epidemiology. 2013 Mar;24(2):212-4. doi: 10.1097/EDE.0b013e318281e856. PMID: 23377090.

The Scientific Basis of the Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water

Peer Review

Reviewer:
Prof Hindrik Bouwman
North-West University
South Africa
February 2022

According to the letter: Additional information for the reviewer search for external scientific peer review of the proposed public health goals for PFOA and PFOS, I have been tasked to review the following aspects and sections of the First Public Review Draft of the document Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water, dated July 2021. This review will cover the entire draft document; however, the focus of this review will be on 1) Environmental epidemiology, and 2) Mammalian toxicology, as set out, following.

Based on my expertise and experience, I am reviewing the findings, assumptions, or conclusions I agreed I could review with confidence:" and list them by number, as they are referred to in Attachment 2 of the review request.

1) Environmental epidemiology (for PFOA and PFOS): OEHHA based the noncancer health-protective concentrations for PFOA and PFOS on liver damage and increased total cholesterol in humans, respectively. For environmental epidemiology, I evaluated immunotoxicity, liver toxicity, lipid homeostasis, and developmental and reproductive effects, to determine whether OEHHA utilized the epidemiology data appropriately in hazard identification and in the derivation of health-protective concentrations for noncancer effects. For this, I focused on the following chapters and sections (and appendices cited therein).

• Chapter 5 (*Evidence of Toxicological Effects*), sections with the headings *Recent Human Evidence* and *Conclusions*.
• Chapter 6 (*Dose-Response Assessment*), Section 6.1 for noncancer dose-response analyses of human studies and acceptable daily dose derivation.
• Chapter 7 (*Health-Protective Drinking Water Concentrations*), Section 7.1 for the calculation of noncancer health-protective drinking water concentrations for PFOA and PFOS.
• Chapter 8 (*Risk Characterization*).

AND

2) Mammalian toxicology (for PFOA and PFOS): OEHHA evaluated the major health effects in humans and laboratory animals associated with exposure to PFOA and PFOS, including liver toxicity, immunotoxicity, thyroid toxicity, developmental and

reproductive toxicity, effects on lipids and cholesterol, and cancer. The proposed PHG for PFOS was based on tumors observed in laboratory animals. For mammalian toxicology, I reviewed OEHHA's conclusions regarding the toxicity of PFOA and PFOS in animals, as well as the decision to evaluate PFOS as a carcinogen, and that there is sufficient rationale to utilize a default linear model for estimating the cancer potency of PFOS for PHG derivation. For this effort, the reviewer should focus on the chapters and sections (and appendices cited therein) listed below.

• Chapter 5 (Evidence of Toxicological Effects), all sections except Recent Human Evidence for each chemical and endpoint in the chapter.
• Chapter 6 (Dose-Response Assessment)
  - Section 6.1.1 for noncancer dose-response analysis of PFOA animal studies,
  - Section 6.1.2 for noncancer dose-response analysis of PFOS animal studies,
  - Section 6.2.2 for cancer dose-response analysis of PFOS animal studies.
• Chapter 7 (Health-Protective Drinking Water Concentrations), Section 7.2.2 for calculation of the PFOS health-protective concentration based on cancer in animals.
• Chapter 8 (Risk Characterization).

REVIEW

General

1) Overall, I found the use of literature excellent. Almost a 1000 papers and documents were directly considered, in addition to the references in the major reports that were used. The use of the literature in 2.1 (p18 and onwards) and in other sections, with tabulated summaries in Appendix 1 (p280 onwards), and with additional literature in Appendix 7 (p319 onwards) is evidence a very wide net cast. The librarians have done excellent work. Together with the additional literature in prior assessments, and actually identifying literature that were missed or not considered in the later, shows that a huge amount of work has been done. There were also instances of literature added that came out after the cutoff date, showing initiative and thoroughness.

Key documents and papers were rigorously selected and considered. It is very unlikely that any one or few papers would suffice for the aims of establishing Public Health Goals, but the combinations of papers selected, and the careful consideration of those that were not add tremendously to this work.

Positive, negative, ambivalent, and contradictory studies were considered and included in all category assessments. Contradictions particularly were well argued throughout and clear reasons for selection or rejection, for the current purpose, given.

I appreciate the bolding of relevant rows in the key tables and corresponding text.

2) The consistency in style, grammar, and layout throughout was remarkable. This made it easy to read and anticipate what would be coming. I indicate a couple of instances for improvements, following in *Specific Comments* below. All too often, style,

layout, and grammar of review documents vary due to multiple authors and various editorial input.

3) I am not aware of any scientific issues not mentioned in this document that should have been considered.

4) Suffice to say, that after reviewing the entire front text, I have found no problems with the approach, analyses, selection of methods, consideration of weak and strong papers and reports for the current purpose, impacts that these weak and strong points have had on conclusions, sample size, selection bias, participation rates, confounder identification and adjustments, consistent findings between human, animal, and mechanistic studies, the influence of co-variant pollutants, and reverse causality (amongst a host of others) were convincingly presented.

Therefore, I have no hesitation in saying that the best has been done with the data and information available at the time that the Public Health Goals were derived for this Draft.

Undoubtable, there will be inputs and comments on the methods, assumptions and results of calculations, but when reading the argumentations and considerations of how each PHG was eventually calculated (especially for the noncancer issues, but equally for the cancer issues that I also examined), these are unlikely to have any major influence, if at all. This report assessment was not a 'hunt-for-the-lowest', but a 'search-for-the-best".

Specific comments

- From my background, I find it strange that both ppt and ng/L (or similar) were used inconsistently and interchangeably. Table S1 on p10 for instance, is probably the table that will be read most and quoted. There might be reasons for using ppt that I'm not aware of, but I suggest that wherever ppt is used, the equivalent mass-based concentration unit is provided to avoid ambiguity.
- The Uncertainty Factors on P20 are good (+Appendix 2).
- RSC on p20 and Appendix are 4 good.
- The argumentation on why dermal uptake as part of DWI (p21) was not considered should be better argued, and also brought in relation with Section 4.2. Specifically, the exclusion of dermal uptake via washing and swimming needs to be addressed. I understand the aim is purely related to PHG for drinking water, but clear reasoning and motivation should be given for excluding uptake from other purposes that may affect uptake from use of the same water.

Chapter 3, no comments other than, excellent.

Chapter 4

- The last two complete sentences on P37 are not clear to me.
- Excellent discussion and comparisons of a good number of PBPK models
- P45, #4, 1st sentence, include "….<u>infant</u> life…".

- P46, #4, 1st line, "…species with PFOA and PFOS half-lives…".
- Section 4.7, very good comparisons between models.

- Chapter 5
  - 5.1.4 Good argumentation and critical assessments.
  - Consistent independent reinforcement from the various papers provides confidence of associations at appropriate levels of exposure.

Chapter 6

- Good consideration and weighing of the strengths of each study.
- Good selection of NOAEC/LOAEC values rather BMD methods in most cases.
- Confounding, selection bias, consistency, reverse causality, sample size, study design, relevant clinical outcomes, alternative explanations, and multiple studies were considered and compared.
- P182, the reasoning for the use of √10 to calculate UF for ADD was well done.
- P183, make clear that the calculation was for PFOA. Suggest you add "The ADD for PFOA was calculated as:", as for PFOS on p197.
- The consideration of children in this regard was excellent and convincing.
- Section 6.2 (cancer text) I only scanned, as this was a bit out of my prevue. But, from what I read, seems to be the same quality as the prior text.

Chapter 7

- The way that relative source contributions were considered and worked in was exhaustive and convincing.
- P225, state clearly that the first calculation is for PFOA, and the second for PFOS (here, mass-based units and ppt within the same formulas are striking, and I am not sure why ppt is used).
- Table 8.1
  - I suggest adding the date each of these regulations or guidelines were published.
  - I suggest adding the proposed (draft) PHG here as well, for comparison.

Overall assessment

Based on the above, it is my opinion that the text provides a balanced and unbiased assessment with all due qualifications and respect given to all the authors and institutions, and that the PHG values have been derived with utmost care and consideration, and applicable for the intended purpose.

Note on abbreviations

I noticed the following that were not in the list, acknowledging that the heading refers to commonly used abbreviations.

- PECO
- C? (a single letter abbreviation?)
- MLE
- MMR vaccine

The opinions and conclusion presented in this review are my own, based on my knowledge and experience. I have had no contact with anyone on this matter. I have no conflicts of interest to declare.

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"Based on my expertise and experience, I am reviewing the findings, assumptions, or conclusions I agreed I could review with confidence:"

Cancer epidemiology

Chapter 5 (Evidence of Toxicological Effects), Section 5.7.1 for human evidence of cancer and Section 5.7.4 for conclusions regarding cancer.

• Chapter 6 (Dose-Response Assessment), Section 6.2.1 for cancer dose-response analysis of PFOA human epidemiologic studies.

• Chapter 7 (Health-Protective Drinking Water Concentrations), Section 7.2.1 for calculation of the PFOA health-protective concentration based on kidney cancer in humans.

• Chapter 8 (Risk Characterization). and list them by number, as they are referred to in Attachment 2 of the review request.

Environmental epidemiology

Chapter 5 (Evidence of Toxicological Effects), sections with the headings Recent Human Evidence and Conclusions.

• Chapter 6 (Dose-Response Assessment), Section 6.1 for noncancer dose-response analyses of human studies and acceptable daily dose derivation.

• Chapter 7 (Health-Protective Drinking Water Concentrations), Section 7.1 for the calculation of noncancer health-protective drinking water concentrations for PFOA and PFOS.

• Chapter 8 (Risk Characterization).

Cancer epidemiology
Chapter 5 (Evidence of Toxicological Effects), Section 5.7.1 for human evidence of cancer and Section 5.7.4 for conclusions regarding cancer.

General comments:
-Presentation of Results: Please consider organizing each section by: 1) study design (cross-sectional, case-control, prospective cohorts), 2) exposure levels (occupational cohorts, high exposure population studies, general population studies), 3) area of research (e.g. number of studies performed in the US, Europe, Asia etc.), 4) consider describing exposure range in the studies included in the review, 6) consider describing quality assessment for studies included in the review, 7) consider performing a meta-analysis, especially for the sections which include many studies with conflicting results.
-Assessment of ecological studies: Results from ecological studies should be interpreted consistently across different cancer types. For example, the study of Mastrontonio et al is presented as "a study based on an ecologic design with very limited information on potential confounders", however, for testicular cancer, the same study is presented as "an ecologic study, but there is no reason to suspect that ecologic fallacy or exposure misclassification caused this elevation, and no major confounders are obvious."
- **Assessment of cross-sectional studies**: Appendix 7 (page 430) states that "although the results of some cross-sectional studies are discussed in this review, OEHHA excluded cross-sectional studies of cancer from its main analyses given the long latency usually associated with environmentally caused cancer, and the possibility that cancer diagnosis or treatment could lead to medication use or a change in behaviors that could change PFOA or PFOS exposure levels."
Given that serum PFOA and PFOS measurements are generally thought to represent several years of exposure, the rationale for excluding cross-sectional studies should be described in more detail.
- **Comparability of studies and dose-response:** Discussion of quantile cut-offs used across studies is inconsistent. Especially for type of cancers where studies show unusual dose-response relationships or have inconsistent findings, additional information about quantiles used and number of cases within each quantile should be included as this might offer a partial explanation.
-Follow up: For prospective studies, please describe the amount of time between exposure assessment and cancer diagnosis.
-Focus only on 2 chemicals (PFOA/PFOS): Current chemical regulation depends on one-chemical-at-a-time risk assessment. It has been suggested that this approach provides inadequate protection against human exposure to PFAS because it fails to consider the mixture effects of exposure to multiple PFAS simultaneously. It would be useful to add a section on the perspective of other PFAS chemicals or PFAS mixtures in the discussion of main conclusions for cancer outcomes.
- Quality Assessment: Please provide some additional information on how this was completed, including what the possible ratings were (i.e., "low," "probably low"), domains/characteristics evaluated, and an example of how they were determined (e.g., "For a study to be rated "low" risk of bias from confounding, the analysis must have evaluated the following confounders:….).

In addition, there is no discussion on the quality of evidence overall, publication bias, or mention of any studies that were excluded.
-Sex specific effects: Did any studies provide different estimates by sex? If so, please describe.
-Differential effects by ethnicity/race: Did any studies provide different estimates by race/ethnicity? If so, please describe.
-Please comment on whether studies were specifically designed to look at a specific cancer type, or if many/all cancers were considered. Studies that looked at many cancers and did not control for multiple comparisons may have significant findings that occurred by chance. This could be noted in the Appendix tables or in the text.
-Appendix Tables:

- Please describe median/mean exposure levels and range of exposure levels for each one of the studies included in the table.
- Please indicate whether the study examined only the specific type of cancer or many other cancer types.
- Some tables list "weaknesses" instead of "potential weaknesses" in the notes column. Does this distinction mean anything?

-Appendix-New Figures: I would strongly advise to create coefficient plot figures for the studies that have the most conflicting results or even better to conduct a meta-analysis and include the results of the meta-analysis in the appendix.

Specific comments:

1. Bladder cancer:
-Please present summary of results by type of studies and exposure groups (occupational vs general population).
- A new study Li et al 2022 shows modestly elevated hazard ration for bladder cancer (HR 1.32; 95%CI 1.01–1.72).
-Please describe the discrepancy in results between the two studies published in the general population (Eriksen et al 2009 and Li et al 2022).

2. Breast cancer:
-Please describe study area and PFAS levels. For example, the study by Bonefeeerl-Jorgensen et al was conducted in the Inuit population in Greenland. Another study that was conducted in the same population (Wielsoe et al 2017) is not reported in the review summary.
Wielsoe M, Kern P, Bonefeld-Jorgensen EC. Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study. Environ Health. 2017;16(1):56.
-Please describe differences in exposure levels between studies**.** The two studies conducted in an area near a chemical plant (DuPont, West Virginia), although reported in Table A7.22, are not discussed in the summary of human evidence for breast cancer (page 140):
Barry V, Winquist A, Steenland K. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ Health Perspect. 2013;121(11-12):1313-8.

Vieira VM, Hoffman K, Shin HM, Weinberg JM, Webster TF, Fletcher T. Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. Environ Health Perspect. 2013;121(3):318-23.:

-Report on studies conducted in Asia. One hospital-based case-control study from Taiwan which showed null results between PFOA exposure and breast cancer is missing from the summary of human evidence for breast cancer. The authors claim that they did not include it due to its cross-sectional design, but the design is case-control and other studies without prediagnostic samples for PFAS measurements were included in the review.
Tsai MS, Chang SH, Kuo WH, Kuo CH, Li SY, Wang MY, et al. A case-control study of perfluoroalkyl substances and the risk of breast cancer in Taiwanese women. Environ Int. 2020;142:105850.

-Discussion of the use of pre-diagnostic vs samples collected after diagnosis: Please describe how many studies utilized pre-diagnostic samples.

-Please compare year of sample collection between studies and how this may affect the reported results.

-Are there any studies conducted in African American or Hispanic populations? If yes, please describe effect estimates for these populations.

3. Kidney cancer
This section should go into more detail, even though is discussed in Chapter 6. The authors could provide here a brief overview of the most significant findings and discussion of any conflicting results.

4. Liver cancer
The summary paragraph should discuss all studies reported in Table A7.24, and not only the results of the high exposure occupational studies.

5. Prostate cancer
Two studies are discussed in this section, but there are several others listed in Table A7.26. Do these two best represent the all the available research on this subject? Are they in agreement with the conclusions of the other studies in Table A7.26?
Additional justification should be given for why cancer screening might be a confounder. Is screening related to PFOA exposure? Were some people more likely to be screened than others? If cancer screening is likely to confound the PFOA-prostate cancer relationship, we might have this concern for other cancers as well (especially breast cancer).

6. Testicular cancer
Although the 3 studies are consistent in finding increases of testicular cancer among the highest PFOA exposed groups, two of these studies rely on overlapping populations based on the C8 Science Panel study (Barry et al. 2013; Vieira et al. 2013).
No cancer studies of general populations have been published on PFOA and testicular cancer. Additionally, the ecological study results (Mastrantonio et al. 2018) could not be used to investigate dose-response relationship and due to lack of quantitative exposure

assessment, it is not possible to convert exposure from this study in a common exposure scale.

PFOS

1. Liver cancer

Rather than stating that there was “no clear association,” please describe what they study did or did not find – was the relationship not statistically significant? Was the effect very small, or was there no dose-response relationship?

2. Other cancer types

Please describe (or list) which types were examined.

• Chapter 6 (Dose-Response Assessment), Section 6.2.1 for cancer dose-response analysis of PFOA human epidemiologic studies.

Overall, this chapter is well-written and describes in detail the two selected studies (Vieira et al. 2013 and Shearer et al. 2021) for dose-response assessment and cancer slope factor (CSF) calculations. Some minor comments are listed below:

Page 204, Selection bias: Shearer et al. 2021 being a convenience sample should not, on its own, introduce selection bias. Characteristics of participants selected for a prospective cohort would not compromise the internal validity of the study, though the results may or may not be generalizable to a general population with different characteristics.

Page 209, Exposure misclassification: Residential addresses were only available at the time of diagnosis for the Vieira et al. study, so there is the assumption that participants had been living at the same address for several years prior to diagnosis. Please describe how this can or cannot affect misclassification of exposures. Most likely this results in non-differential exposure misclassification, but this needs to be in the report.

Exposure levels: Shearer et al and Vieira et al do not find effects at the same exposure levels. For example, the “medium” exposure level in Vieira included PFOA levels between 12.9 and 30.7, similar to the fourth quartile in Shearer (7.3-27.2), but the magnitudes of association (OR=1.2 in Vieira and OR = 2.6 in Shearer) and statistical significance (non-significant in Vieira and significant in Shearer) are different. Any implications for the PFOA-RCC relationship and/or discussion of differences in study design that would account for this should be included.

Chapter 7 (*Health-Protective Drinking Water Concentrations*), Section 7.2.1 for calculation of the PFOA health-protective concentration based on kidney cancer in humans.

No comments.

Chapter 8 (Risk Characterization).

No comments

Environmental Epidemiology

- Chapter 5 (Evidence of Toxicological Effects), sections with the headings Recent Human Evidence and Conclusions.

General comments

Organization: Please consider organizing each section in this chapter by 1) study design (cross-sectional, case-control, prospective cohorts), 2) exposure levels (occupational cohorts, high exposure population studies, general population studies), 3) area of research (e.g. number of studies performed in the US, Europe, Asia etc.), and/or 4) Age of study participants (adult studies vs studies conducted in children). Please also describe exposure range in the studies included in the review (e.g. median exposure levels for PFOA and PFOS per type of study).

Meta-analysis: Consider performing a meta-analysis, especially for the sections which include many studies with conflicting results. Coefficient plots may also help with these cases if a meta-analysis is not possible. Systematic reviews with meta-analyses have already been published for some outcomes and citing these findings may also support the OEHHA's conclusions.

Studies identified after the initial literature review (Table A7.29): Some of these studies are presented in subsections (for example the study of Abraham et al, 2020 for immunotoxicity) but not all of them. This should be consistent throughout the review.

Quality assessment: Please provide more information about quality ratings (e.g., how a study earns a 'high quality rating', or if any studies were excluded due to quality). The format of Table A7.12. is a good representation and can be used for all health outcomes in Chapter 5.

Appendix 7: Please consider organizing the Appendix 7 tables in a harmonized format for all health outcomes, so that all tables present information in the same way (eg: Authors and year, Location, Type of Study, Study population, Age of Study participants, No of subjects, Exposure assessment, Disease Outcome/Outcome assessment, Covariates, Results, Notes). In particular, for sections with many papers that each look at several outcomes (e.g., immunotoxicity), it is difficult to reference the tables when they are organized by outcome, and there are multiple rows per study. It may help to create separate tables for each outcome in this case. It may also help to group the studies by design (cross-sectional, case-control, cohort, etc.) where possible. Also, please be consistent in adding "potential weaknesses" to the notes section. If these notes are present for some studies and missing for others, it implies that those studies had no weaknesses.

Immunotoxicity

5.1.1 – Recent Human Evidence

Antibody response: When discussing results, be clear which studies are prospective vs. cross-sectional, and describe studies by age group (eg <=1 years of age, 1-5 years, >5 years).

Please consider adding/describing the study by Timmermann et al (2020), which examined the association of PFAS exposure and antibody response to measles vaccination among children from Guinea-Bissau.

Please also consider adding or describing Abraham et al (2020), which showed a consistent inverse association between levels of vaccine antibodies for tetanus and diphtheria (IgG) in relation to PFOA blood serum concentrations among 1-year-old children.
In Table 5.1.1, values of the outcome and exposure should be included. Please also consider including confidence intervals, and the difference between the 5 years pre and 5 years post needs to be further explained (are these the same children, before and after vaccination? Or different groups? It is not clear from the table notes).
A meta-analysis might also help understand findings for overlapping study conditions.
In table 5.1.3, please indicate in some way which studies are prospective.

Infectious disease: Please specify how many new studies have been published since NTP 2016, their characteristics, and whether anything new information is added.
For lower respiratory tract infections, please discuss the magnitudes of these associations.
Additionally, if 3 out of 4 studies found statistically significant results, these findings should be discussed further unless there is some reason not to (such as extremely small effect sizes, study quality issues, or conflicting directions of association).
In general, the statistical significance will mean little if the magnitude of the association is not described.
This section should take into account study quality and design, with more focus on high quality/well designed studies, while low quality studies may be summarized briefly.
Please also discuss the methodology in how health outcomes (infectious diseases) were measured, as this may affect the quality of the studies, and what efforts were made to control for known confounders and effect modifiers that may contribute to infectious disease susceptibility (e.g. family history, BMI, nutrition, stress).
Consider adding the study by Dalsager et al. (2021) which showed that prenatal PFAS levels were associated with increased hospitalization rates and higher risk of lower respiratory tract infections in childhood.

Hypersensitivity: In addition to the organization suggestions described in the general comments, consider distinguishing between exposure assessment methods (maternal blood samples vs. cord blood samples).
Please be specific on the magnitudes of association.
For asthma, even if all studies are all cross-sectional, an attempt should be made to describe effects and consistency of the effect estimates across studies. Additionally, the text implies that all or most studies are cross-sectional but goes on to describe several prospective studies (PFOA concentrations at 5 years with asthma up to age 13, PFOA in maternal/cord blood and asthma in childhood). This should be clarified.
It may also be useful to describe the potential effects of PFAS on asthma by various age groups and sex (if information is available) since sex disparities and changes by age are frequently reported in asthma research.
For eczema, how many studies were available, and how many of those were not clear or inconsistent? What were their designs, quality?

Other outcomes: A table would be helpful to summarize these findings (number of studies, direction of effect & magnitude, quality/confidence assessment).

5.1.4 - Conclusions
When discussing results from studies identified since the NTP (2016) review, it's helpful to include in the text the number of studies that actually is (ie, page 69: "For other outcomes such as colds or gastroenteritis, studies published since the NTP review did not identify clear associations" – how many studies is this?). This would allow readers to understand how consistent the literature is, especially in recent publications. The discussion of potential for bias/sources of possible bias should also expand to outcomes other than decreased antibodies responses (e.g., asthma, infections etc.), at least briefly.

Appendix 7, Immunotoxicity
What makes the studies in Table A7.1 "more informative"? Page 322: "A brief review of the results of a few of the more informative studies is shown in Table A7.1."

There are several typos in the confidence intervals. A non-exhaustive list:
-Stein et al., 2016a for PFOA and FluMist antibody response: A typo in the results column - OR = 1.8 (0.7-48.1) p- trend=0.27 for IHC (Table A7.3)
-Timmerman et al., 2017, asthma: results column for PFOA in maternal serum, age 13 (OR = 1.12 (0.67-188)), PFOA at age 5, age 5 (OR = 10.4 (1.06- 102)), and PFOA at age 5, age 13 (OR = 9.92 (1.06- 93)) (Table A7.3)
-Timmerman et al. 2017, eczema: typo in results column (OR = 8.94, 0.27-299) (Table A7.4)

Liver Toxicity
5.2.1 – Recent Human Evidence
There should be more discussion of the findings in children, given the diversity of study populations (the Khalil study was in a very small group of obese children, Attanasio was teens and general population, Mora was younger birth cohort). These differences might have implications for the interpretation of inconsistent results.

5.2.4 – Conclusions
There should be additional discussion about differences in results between children and adults. Most studies in children don't show clear relationships between PFAS and liver enzymes, while many adult studies do. The study in children with NAFLD (Jin et al. 2020) shows that the severity of liver disease may be associated with PFAS exposure and may add to the discussion.
Regarding the small effect sizes for PFOA and liver enzymes: most studies transformed the exposure and/or outcome, which makes it difficult to understand the actual magnitude of the association. Please describe further how much of an increase (in liver enzyme levels, or only ALT) would be significant for health. The main conclusion is that a doubling of PFOA results in a 6.8% increase in ALT, but the text should give an example of 1) how much of an increase that would be for an average person, in units of ALT; and 2) how much PFOA represents a 'doubling' for an average person. This would

emphasize the significance of the exposure, especially given the differences in study designs (occupational vs. population based).
There is also consistent evidence for a relationship between PFOS and ALT, even though the studies are primarily cross-sectional and there is no huge prospective study like Darrow et al (2016). The animal evidence also supports this relationship. This should be emphasized more, even if it does not allow an absolute determination of causality.

Appendix 7, Liver Toxicity
The liver toxicity search did not include liver disease terms like NAFLD, steatosis, inflammation, etc. This should be justified, if there was a reason for it.
Rantakokko et al (2015) is listed in the table of excluded studies, because it was conducted in bariatric surgery patients (Table A7.28). There should be additional justification for excluding this population. Obesity, including severe obesity and related complications, is common worldwide, and any reason why the relationship between PFOA/PFOS exposure and liver disease would be substantially different in this population should be described.
In Table A7.5 and A7.6, the type of log transformation (or lack thereof) should be described for continuous exposures and outcomes. This is done inconsistently in the Tables, and these studies made many different choices.
There is another study by Jain (2018) that was not listed in the liver tables and was not in the table of excluded studies. It overlaps with other NHANES populations (including Jain & Ducatman 2018a) but the analysis is different and may be relevant.

- Jain RB. Concentration of selected liver enzymes across the stages of glomerular function: the associations with PFOA and PFOS. Heliyon. 2019 Jul 29;5(7):e02168. doi: 10.1016/j.heliyon.2019.e02168. PMID: 31388590; PMCID: PMC6667701.

Perturbation of Lipid Homeostasis
5.3.1 – Recent Human Evidence
Study quality is given a lot of attention in Tables A7.11 and A7.12. Please consider describing the summary of these findings in this section.
More discussion should be provided for the PFOS findings. Specify the number of studies that examined PFOA vs. PFOS, and the number of studies that were cross-sectional vs. longitudinal. Please see my previous comment regarding section organization and modify accordingly.
5.3.4 – Conclusions
This section is very well organized and provides a very thorough bias discussion. Consider using this outline in all other conclusions' sections of this chapter.

Appendix 7, Lipid Homeostasis
The relevant appendix/tables for lipids should come before the relevant appendix/tables for thyroid toxicity.
This is the only appendix for human epidemiological studies that provides detailed information about study quality. All other sections of Chapter 5 could benefit for this kind

of analysis, especially the chapters on immunotoxicity, liver toxicity and developmental/reproductive toxicity.

Thyroid Toxicity

5.4.1 – Recent Human Evidence

PFOA and thyroid hormone levels: Please describe the study designs for these 19 results, and whether the conclusions differ for cross sectional vs prospective studies. This is only discussed for the 17 studies in older children but should be considered for all. Please also describe the magnitudes of these associations, regardless of statistical significance as well as sample size (statistical power could explain large but non-significant effects). It is stated that study quality did not differ, but it should be clear whether the studies were of generally high or low quality.

PFOS and thyroid hormone levels: Please add a call-out to Table A7.7, otherwise it appears that there are no separate appendix tables for PFOA and PFOS. The earlier comments regarding magnitudes of association, discussion of study design and quality, and sample size also apply here.

Thyroid diseases:

Please add an appendix table for thyroid disease. It would be easier to understand and compare the specific study characteristics in a table, even if there are only four studies. Additionally, this section only summarizes the four identified studies, and does not make any comparison of their results. Please expand this section to discuss the consistency of findings and compare results across study populations and designs.

5.4.4 – Conclusions

Please describe the magnitude of association for PFOA and TSH, and whether it might be of clinical concern.

Developmental and Reproductive Toxicity

5.5.1 – Recent Human Evidence

This section reports effect sizes more often than the others. This is helpful to visualize the magnitude of the effect but could probably be pared down to only select or representative associations.

Pregnancy-related hypertension and preeclampsia: The two studies here are discussed at length, which may not be necessary since they do not contribute to the ADD. Most of this information could be presented in tables, with only the main findings and relevant characteristics summarized in this section.

There are studies missing from the report on gestational hypertension. For example:

-Borghese, M.M., Walker, M., Helewa, M.E., Fraser, W.D., Arbuckle, T.E., 2020. Association of perfluoroalkyl substances with gestational hypertension and preeclampsia in the MIREC study. Environ. Int. 141, 105789.

-Rylander, L., Lindh, C.H., Hansson, S.R., Broberg, K., Kallen, K., 2020. Per- and polyfluoroalkyl substances in early pregnancy and risk for

preeclampsia: a case control study in southern Sweden. Epub 2020/06/21 Toxics 8 (2). PubMed PMID: 32560030; PMCID: PMC7355444.
- Birukov, A., Andersen, L.B., Andersen, M.S., Nielsen, J.H., Nielsen, F., Kyhl, H.B., Jørgensen, J.S., Grandjean, P., Dechend, R., Jensen, T.K., 2021. Exposure to perfluoroalkyl substances and blood pressure in pregnancy among 1436 women from the Odense Child Cohort. Epub 2021/02/21 Environ. Int. 151, 106442. PubMed PMID: 33610053.

Measures of fetal growth – birth weight
It is not clear why these few studies were selected for detailed discussion out of the many in Table A7.15. Please describe the reasons for selecting these or expand the discussion to include others. Since there are many studies with conflicting results consider synthesizing results by performing a meta-analysis or reference published meta-analysis on this topic (Cao et al 2021, Gao et al 2021, Negri et al 2017).

Measures of fetal growth – small for gestational age
The comments for the birth weight section also apply here.

Pubertal development
Please summarize the results on puberty development by child sex. The report should also include studies on changes in sex hormone levels associated with PFOA/PFOS exposure.

Fertility and fecundity
The review states that there are only four studies published up to date on woman's exposure to PFOA/PFOS and fertility or fecundity but there is one that was not included in either the EPA (2016) review or in this one:

- Lum KJ, Sundaram R, Barr DB, Louis TA, Buck Louis GM. Perfluoroalkyl chemicals, menstrual cycle length, and fecundity: Findings from a prospective pregnancy study. Epidemiology. 2017;28(1):90-8.

All subsections dedicate far more time to the strengths and weaknesses of individual studies than other sections do. This may not be necessary, unless the other sections will do the same. Discussion of possible bias/confounding should be moved to conclusions, and if a concern applies to only one study, it can be noted in the Appendix tables.

5.5.4 - Conclusions
The structure of the conclusions section should follow a similar structure of the conclusions in "Perturbation of Lipid Homeostasis". More specifically, there should be a summary of the study findings for the different outcomes, and a detailed discussion of possible confounders, study quality, and reasons for inconsistencies. A meta-analysis or coefficient plot would help synthesize the results from various cohorts on fetal growth.

Chapter 6

6.1.1 PFOA

The Faroe Islands NOAEC is much lower than the Abraham (2020) NOAEC and OEHHA's BMD calculation (~4.75 ng/mL rather than 16-20 ng/mL). Reasons for this should be described. Is it because a 5% decrease was used (rather than 10%)? Please also describe why a 5% decrease in antibody levels would be used to calculate the BMD, when 10% was used in the other analysis, as well as how these differences should be interpreted.

In the liver toxicity discussion, Darrow et al. (2016) should not be described as a cross-sectional study, when their exposure is modeled lifetime PFOA exposure. Table A7.5 also categorizes this study as a prospective cohort.

For the ADD calculation, the choice to use the ALT NOAEC (9.8 ng/mL) rather than one of the lower values for immunotoxicity (BMD) is not well justified. There is an indication that the NOAEC may be lower than 9.8, even if the exact value is unknown. The animal-study derived NOAEL should also be compared to the human NOAEC/BMD, and any differences or agreement between them should be discussed.

6.1.2 PFOS

No comments.

Chapter 7

No comments.

Chapter 8

International regulations could also be included in Table 8.1, or discussed in the text.

Name

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Date

February 14, 2022

Item reviewed

Product – Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water

CalEPA Scientific Review Program

Based on my expertise and experience, I am reviewing the findings, assumptions or conclusions I agreed I could review with confidence. I am reviewing Findings, Assumptions, and Conclusions #1 and #2 as outlined in ***Attachment 2***. With respect to the Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water (the "Product"), my review is specific to the areas of 1) mammalian toxicology, 2) cancer epidemiology, and 3) toxicology and risk assessment. While I am reviewing Attachment 2, I also am reviewing the entire product and the sections of the product most relevant to my review included in Chapters 5-8.

Brief summary of approach to external peer-review by the peer-reviewer

In addition to the specific issues presented ***Attachment 2*** and the Product, I also will be addressing the following questions:

(a) Are there any additional subjects that should be considered as part of the scientific basis of the proposed rule?
(b) Taken as a whole, is the proposed rule based upon sound scientific knowledge, methods, and practices?
(c) For each proposed PHG, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in the derivation of PHGs based on cancer.
(d) For the proposed noncancer health-protective concentrations, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in the derivation of each health-protective concentration.
(e) For each chemical reviewed comment on whether a relevant study useful for assessing dose-response relationship or otherwise informing the PHG development was missed.
(f) PHGs must be protective of known sensitive populations. Please comment on whether each PHG is health protective of sensitive populations.

In reading the ***Attachment 2*** and the Product, there did not appear to be additional subjects that were part of the scientific basis that were not described in the Product or ***Attachment 2***.

Taken as a whole, the Product and ***Attachment 2*** appear to be based upon current and sound scientific knowledge, methods, and practices. Some areas of the Product or ***Attachment 2*** were identified as needing additions and/or clarifications. These are included in each point, below.

Each point, below, reflects the application of these questions to Findings, Assumptions, and Conclusions #1 and #2 in ***Attachment 2*** and the Product. References cited also are included to support the external peer-review. Throughout the peer review, I will refer to the "Product – Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water" as the Product.

Attachment 2-specific points addressed by the peer-reviewer

(1) Assumptions, Findings, and Conclusions – Perfluorooctanoic acid.

(a) Are there any additional subjects that should be considered as part of the scientific basis of the proposed rule?

A variety of health endpoints were reviewed in the Product, leading to the assumptions, findings, and conclusions in ***Attachment 2*** that cancer is the primary adverse health effect associated with human exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). For PFOA, the primary adverse health effect is kidney cancer in exposed humans and it is considered to be the most sensitive endpoint for derivation of the Public Health Goal (PHG). For PFOS, the primary adverse health effect is cancer and this comes from data indicating that liver and pancreatic tumors arise from PFOS exposure in rats.

Several epidemiological studies support the link between kidney cancer in humans and PFOA exposure, including studies of the general human population, people living in areas of high environmental exposure, and people exposed to PFOA through their occupations.

The epidemiological data for PFOS do not appear to be as robust as for PFOA, with either fewer studies or no clear or consistent associations in the existing studies. The one animal cancer bioassay produced positive evidence for liver and pancreatic tumors and was considered sufficient by OEHHA for selecting cancer as the primary adverse health effect.

It appears as if relevant subjects were included as part of the scientific basis for the proposed rule.

A recently published critical review and meta-analysis of epidemiological literature for PFOA was not included in the Product. This study by Bartell and Vieira (2021) concluded that associations between PFOA and kidney cancer (and testicular cancer) were likely causal. While Bartell and Vieira (2021) did note that the number of studies was limited and that larger cohort studies were needed to support their conclusion, this critical review and meta-analysis should be included in the Product as a supporting study.

Bartell SM and Vieira VM. 2021.Critical review on PFOA, kidney cancer, and testicular cancer. J Air Waste Manag Assoc. 71:663-679.

(b) Taken as a whole, is the proposed rule based upon sound scientific knowledge, methods, and practices?

It appears as if the proposed rule is based upon sound scientific knowledge, methods, and practices. Throughout the Product and ***Attachment 2***, rationale/justification for choices made appears to be transparent and complete. A particular strength of the Product and by extension, ***Attachment 2***, is in-depth analyses of the strengths and limitations of studies included in

Chapters 5 and 6. Additionally, Chapters 7 and 8 contain clear descriptions and justifications for the choices made.

One area of the Product and ***Attachment 2*** could be improved. The derivation of PHGs based on cancer and the proposed noncancer health-protective concentrations are based on the "most sensitive" health effects. However, neither the Product nor ***Attachment 2*** (or ***Attachment 1***, the Plain English Summary) define "most sensitive" with respect to health effects. As "most sensitive" could be interpreted in more than one way, it is recommended that the operative definition for this phrase be included in a revised version of the Product and ***Attachment 2***.

(c) For each proposed PHG, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in the derivation of PHGs based on cancer.

The US Environmental Protection Agency (US EPA) recently released Approaches to the Derivation of an MCLG for PFOA in Drinking Water (US EPA, 2021a). The US EPA interpretation of the data on the carcinogenicity of PFOA was that PFOA is "likely to be carcinogenic to humans." While the US EPA guidelines for assessment of carcinogenic risks and the guidelines followed in the Product are not identical, a similar data set was evaluated. The Product considered seven human studies that included linkages between PFOA exposure and kidney cancer and when combined with animal and mechanistic data, OEHHA determined that these studies provided evidence that PFOA is a cause of kidney cancer. The US EPA (2021a) considered the quality of the Shearer et al. (2021) study to be of medium confidence and when combined with the remaining dataset of epidemiological, animal, and mechanistic studies, determined that the evidence was for a plausible linkage rather than a causal linkage. Therefore, the potential Federal carcinogenicity designation for PFOA may ultimately differ from the OEHHA designation.

Similarly, the US EPA also recently released Approaches to the Derivation of an MCLG for PFOS in Drinking Water (US EPA, 2021b). The US EPA interpretation of the data on the carcinogenicity of PFOS was that the data were suggestive of carcinogenicity. While the US EPA guidelines for assessment of carcinogenic risks and the guidelines followed in the Product are not identical, a similar data set was evaluated. The Product and the US EPA MCLG document for PFOS appear to agree with respect to the weight of evidence for epidemiological studies, but the US EPA (2021b) did not regard the single animal cancer bioassay as supportive for a higher designation as it was a single study and tumors did not appear to occur in a dose-responsive pattern. Section 6.2.2, which includes the cancer dose-response analyses for PFOS, discusses the level of evidence for PFOS carcinogenicity in detail. In this section, data from studies of PFOA are used to support the conclusion that cancer is a sensitive endpoint for PFOS based on similarities in chemical structure and noncancer toxicity profiles for PFOA and PFOS. It is recommended that additional supporting data for similarities between PFOA

and PFOS be included in this section in a revised version of the Product, i.e., similarities in modes and/or mechanisms of action. Additionally, it is unclear why the Shearer et al. (2021) study is not included in Section 5.7.1 on human evidence for PFOS as PFOS was one of the analytes measured in the Shearer et al. (2021) study.

The intent of comparing the Product to the MCLG documents by the US EPA is not to indicate that one is "better" or "more correct" than the other but to point out inconsistencies between the two documents in terms of the assessment of the available science. In a revised version of the Product, it may be worthwhile to make note of the US EPA MCLG documents.

US EPA. 2021a. Proposed approaches to the derivation of a draft maximum contaminant level goal for perfluorooctanoic acid (PFOA) in drinking water. External peer review draft. EPA Document No. 822D21001.

US EPA. 2021b. Proposed approaches to the derivation of a draft maximum contaminant level goal for perfluorooctane sulfonic acid (PFOS) in drinking water. External peer review draft. EPA Document No. 822D21002.

(d) For the proposed noncancer health-protective concentrations, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in the derivation of each health-protective concentration.

Chapter 6 of the Product contains the dose-response assessment for the identification of points of departure (PODs) for derivation of the proposed noncancer health-protective concentrations. This Chapter contains detailed rationale/justification for the selection of specific PODs. Scientific judgment plays a key role in the process of health risk assessment, which can lead to differences in PODs and other values selected by groups of scientists performing the assessment(s). The detailed rationale/justification for the selection of specific PODs for noncancer health-protective concentrations makes it very clear that OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in the derivation of each health-protective concentration.

(e) For each chemical reviewed comment on whether a relevant study useful for assessing dose-response relationship or otherwise informing the PHG development was missed.

An epidemiological study by Li et al. (2022) just published may be informative for PFOS. It concerns kidney cancer and the study population is exposed to a mixture of PFAS that appear to be dominated by PFOS and one other PFAS. Otherwise, it does not appear as if relevant studies useful for assessing dose-response relationships or otherwise informing the PHG development were missed.

(f) PHGs must be protective of known sensitive populations. Please comment on whether each PHG is health protective of sensitive populations.

Within the Product and ***Attachment 2***, the only specific mention of "sensitive populations" is in the Populations definition for PECO criteria. ***Attachment 1***, the Plain English Summary, does include the statement: "The PHGs and noncancer health-protective concentrations are based on comprehensive analyses of information on the toxicology of each compound and include consideration of sensitive populations, such as infants and children." However, it does not appear as if a similar statement is explicitly included in the Product or ***Attachment 2***.

In a revised version of the Product and ***Attachment 2***, it is recommended that sensitive populations be defined and that a rationale for why the chosen PHGs will be health protective of identified sensitive populations.

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References (references not cited in the proposed rule are listed here)

Bartell SM and Vieira VM. 2021.Critical review on PFOA, kidney cancer, and testicular cancer. J Air Waste Manag Assoc. 71:663-679.

Li H, Hammarstrand S, Midberg B, Xu Y, Li Y, Olsson DS, Fletcher T, Jakobsson K, and Andersson EM. 2022. Cancer incidence in a Swedish cohort with high exposure to perfluoroalkyl substances in drinking water. *Environ Res.* 204:112217.

US EPA. 2021a. Proposed approaches to the derivation of a draft maximum contaminant level goal for perfluorooctanoic acid (PFOA) in drinking water. External peer review draft. EPA Document No. 822D21001.

US EPA. 2021b. Proposed approaches to the derivation of a draft maximum contaminant level goal for perfluorooctane sulfonic acid (PFOS) in drinking water. External peer review draft. EPA Document No. 822D21002.

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February 8, 2022

First Public Review Draft July 2021
Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water
Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water

Based on my expertise and experience, I am reviewing the findings, assumptions, or conclusions I agreed I could review with confidence, as indicated below.

Chapter 4: Toxicokinetics

Section 4.1: Species differences in serum half lives

Comments:

I would not include the PFOA half-life estimate in female mice, repeatedly dosed with PFOA (from Lou et al., 2009). The estimate is questioned as “contradictory” in the original paper, and there is no support for a 1.2d half-life for PFOA in other publications.

I also question the comparison of rat PFOA pharmacokinetic data with other species. The rat is an outlier in terms of having strong sex differences in elimination. Although, I agree that the sex difference in rat PFOA half lives did reveal an interesting transporter protein-based mechanism.

Section 4.2: Absorption

Comments:

1) Oral

Human data from the clinical trial reported in the Convertino study (2018) should not be considered. The people in the study were extremely ill with confounding underlying pathology that negates comparison to healthy people.

Also note: Data from the same clinical trial reported in Convertino, 2018 were used in the human half-life estimate in Dourson, 2019 and appears to be included in the human PFOA half-life estimate in Table 4.7.1. Again, I argue that data/estimates from that study are highly confounded by underlying pathology and should NOT be used in estimating PFOA half-life in healthy people.

I concur with the assessment that Trudel, 2008 should not be used in the estimate human absorption.

2) Dermal

A critical point not considered with regard to dermal absorption of PFOA and PFOS is their ionizability. These chemicals will be ionized at physiological pH. Dermal absorption

occurs only via diffusion and thus would be limited for an ionized chemical. This was shown by Franko et al., 2012.

3) Inhalation

The one animal inhalation study in an animal model that I found (Hinderliter (2003)) was included in this analysis. There is evidence in humans (particularly in occupational exposures) to support the conclusion that inhalation exposure can lead to absorption in PFOA. Little is known about this route of exposure, however.

Section 4.3: Distribution

Comments:

I do not understand the point is being made with the following statement, “However, in a cross-sectional study of 300 children in Texas, plasma concentrations of PFOA or PFOS steadily increased for 0-3, 3-6, 6-9 and 9-13 years of age groups, indicating that a possible early life spike in plasma concentrations would have dissipated by 3 years of age (Schecter et al., 2012).” The study cited shows that multiple PFAS continue to increase in blood concentration up to the oldest age group, so I do not see how this supports dissipation of an “early life spike.”

I do not think that data support the following statement, “Displacement of endogenous ligands from carrier or transporter proteins has been hypothesized as one of the possible mechanisms of action in PFOA/PFOS toxicity.” The mass balance between fatty acids and PFAS is more likely to displace PFAS from shared binding proteins than the other way around.

The following is an overstatement, “At physiological pH, PFOA and PFOS are charged and therefore, would not be able to cross membranes via passive transport.” First, diffusion is limited but does occur (Kimura et al., 2017). Second, there are portions of the GI tract with pHs below the pKa of PFOA and PFOS (e.g., stomach and upper small intestine). I do agree that active transport is the major mechanism of movement across membranes.

Kimura, O., Fujii, Y., Haraguchi, K., Kato, Y., Ohta, C., Koga, N., & Endo, T. (2017). Uptake of perfluorooctanoic acid by Caco-2 cells: Involvement of organic anion transporting polypeptides. *Toxicology Letters*, *277*, 18–23. https://doi.org/10.1016/j.toxlet.2017.05.012

Section 4.4: Metabolism

Comments:

I agree; PFOA and PFOS are inert to biotransformation.

Section 4.5: Excretion

Comments:

The following introduction is not consistent with the data presented:

“Excretion pathways of PFOA and PFOS include:

1)Renal or urinary excretion, which occurs in all mammalian species and appears to be dominant in fast eliminators, e.g., in the case of PFOA elimination in the female rat.

2)Fecal or gastrointestinal excretion appears to play a more important role in slow eliminators, such as humans; likely subject to enterohepatic circulation.

3)Elimination pathways via pregnancy and lactation in human females (Wong et al., 2014).”

Renal/urinary and biliary/fecal excretion occur in all mammals (see Hundley et al., 2006)

Urinary excretion is dominant in humans, in spite of the fact that the biliary clearance rate is higher than the urinary clearance rate. Enterohepatic recirculation limits actual excretion in feces.

Elimination pathways in human females include menstruation, pregnancy and lactation. While Table 4.5.2 suggests that menstruation may play a lesser role in elimination than lactation, studies do show that oral contraceptive use is associated with increased PFOA and PFOS concentrations (e.g., Ngueta et al., 2017).

Chapter 5: Evidence of Toxicological Effects

Section 5.1: Immunotoxicity

Comments:

More human studies have been published supporting a relationship between PFAS and adverse immune endpoints. Two more studies show a decrease in antibody response to vaccination, supporting already strong evidence. Confidence for increased risk of infectious disease was considered “low” by NTP in 2016. But four new studies have been published that likely increase confidence in this endpoint.

Table 1: PFAS and immunotoxicity endpoints.

Study	Endpoint	Association
Reduced antibody response		
(Shih et al., 2021)	Serum antibody concentrations against hepatitis type A and B in adults	PFAS concentrations at 14, 22 and 28 years of age.
(Abraham et al., 2020)	Reduced antibody response to vaccinations for Haemophilus influenza	PFOA level in serum of children

	type b, tetanus and diphtheria.	NOAECs: 12.2, 16.9 and 16.2 µg/L
Increased risk of infectious disease		
(Wang et al., 2022)	Diarrhea in infant	PFAS in maternal serum
(Ji et al., 2021)	Increased risk of SARS-CoV-2 infection in adults	Urinary PFAS
(Bulka et al., 2021)	Increased pathogen (cytomegalovirus, Epstein Barr virus, hepatitis C and E, herpes simplex 1 and 2, HIV, T. gondii, and Toxocara spp) burden score	Serum PFAS, particularly in adolescents.
(Dalsager et al., 2021)	Increase in the risk of hospitalization due to any infection	PFOS in maternal serum
Other immune endpoint		
(Salihovic et al., 2020)	Reduced serum inflammatory proteins	PFAS in serum of elderly

Allergy and autoimmunity are a different type of immune dysfunction (than ability to fight infection). I am thus not surprised that strong associations have not necessarily been found between PFOA/PFOS exposure and increased risk of allergy, delayed type hypersensitivity or autoimmune disease.

Dr. Jamie DeWitt's studies, in particular, have shown consistently shown a reduction in antibody response to SRBCs in animal models, supporting the cause-and effect relationship between PFOA/PFOS exposure and reduced antibody response to vaccination in humans. An important study also to consider is Guruge et al., 2009, which showed a significant reduction in survival of influenza a infection, following 21 day exposure to PFOS (LOAEL 0.005 mg/kg/day; 189 ng PFOS/ml serum). A recent study showed that a 28 day PFOS exposure to 0.0015 mg/kg/day (99 ng PFOS/ml serum) modified distributions of immune cell types and resulted in greater weight loss in response to influenza a infection (Torres et al., 2021).

I agree that the relationship between PFAS exposure and suppressed immune responses is well supported by animal and human data. The data suggesting associations between PFAS exposure and inappropriate activation of the immune system (allergy and autoimmunity) is not strong.

Abraham, K., Mielke, H., Fromme, H., Volkel, W., Menzel, J., Peiser, M., Zepp, F.,

Willich, S. N., & Weikert, C. (2020). Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. *Archives of Toxicology*. https://doi.org/10.1007/s00204-020-02715-4

Bulka, C. M., Avula, V., & Fry, R. C. (2021). Associations of exposure to perfluoroalkyl substances individually and in mixtures with persistent infections: Recent findings from NHANES 1999-2016. *Environmental Pollution (Barking, Essex : 1987)*, *275*, 116619. https://doi.org/10.1016/j.envpol.2021.116619

Dalsager, L., Christensen, N., Halekoh, U., Timmermann, C. A. G., Nielsen, F., Kyhl, H. B., Husby, S., Grandjean, P., Jensen, T. K., & Andersen, H. R. (2021). Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Child Cohort. *Environment International*, *149*, 106395. https://doi.org/10.1016/j.envint.2021.106395

Guruge, K. S., Hikono, H., Shimada, N., Murakami, K., Hasegawa, J., Yeung, L. W. Y., Yamanaka, N., & Yamashita, N. (2009). Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice. In *The Journal of toxicological sciences* (Vol. 34, Issue 6, pp. 687–691). https://doi.org/10.2131/jts.34.687

Ji, J., Song, L., Wang, J., Yang, Z., Yan, H., Li, T., Yu, L., Jian, L., Jiang, F., Li, J., Zheng, J., & Li, K. (2021). Association between urinary per- and poly-fluoroalkyl substances and COVID-19 susceptibility. *Environment International*, *153*, 106524. https://doi.org/10.1016/j.envint.2021.106524

Salihovic, S., Lind, L., Larsson, A., & Lind, P. M. (2020). Plasma perfluoroalkyls are associated with decreased levels of proteomic inflammatory markers in a cross-sectional study of an elderly population. *Environment International*, *145*, 106099. https://doi.org/10.1016/j.envint.2020.106099

Shih, Y.-H., Blomberg, A. J., Bind, M.-A., Holm, D., Nielsen, F., Heilmann, C., Weihe, P., & Grandjean, P. (2021). Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substances: A birth cohort in the Faroe Islands. *Journal of Immunotoxicology*, *18*(1), 85–92. https://doi.org/10.1080/1547691X.2021.1922957

Torres, L., Redko, A., Limper, C., Imbiakha, B., Chang, S., & August, A. (2021). Effect of Perfluorooctanesulfonic acid (PFOS) on immune cell development and function in mice. *Immunology Letters*, *233*, 31–41. https://doi.org/10.1016/j.imlet.2021.03.006

Wang, Z., Shi, R., Ding, G., Yao, Q., Pan, C., Gao, Y., & Tian, Y. (2022). Association between maternal serum concentration of perfluoroalkyl substances (PFASs) at delivery and acute infectious diseases in infancy. *Chemosphere*, *289*, 133235. https://doi.org/10.1016/j.chemosphere.2021.133235

Section 5.2: Liver Toxicity

Comments:

Overall, the analysis is thorough. However, it does not address a critical criticism of studies of PFOA/PFOS liver toxicity in rodent models. Adverse effects in liver are most certainly an outcome of PPARa activation (among other mechanisms), and there are species differences in rodent and human PPARa. This is not to say that humans are not susceptible to PFAS-induced effects that are mediated by PPARa. I suggest that a specific section be added that addresses this issue head on. There are two in vivo studies in mice expressing human PPARa that need to be called out. Nakagawa et al., 2012 shows that hPPARa mice respond to PFOA with increased liver weight, liver triglycerides and plasma Alt. Schlezinger et al., 2020 shows that hPPARa mice respond to PFOA with increased liver weight and liver triglycerides, as well as with increased expression of genes whose expression is controlled by PPAR. It less likely that hPPARa plays a significant role in the liver toxicity induced by PFOS, however, as shown recently in the hPPARa mouse model (Su *et al.,* 2022).

Further, there is ample evidence in human hepatocyte models, including primary human hepatocytes and liver spheroids, for induction of PPARα, CAR and PXR target gene expression by PFOA and PFOS (Wolf *et al.*, 2008; Bjork *et al.*, 2011; Wolf *et al.*, 2012; Buhrke *et al.*, 2013; Peng *et al.*, 2013; Rosen *et al.*, 2013; Buhrke *et al.*, 2015; Behr *et al.*, 2019, Rowan-Carroll et al., 2021).

Overall, the data strongly support that human liver is a target organ of PFOA and PFOS.

Behr, A.C., Plinsch, C., Braeuning, A., Buhrke, T., 2019. Activation of human nuclear receptors by perfluoroalkylated substances (PFAS). Toxicol In Vitro **62**, 104700.

Bjork, J.A., Butenhoff, J.L., Wallace, K.B., 2011. Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. Toxicology **288**, 8-17.

Buhrke, T., Kibellus, A., Lampen, A., 2013. In vitro toxicological characterization of perfluorinated carboxylic acids with different carbon chain lengths. Toxicol Lett **218**, 97-104.

Buhrke, T., Kruger, E., Pevny, S., Rossler, M., Bitter, K., Lampen, A., 2015. Perfluorooctanoic acid (PFOA) affects distinct molecular signalling pathways in human primary hepatocytes. Toxicology **333**, 53-62.

Nakagawa, T., Ramdhan, D.H., Tanaka, N., Naito, H., Tamada, H., Ito, Y., Li, Y., Hayashi, Y., Yamagishi, N., Yanagiba, Y., Aoyama, T., Gonzalez, F.J., Nakajima, T., 2012. Modulation of ammonium perfluorooctanoate-induced hepatic damage by genetically different PPARalpha in mice. Arch. Toxicol. 86, 63–74.

Peng, S., Yan, L., Zhang, J., Wang, Z., Tian, M., Shen, H., 2013. An integrated metabonomics and transcriptomics approach to understanding metabolic pathway disturbance induced by perfluorooctanoic acid. J Pharm Biomed Anal **86**, 56-64.

Rosen, M.B., Das, K.P., Wood, C.R., Wolf, C.J., Abbott, B.D., Lau, C., 2013. Evaluation of perfluoroalkyl acid activity using primary mouse and human hepatocytes. Toxicology **308**, 129-137.

Rowan-Carroll, A., Reardon, A., Leingartner, K., Gagné, R., Williams, A., Meier, M.J., Kuo, B., Bourdon-Lacombe, J., Moffat, I., Carrier, R., Nong, A., Lorusso, L., Ferguson, S.S., Atlas, E., Yauk, C., 2021. High-throughput transcriptomic analysis of human primary hepatocyte spheroids exposed to per- and polyfluoroalkyl substances (PFAS) as a platform for relative potency characterization. Toxicol. Sci. 181(2):199-214

Schlezinger, J., Puckett, H., Nielsen, G., Oliver, J., Heiger-Bernays, W., Webster, T., 2020. Perfluorooctanoic acid activates multiple nuclear receptor pathways and skews expression of genes regulating cholesterol homeostasis in liver of humanized PPARα mice fed an American diet. Toxicol Appl Pharmacol 405, 115204.

Su, S., Billy, L. J., Chang, S., Gonzalez, F. J., Patterson, A. D., & Peters, J. M. (2022). The role of mouse and human peroxisome proliferator-activated receptor-α in modulating the hepatic effects of perfluorooctane sulfonate in mice. *Toxicology*, *465*, 153056.

Wolf, C.J., Takacs, M.L., Schmid, J.E., Lau, C., Abbott, B.D., 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol Sci **106**, 162-171.

Wolf, C.J., Schmid, J.E., Lau, C., Abbott, B.D., 2012. Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARalpha) by perfluoroalkyl acids (PFAAs): further investigation of C4-C12 compounds. Reproductive toxicology (Elmsford, N.Y **33**, 546-551.

Section 5.3: Perturbation of Lipid Homeostasis (Animal data only)

Comments:

Great care needs to be taken in interpreting effects of PFOA and PFOS on serum lipids (triglycerides and cholesterol) in animal models. And the following is an overinterpretation of the data presented:

Pg. 108, “In contrast, some animal studies have shown decreased cholesterol with PFOA and PFOS exposure (Table 5.3.6). Different results in animals and humans may be explained by the stronger activity of PPARα in animals, which is involved in the metabolism of cholesterol and fatty acids.”

First, studies that do NOT report serum PFAS concentrations should not be considered. Second, experimental exposures that result in supra-human serum concentrations should not be considered. It is becoming clear that PFOA, at least, induces a non-monotonic dose response with regard to effects on serum lipids, with increases in serum lipids being observed at low PFOA serum concentrations and decreases in serum lipids being observed at high (non-human-relevant) serum concentrations, likely

a result of the increasing influence of PPARa activity. Studies that support this are outlined below.

Serum triglycerides

In male mice, studies have reported that perfluorocarboxylic acids induced both increases (S E Loveless et al., 2006; Minata et al., 2010; Yan et al., 2014) and decreases in serum TG (S E Loveless et al., 2006; Minata et al., 2010; Pouwer et al., 2019; Qazi et al., 2010; Wang et al., 2015; Xie et al., 2003; Yan et al., 2014) or had no effect (Nakamura et al., 2009; Pouwer et al., 2019). In studies with male rats, perfluorocarboxylic acid exposure also was associated with decreased serum TG (Elcombe et al., 2010; Haughom & Spydevold, 1992; Kudo et al., 1999; S E Loveless et al., 2006; Scott E Loveless et al., 2008; NTP, 2019), increased serum TG (NTP, 2019; Zhang et al., 2008), or no effect on serum TG (NTP, 2019). There are two mouse studies that are important to note, in which full dose response assessments were conducted and serum PFOA concentrations were measured (Pouwer et al., 2019; Yan et al., 2014). Across these two studies, at lower PFOA body burdens, increased serum triglycerides were observed and at high PFOA body burdens (above those measured even in fluorochemical workers in the US), decreased serum triglycerides were observed. Furthermore, in a study with male cynomolgus monkeys, significant increases in serum triglycerides were observed following exposure to PFOA at serum levels less than 90 µg/mL (Butenhoff et al., 2002). Thus, when only studies that used exposure scenarios resulting in human-relevant serum PFOA levels are considered, PFOA exposure consistently results in increased serum triglycerides.

Serum cholesterol

Studying cholesterol biology in rodents has several challenges. Diet influences serum cholesterol levels (Dietschy et al., 1993). Cholesterol homeostasis differs depending on mouse strain and sex (Bruell et al., 1962). Species differ in the distribution of cholesterol among the different cholesterol particle types (Yin et al., 2012). However, with careful model and experimental design, these challenges can be addressed.

Studies using rodents fed a standard, low fat/low cholesterol rodent diet and exposed to PFOA for 6 weeks show decreased serum cholesterol levels (reviewed in (Rebholz et al., 2016)). However, when mice are fed a cholesterol and fat-containing diet, PFOA does increase serum cholesterol levels (Rebholz et al., 2016), particularly in males and in C57BL/6 mice at a serum concentration of approximately 30 µg PFOA/mL. In a dose response analysis in male APOE*3-Leiden.CETP mice treated with PFOA for 4 weeks, serum cholesterol concentrations were only decreased in mice with a PFOA serum concentration of 144 µg/mL (Pouwer et al., 2019). Importantly, in mice expressing human PPARα, PFOA increased serum cholesterol. Nakamura et al (Nakamura et al., 2009) exposed male hPPARα (Sv/129 strain) to 0.3 mg PFOA/kg/day for 2 weeks (no serum PFOA concentration was reported) and Schlezinger et al. (J. Schlezinger et al., 2020; J. J. Schlezinger et al., 2020) exposed male hPPARα mice to 0.7 mg PFOA/kg/day for 6 weeks (47 µg PFOA/mL serum). In both studies, PFOA exposure was associated with increased serum cholesterol, particularly low density lipoprotein cholesterol (Nakamura et al., 2009; J. Schlezinger et al., 2020; J. J. Schlezinger et al., 2020). No study in rodents to date has investigated the relationship of PFAS at steady

state exposures to effects on serum lipids; however, increased serum cholesterol with associated with serum PFAS concentrations in household cats (Weiss et al., 2021). When studies used human-relevant diets and exposure scenarios, PFOA exposure consistently results in increased serum cholesterol in sensitive rodent strains, including one that expresses human PPARα.

Mechanisms of action

I reiterate that the PFAS-induced effects are likely a result of the actions of PFAS in the liver. Thus, regulators need to be aware that species differences in PPARa are likely to be important. With that said, evidence discussed above with regard to liver toxicity, also supports the ability of PFOA and PFOS to perturb serum lipids via alterations in signaling in the liver in a human-relevant manner.

Overall, the human epidemiological data and data derived from animal models that are human relevant in dose and diet strongly support the association between PFAS exposure and dyslipidemia. Further, data from the humanized PPARα mouse model also support the association.

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Butenhoff, J., Costa, G., Elcombe, C., Farrar, D., Hansen, K., Iwai, H., Jung, R., Kennedy Jr., G., Lieder, P., Olsen, G., & Thomford, P. (2002). Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. *Toxicol Sci*, *69*(1), 244–257. http://www.ncbi.nlm.nih.gov/pubmed/12215680

Dietschy, J. M., Turley, S. D., & Spady, D. K. (1993). Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *Journal of Lipid Research*, *34*(10), 1637–1659.

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Kudo, N., Mizuguchi, H., Yamamoto, A., & Kawashima, Y. (1999). Alterations by perfluorooctanoic acid of glycerolipid metabolism in rat liver. *Chem Biol Interact*, *118*(1), 69–83. http://www.ncbi.nlm.nih.gov/pubmed/10227579

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Section 5.4: Thyroid Toxicity

Comments:

Overall, the results from the human epidemiology do not show a strong or consistent association between PFOA and/or PFOS in increased TSH, which is used for clinical diagnosis of hypothyroidism. I am concerned that the table with the results from the NTP study in rats does not include serum PFOS concentrations. Studies in rats are fairly consistent in showing negative associations between PFOA and PFOS exposure and decreases in T3 and T4, but they are not accompanied by increases in TSH. Thus, it is hard to determine the biological significance of the observations. The evidence is strong that PFAS can bind to TTR, which could reduce serum hormone levels. But, it is not clear that these effects are occurring at human-relevant PFAS serum concentrations.

Section 5.5: Developmental and Reproductive Toxicity

Comments:

On pg. 118, it states, “Preeclampsia is a condition in which the pregnant woman is hypertensive because of reduced renal excretion associated with a decrease in GFR (US EPA, 2016b).” This is not correct. Preeclampsia is a disease of the placenta (Rana et al, 2019).

I agree that epidemiological data support and association between PFOA and PFOS exposure and increased risk of hypertensive disorders of pregnancy. The strength of the association is limited as results across studies remain inconsistent, particularly for gestational hypertension. I also agree that the results are inconclusive for fetal growth, pubertal development and fertility/fecundity for humans.

An important developmental endpoint in humans does not appear to have been considered, which is bone quality. Analyses of early life exposure to PFAS, including PFOA and PFOS, are consistently associated with reductions in bone quality in childhood, adolescence and early adulthood (Buck Louis et al., 2018; Buckley et al., 2021; Cluett et al., 2019; Di Nisio et al., 2020; Jeddy et al., 2018; Khalil et al., 2018). This endpoint is important, in particular, for three reasons. 1) PFAS have been found in human bone (Koskela et al., 2017). 2) Reduced ossification in rodent pups exposed in utero (Lau et al., 2006) is the endpoint used for the candidate RfD for PFOA (https://www.federalregister.gov/documents/2020/03/10/2020-04145/announcement-of-preliminary-regulatory-determinations-for-contaminants-on-the-fourth-drinking-water). 3) Failure to reach peak bone mass in early adulthood is a significant risk factor, and perhaps the most important factor, in determining osteoporosis risk (Hui et al., 1990; Klibanski et al., 2001).

Results from animal studies strongly support a cause/effect relationship between PFOA and PFOS exposure and adverse developmental and reproductive health outcomes. First, the analysis of recently published studies includes two that support bone as a target organ of early life exposure to PFOA (Koskela et al., 2016; van Esterik et al., 2016). Second, a newer experimental study and a meta-analysis continue to support that conclusion that PFOA is a male, reproductive, developmental toxicant, which leads to disruption of testis function and testosterone production (Bao et al., 2021; Wang et al., 2021). Fewer studies have investigated the effects of PFOS on male reproductive development, but effects seem to be similar to PFOA. In females, the data support the conclusion that PFOA and PFOS are placental toxicants, including newer studies (Jiang et al., 2020; Li et al., 2021, 2022; Wan et al., 2020).

I was surprised to see that activation of nuclear receptors beyond PPARγ (i.e., PPARα, CAR and PXR) received little attention as mechanisms of action for developmental and reproductive toxicity. Although, after from searching on PubMed, this seems to be a result of the state of the science. Bone (developmental toxicity), placenta (reproductive toxicity) and testis (developmental and reproductive toxicity appear to be important target organs for PFAS.

Overall, effects on bone should be included in the epidemiological and animal model analyses and conclusions.

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Section 5.6: Neurotoxicity

Comments:

The data on the potential neurotoxicity of PFOA and PFOS are very limited. In Guo et al., 2019, it appears that the effect on protein expression the brain occurs downstream of effects in the liver. Given that there was significant weight loss in the mice with this effect (and the serum level of PFOA was at a high occupational level), it is unclear if the effect is specific to the brain or just a downstream result of overt toxicity.

Section 5.7: Cancer

Comments:

Human Epidemiology

I concur that the strongest epidemiological evidence for PFOA-induced cancer is for kidney and testicular cancers. It is important to point out that there also is evidence of non-cancer endpoint related effects in testis, which point to testis as a sensitive target organ of PFAS. These target organs were supported in a recent meta-analysis, as well (Bartell & Vieira, 2021)The epidemiological evidence of increased risk of pancreatic cancer is limited because this is a rare cancer, but should not be dismissed because of the very low survival rate and the strong evidence of pancreatic cancer in animals studies. Confirmation of risk of breast and liver cancer needs to be generated from studies of more cohorts. There does not appear to be epidemiological evidence to support prostate as a target organ for cancer induction.

Animal Studies

I concur that there is significant, multi-study evidence of liver and pancreatic tumors induced by PFOA and PFOS and testicular cancer induced by PFOA. A single study showing increased risk of uterine tumors induced by PFOA is insufficient to make conclusions. I have two concerns with the analysis, however. First, where serum PFAS data are provided, it is clear that carcinogenesis only occurs are concentrations that are not experienced by humans. Second, no studies of humanized PPARa mice appear to have been included, see below.

Mode of Action

Human PPARα is less responsive to PFOA than rodent PPARa (Nakamura et al., 2009), and the human PPARα response to PFOS is even less efficacious (Su et al., 2022). This is also evident in vitro (Nielsen et al., 2022). While PFOA and PFOS have not been studied for their carcinogenic potential in humanized PPARa mice. There is sufficient evidence with other ligands to show that hepatocellular carcinogenesis (HC) is unlikely to be induced in a human relevant manner. HC induced by a modestly potent and efficacious PPARa ligands (WY and bezafibrate) is completely absent in the humanized PPARa mouse (Cheung et al., 2004; Hays et al., 2005; Morimura et al., 2006). HC induced by a highly potent/efficacious PPARa ligand (GW7647) is diminished

in the humanized PPARa mouse and likely occurs via a different mechanism than in the mice expression mouse PPARa (Foreman et al., 2021a) Perinatal exposure to a high affinity PPARa in humanized PPARa mouse does not result in enhanced hepatocellular carcinogenesis (Foreman et al., 2021b).

Other mechanisms of action are most certainly at play for PFOA and PFOS (see liver toxicity and dyslipidemia discussions). Activation of CAR, which also is an MIE of PFOA and PFOS, can lead to carcinogenesis. But, again this appears to be specific to rodent CAR (Yamada et al., 2021).

Overall, the human data support kidney and testis as targets for PFOA/PFOS-induced carcinogenesis. Although I have found little evidence for biological effects on kidney in rodent models. Most data on liver carcinogenesis in animals should not be considered because this effect is dependent up rodent PPARα and is not a function human PPARα. With that said, evidence from humanized PPARa and CAR models should be taken into account (although little of data has been generated in these models). The increase in pancreatic carcinogenesis in animals is a concern, but it is unclear if this also is dependent upon the presence of mouse, rather than human, PPARa.

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Section 5.8: Other Toxic Effects

Comments:

Reduction in weight gain

This is a logical endpoint, given that PFOA has been shown to increase energy consumption (shown by indirect calorimetry) (Zheng et al., 2017). However, the analysis does not include what serum concentrations of PFOA and PFOS are associated with reduced weight gain. Also, sex as a variable was not discussed, which can influence this endpoint. Thus, it is difficult to know if reduced weight gain is a sensitive endpoint.

Adipose effects

It is not surprising that chemicals that activate PPARa have an effect on adipose, as PPARa activation plays an important role in determining the white vs brite adipocyte phenotype (Chen & Chao, 2017). However, the studies analyzed do not take into account the fact that mouse PPARa is more efficiently activated by PFOA than human PPARa. Again, whether a human-relevant serum PFAS concentration leads to these effects is not discussed.

Reduced bone quality

This is an important adverse endpoint, shown by both human epidemiology and a growing number of animal studies. Please see my comments in the “Developmental and Reproductive Toxicity” section.

Increased blood glucose

A number of very recent epidemiological studies (2019-present) supporting an association between aspects of glucose homeostasis and PFOA/PFOS/PFAS body burden have been published. PFOA-related increases in blood glucose in animal models appear to be more consistent than those reported for PFOS.

Mechanistic evidence

For the most part, what is presented as potential mechanisms of action are downstream of the molecular initiating event (MIE). What needs to be identified is/are the MIE(s) that occur at the lowest exposure levels (lowest concentrations in in vitro assays). These are most likely going to be activation of nuclear receptors (as discussed above), which are "designed" to respond specifically to very low concentrations of ligands. There is ample evidence that perfluorocarboxcylic acids such as PFOA are ligands for human PPARa and that perfluorosulfonic acids such as PFOS are at best, partial ligands, for human PPARa (e.g., shown and reviewed in Nielsen et al 2022). There is growing evidence that PFAS are ligands for PPARa, as well. These two nuclear receptors play important roles in regulating metabolic, adipose and bone homeostasis, thus it is logical that weight gain, adipose phenotype and glucose homeostasis can be altered by PFAS. Given species differences in PPARa in particular, the ability of PFAS to induce adverse health effects via human PPARa need to be demonstrated. With that said, again there is ample evidence of other nuclear receptors (CAR, PXR and PPARg) being activated by PFAS. CAR and PXR also play important roles in metabolic homeostasis (Gao et al., 2009; Spruiell et al., 2014).

The ToxCast database (accessible via the CompTox website) is an important resource for in vitro data on the biological activities of PFOA and PFOS. Care needs to be taken when determining the biological relevance of certain assays within the database. For instance, many of the reporter assays used to describe chemical interactions with nuclear receptors are Gal4 systems in which only the ligand binding domain of the receptor is present in the reporter system. While this can be useful in identifying a ligand for a nuclear receptor, it does not necessarily translate to biological activity given that the response element is not a nuclear receptor response element and that RXR is not required for DNA binding. The most relevant assays are those which use native, human receptors (discussed in Nielsen et al., 2022). With that said, there are number of analyses with PFOA and PFOS in HepaRG cells, in which the endpoint measured is endogenous gene expression. These support the activation of human PPARa, PPARg, CAR and PXR by PFOA and PFOS and demonstrate differences in potency and efficacy with which they activate these receptors.

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Zheng, F., Sheng, N., Zhang, H., Yan, S., Zhang, J., & Wang, J. (2017). Perfluorooctanoic acid exposure disturbs glucose metabolism in mouse liver. *Toxicology and Applied Pharmacology*, *335*, 41–48. https://doi.org/10.1016/j.taap.2017.09.019

Chapter 6: Dose response assessment

Section 6.1: Non-Cancer Dose Response Analyses and Acceptable Daily Dose Derivation (Animal data only)

Comments:

My only comment with regard to the analysis of human data is that there may be a missed opportunity to consider bone quality as a sensitive outcome of PFAS exposure.

Animal data

PFOA: Yan et al., 2014 (PMID: 24459700) is not included as a candidate critical study. I think this is an oversight. It is a 28 day study, similar to those listed, with LOAEL = 0.31 mg/kg/day and a NOAEL = 0.08 mg/kg/day based on multiple liver and serum endpoints. There is no comparison to the data supporting the candidate RfD, which is based on reduced ossification during development and a LOAEL 0f 1 mg/kg/day with a 16 day exposure (Lau et al., 2006). Both of these studies also report serum PFOA concentrations.

PFOS: Guruge et al., 2009 (LOAEL: 0.005 mg/kg/day) should also be considered for the immunotoxicity endpoint. A recent study showed that a 28 day PFOS exposure to 0.0015 mg/kg/day modified distributions of immune cell types while causing a less severe effect on influenza a-induced death (Torres et al., 2021), suggesting a closer estimate of the PFOS LOAEL. Overall, immunotoxic endpoints appear to be more sensitive endpoints that gross changes in liver weight or thyroid toxicity. They also suggest that the POD determined from Dong et al., 2009 is too high.

Guruge, K. S., Hikono, H., Shimada, N., Murakami, K., Hasegawa, J., Yeung, L. W. Y., Yamanaka, N., & Yamashita, N. (2009). Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice. In *The Journal of toxicological sciences* (Vol. 34, Issue 6, pp. 687–691). https://doi.org/10.2131/jts.34.687

Lau, C., Thibodeaux, J. R., Hanson, R. G., Narotsky, M. G., Rogers, J. M., Lindstrom, A. B., & Strynar, M. J. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicological Sciences : An Official Journal of the Society of Toxicology*, *90*(2), 510–518. https://doi.org/10.1093/toxsci/kfj105

Torres, L., Redko, A., Limper, C., Imbiakha, B., Chang, S., & August, A. (2021). Effect of Perfluorooctanesulfonic acid (PFOS) on immune cell development and function in mice. *Immunology Letters*, *233*, 31–41. https://doi.org/10.1016/j.imlet.2021.03.006

Yan, S., Wang, J., Zhang, W., & Dai, J. (2014). Circulating microRNA profiles altered in mice after 28 d exposure to perfluorooctanoic acid. *Toxicol Lett*, *224*(1), 24–31. http://www.ncbi.nlm.nih.gov/pubmed/24459700

Chapter 7: Health Protective Drinking Water Concentrations

Section 7.1 Non-Cancer Health Protective Drinking Water Concentrations

Comments:

PFOA: I concur with justification of the values for RSC and DWI used in the equation. I cannot comment on the choice of the ADD, as I did not review the human data analysis for Chapter 6 given a COI.

PFOS: I concur with justification of the values for RSC and DWI used in the equation. I cannot comment on the choice of the ADD, as I did not review the human data analysis for Chapter 6 given a COI.

Chapter 8: Risk Characterization

Comments:

The analysis found that drinking water concentrations that would be protective of PFOA- (0.007 ppt) and PFOS- (1 ppt) induced cancer were lower than non-cancer endpoints, and thus would be protective for both. My only caution is that PFOA-induced bone toxicity and PFOS-induced immunotoxicity may not have been sufficiently considered and could lower the non-cancer-endpoint-based protective concentration.

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Review of The scientific basis of the proposed public health goals for perfluorooctanoic acid and perfluorooctane sulfonic acid in drinking water

Based on my expertise and experience, I am reviewing the findings, assumptions, or conclusions with confidence to i. Mammalian toxicology (for PFOA and PFOS) and ii. Environmental epidemiology (for PFOA and PFOS). Since cancer endpoints were the selected point of departure values used to set the public health goal, the assumptions and recommendations were also briefly evaluated.

General Introduction

Although there are a number of structurally related Per- and Polyfluoroalkyl Substances (PFAS) found in the environment and in drinking water, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) specifically were widely used and are the most studied PFAS from a health effects stand point. Although the intentional production of PFOA and PFOS in the US was phased out, their legacy persists as both PFOA and PFOS are still found in the environment and drinking water sources posing potential risks to human health. It is noteworthy that there are numerous structurally related PFAS compounds in the environment, but there is insufficient information to understand if these chemicals behave similarly in biological systems. This is an area of significant uncertainty for the commercially introduced PFOA and PFOS replacements.

Assessment of the Systematic Literature Review

An important aspect of the scientific basis of the proposed public health goals for perfluorooctanoic acid and perfluorooctane sulfonic acid in drinking water is the rigorous systematic literature review of the toxicity of PFOA and PFOS in humans and animals models. The review for this assessment report was inclusive of peer-reviewed journal articles, books, reports, and other potentially relevant sources. Since recent systematic reviews were available from the US EPA, New Jersey Drinking Water Quality Institute, and ATSDR (2016-2018 reports) the Office of Environmental Health Hazard Assessment (OEHHA) added searched for additional information available from January 2016 to September 2019. Overall, the literature review is considered systematic and thorough.

Drinking water intake rate.

Although there is some uncertainty in calculating lifetime average drinking water intake rates, the OEHHA used the most up to date water intake estimates based on US EPA, NHANES, and OEHHA studies. For the health protective goals calculations, the 0.053 L/kg-day is considered appropriate and protective for infants due to their greater exposure to drinking water contaminants.

Relative Source Contribution.

A critical factor is establishing a drinking water health protection goal is to clearly understand the proposition of chemical exposure in the drinking water relative to other sources of chemical exposure. Numerous studies indicate that the major exposure to PFAS compounds is through contaminated food, and drinking water under most situations will contribute a relatively minor and variable fraction of PFOS and PFOA exposures depending on lifestyle and water consumption sources. The OEHHA recognized this challenge and concluded that it is currently not possible to accurately estimate relevant sources of PFOA or PFOS exposure for California residents, and used a default of 20% for both chemicals. In my view, this is a conservative, but reasonable estimate for the public health goal calculations.

Toxicokinetics

Oral Absorption

PFOA and PFOS are efficiently absorbed following oral administration in human and animal studies. For all reviewed animal studies oral efficiency exceeded 90% and approached 100% for some doses. There are limited PFOA and PFOS absorption studies in humans, but exposure modeling predicts a somewhat lower absorption rate for PFOA.

Estimation of Elimination of Half Life

There are well established differences in the bioaccumulation of both PFOA and PFOS between humans and animal models. Measured serum half-lives in animal models range between weeks and months, and is estimated at 2.3 years for PFOA and 5.4 years for PFOS in humans. With these differences, it is most appropriate that OEHHA used human half-life data to reduce uncertainty for calculation of the human protection goal values.

PFOA - Regression analysis of available human epidemiological PFOA exposure data from several locations and exposure scenarios, the PFOA clearance rate of 2.8x10-4 L/kg-day was applied to convert serum levels to applied dose. The considered scenarios, assumptions, and approaches were systematically compared and the final recommendation was well justified in the report.

PFOS - OEHHA used newly available human exposure data from a high PFOS exposure in contaminated drinking water that occurred in Ronneby, Sweden to derive the clearance rate of 3.9x10-4 L/kg-day as a conversion of serum levels to applied dose. The considered scenarios, assumptions, and approaches were systematically compared and the final recommendation for PFOS was well justified in the report.

Noncancer Endpoints

Immunotoxicity

PFOA – This authors quote the National Toxicology Program (NTP) from the 2016 document “The NTP concludes that PFOA is presumed to be an immune hazard to humans based on a high level of evidence that PFOA suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. Although the strongest evidence for an effect of PFOA on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from epidemiological studies that PFOA reduced infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence. The evidence indicating that PFOA affects multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans.”

PFOS –The authors quote the NTP 2016 conclusion “The NTP concludes that PFOS is presumed to be an immune hazard to humans based on a high level of evidence that PFOS suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. Although the strongest evidence for an effect of PFOS on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from studies in experimental animals that PFOS suppresses disease resistance and natural killer (NK) cell activity. The evidence indicating that PFOS suppresses multiple aspects of the immune system supports the overall conclusion that PFOS alters immune function in humans.”

Overall, the OEHHA identified clear evidence from human epidemiologic data and from animal data that that both PFOA and PFOS are strongly associated with decreased antibody response. Some of the other exposure related changes in functional immunological responses were suggestive in the human epidemiological literature, and the animal data further suggest the immune system is susceptible to PFAS exposures. The final recommended public health goal will thus also set protective levels for the immune system.

Liver Toxicity

PFOA – A careful review of the epidemiologic literature, indicates that PFOA exposures are associated with human hepatotoxicity. The most consistent endpoint is increases in levels of liver enzyme. There are somewhat inconsistent associations between adult PFOA exposures and total cholesterol or low density lipoproteins. There is strong concordant liver toxicity from rat and murine studies. Rodent data consistently identified increased liver weight, histopathological responses, and increased serum enzymes indicative of liver damage.

PFOS – The human epidemiological data for PFOS exposures are less clear, but PFOS exposure studies in rodents indicate clear hepatotoxicity with effects similar to that of PFOA including increased liver weight, liver enzymes, and histopathology. There are somewhat inconsistent associations between human adult PFOS exposures and total cholesterol or low density lipoproteins.

Thyroid Toxicity

PFOA - OEHHA did not find consistent effects related to the thyroid in the epidemiologic literature. PFOA associated thyroid effects have been reported in environmentally exposed animals, and controlled laboratory studies find positive associations between PFOA and changes in thyroid gland weights and thyroid hormones.

PFOS OEHHA also did not see consistent associations between PFOS and thyroid hormone levels in humans. It is noteworthy that the US EPA had identified three epidemiologic studies that reported positive associations. Subacute rat NTP studies produced decreases in T3 and T4 were observed in both sexes and these effects were observed only in high plasma concentrations. Decreased thyroid weight was reported only in males.

Developmental and Reproductive Toxicity

Measures of human fetal growth – birth weight

PFOA – A summary of the human epidemiological literature looking for an association between prenatal PFOA exposure and lower birth weight revealed inconsistent results with no clear trends. Although a few small studies found positive association between PFOA and low birth weight, the majority of studies found no statistically significant associations between PFOA and birth weight. In my view this analysis was compressive and I concur with the authors that there is no clear relationship between prenatal PFOA exposures and decreased birth weight.

PFOS - Although a few studies reported prenatal PFOS exposure related decreases in birth weight, more recent large scale prospective studies failed to report statistically significant associations between prenatal exposure to PFOS and birth weight. In my view the authors were systematic, thorough, and used the most recent data and I agree with the current report conclusion that there is no clear relationship between prenatal PFOS exposures and risk of decreased birth weight.

Fertility and fecundity:

PFOA – In review of the human epidemiologic literature, OEHHA did not identify consistent evidence that PFOA decreased fertility or fecundity. A large number of animals studies identified reproductive effects in male and female rodents indicating that PFOA is a developmental and reproductive toxicant.

PFOS – A review of the PFOS human exposure data also did not find clear epidemiologic evidence that PFOS exposure decreased fertility or fecundity. However, the recent animal data is consistent with previous findings that PFOS adversely affects reproduction and development systems in rodents.

Developmental Outcomes from Animal Studies

PFOA- OEHHA comprehensively collected and summarized the recent animal developmental and reproductive toxicity studies. There is strong evidence that PFOA produces adverse developmental and reproductive outcomes in pups. The study by Esterik et al. (2016) is noteworthy as the two highest doses decreased litter sizes and several developmental effects were reported in male and female pups. For example, the OEHHA determined a NOAEL of 0.003 mg/kg-day based on decreased body weight in female pups on PND 4 representing a sensitive PFOA developmental endpoint.

PFOS- OEHHA comprehensively collected and summarized the recent animal developmental and reproductive toxicity studies. There is strong evidence that PFOS produces adverse developmental outcomes in pups. The adverse outcomes were similar to those produced by PFOA, but there is no evidence that PFOS exposures effect litter size.

Recent Animal Neurotoxicity Evidence

PFOA – Investigations of the potential neurotoxicity of PFOA are surprisingly limited. Two recent rodent studies (Guo et al. (2019)) reported that high doses decreased brain glutamic acid content and increased glutamate synthetase and in utero exposure to PFOA (5 mg/kg-day) increased cortical nerve cells numbers in Kunming mouse pups (Qin et al., 2018). I agree with the report authors that more studies are needed to conclusive determine if PFOA is a neurotoxic to animals.

PFOS – The most recent PFOS animal studies are highly suggestive of exposure-induced neurotoxicity in vivo and in vitro. There is increasing evidence that PFOS produces neurotoxicity via multiple potential modes of action.

Cancer Health-Protective Drinking Water Concentrations for PFOA

This reviewer is not an expert in cancer risk assessment. Following a thorough review of the human and animal cancer data OEHHA has determined that that PFOA should be evaluated as a carcinogen. The strongest human association is for kidney cancer and thus cross species extrapolations for PFOA is not necessary to calculate the cancer slop factor for PFOA. PFOA also produces liver and pancreatic tumors adding confidence to this determination. Since exposure to PFOA is primarily via ingestion the decision of the OEHHA to calculate a cancer-based health-protective concentration based solely on drinking water consumption is appropriate. The calculated cancer health-protective concentration of 0.007 ppt was selected as the public health guidance using appropriate assumptions.

Cancer Health-Protective Drinking Water Concentrations for PFOS

Following a thorough review of the animal cancer data has determined that determined that PFOS should also be evaluated as carcinogen. PFOS exposures produced liver and pancreatic tumors in male and female rats. Since exposure to PFOS is primarily via ingestion the decision of the OEHHA to calculate a cancer-based health-protective concentration based solely on drinking water consumption is appropriate. The

calculated cancer health-protective concentration of 1 ppt was selected as the public health guidance using appropriate assumptions.

Overall Assessment for the Drinking Water Public Heath Goal for PFOA

A thorough review of the available literature for PFOA indicates that exposures to this chemical is associated with several adverse health effects in humans. Although some of literature of the noncancer endpoints are not congruent, there is reasonable evidence that environmental exposures to PFOA is associated with kidney cancer, reduce immune system and liver functions. Importantly, there is ample experimental animal data that also reports kidney cancer, immunological and liver toxicity. Since human epidemiological studies for PFOA are more sensitive than the animal model data for these endpoints, it is most appropriate to use the currently available human risk of kidney cancer as the endpoint driver. The calculated public health goal for PFOA is drinking water of 0.007 ppt would also be protective for the currently known noncancer endpoints.

Overall Assessment for the Drinking Water Public Heath Goal for PFOS

A review of the available literature for PFOS indicates that exposures to this chemical is associated with adverse health effects in humans. Although some of literature of the noncancer endpoints are inconsistent, there is evidence that environmental exposures to PFOS is associated with immunological and liver toxicity. The observed elevated total cholesterol association with PFOS in humans is the strongest adverse outcome. Similar to PFOA, the major noncancer effects of PFOS in experimental animals are liver, immune, developmental and reproductive systems. The decision to use the observed increased liver and pancreatic tumor incidence in a two-year rat study to calculate the PFOS public health goal of 1 ppt for PFOS is appropriate with the currently available information. This level should protect against all currently known noncancer toxicities.

Concluding Comments

The OEHHA has adequately reviewed and addressed the available information for both PFOA and PFOS. The methods applied in the derivation of protective health goals based on human kidney cancer for PFOA, and the two-year cancer rat study for PFOS appeared appropriate. In my view the OEHHA adequately addressed all of the important scientific issues relevant for both PFOA and PFOS and the methods applied in the derivation of each health-protective concentration. Although the derived health protective goals for cancer are lower than any of the noncancer endpoints, the proposed noncancer health-protective concentrations were well justified. I am not aware of any missing critical data that that would impact the conclusions made in this report. Finally, based on the available human and animal model data, the recommended public health goals would expect to be protective to vulnerable and sensitive populations.