

REVISED AND PRELIMINARY
INDIVIDUAL COMMENTS

SAB PFAS REVIEW PANEL
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REVISED COMMENTS

Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Study Identification and Inclusion

1. EPA used systematic review methods consistent with the current ORD systematic review practice to ensure transparency and completeness of literature identification, sorting, and study quality evaluation. Is the process clearly described? Please identify additional peer-reviewed studies that the panel is aware of that could inform toxicity value derivation.

CHIU: Defer to assigned reviewers.

Noncancer Hazard Identification

1. Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

CHIU: The hazard identification process is not fully transparent, either in the evaluation of different evidence “streams” (human, animal, mechanistic), or in their integration. For instance, although study quality/risk of bias is discussed, the “synthesis” within an evidence stream does not appear structured. Examples of how to structure “synthesis” include the Hill criteria (see IRIS Handbook and NASEM review of the IRIS Handbook), GRADE, NTP/OHAT, and the Navigation Guide. Similarly, there is no framework for integration of human, animal, and mechanistic data, which is also addressed in several of these examples. These frameworks include templates for tabular summaries of evidence both “within” and “across” evidence streams that could be adapted to provide more transparency.

A second issue is that the document is not clear as to the demarcation between the process of hazard identification and the process of dose-response assessment. For instance, the evidence integration sections discuss POD derivations as part of evidence synthesis. Separating the steps of hazard identification and identifying PODs would provide greater transparency. In particular, a conclusion as to hazard should not depend on whether the data can provide PODs – indeed, the dependency should be the other way around, where only health effects with a certain level of confidence in “hazard” should be considered for dose-response. This separation of hazard and dose-response is important because studies that provide strong evidence for hazard do not necessarily have to be amenable to POD derivations (e.g., epidemiologic studies with semi-quantitative exposure metrics), and conversely, the availability of studies amenable to POD derivations is not sufficient to provide strong evidence of hazard.

One approach would be to follow existing frameworks whereby for each health effect, a “synthesis” conclusion is made for each evidence stream (animal, human, mechanistic), and then

an overall “hazard” conclusion is made by integrating across evidence streams. This would provide additional transparency as to the hazard conclusions, as well as clarify the reasons why a particular health endpoint is chosen for candidate RfD derivations. Selecting studies for POD derivation would be a subsequent step, and consider both whether studies are amenable to POD derivation (especially BMD analysis) as well as study confidence/quality indicators.

Of particular importance is to make more transparent the strength of evidence conclusion with respect to serum lipids and CVD, given the prominence of that endpoint in the CVD document separately reviewed. It appears that the data on total cholesterol in humans, although showing an effect in the adverse direction, have high heterogeneity. Indeed, the meta-analysis from the CVD document could well be included to support the hazard identification conclusions. However, there does not seem to be strong evidence on “hard” outcomes; and moreover, the experimental animal studies show effects in the opposite direction – decreases in serum lipids. Additional discussion as to this lack of concordance would be helpful (e.g., are there known “positive” controls in humans that increase serum lipids in rodents?).

2. Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.
3. Does the SAB panel agree with EPA’s rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.
 - A. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.
 - B. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

CHIU: The main concern here is consistency across endpoints. The two reasons cited – “1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease” – could arguable also apply to some of the other endpoints.

I defer to subject matter experts as to interpreting whether the endpoints and BMR levels for specific health effects reflect a “minimally adverse” level of effect.

Cancer

1. Cancer classification for PFOA/PFOS

- A. PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a ‘likely carcinogen’ categorization which is a change from ‘suggestive’ in the 2016 HA. Does the panel agree with the ‘likely’ designation based on the new evidence? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.
- B. PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a ‘suggestive’ categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

CHIU: Similar comments as above regarding Hazard Identification for carcinogenicity apply, except that the EPA Cancer Guidelines provide a structured approach (though not as formal as other more recent frameworks) that can enhance transparency. Included in this would be a discussion of epidemiologic data structured along the Hill criteria, an assessment of evidence from animal studies, and assessment of MOA, and a “weight of evidence narrative” that justifies why the evidence fits into one of the “descriptors” defined by the Cancer Guidelines.

2. Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends.

CHIU: One concern about these epidemiologic studies is whether other PFAS may be contributing to the effect because they are correlated with PFOA, but not measured. If this were the case, then the actual potencies of PFOA would be lower than implied by the studies. This possibility needs to be addressed more explicitly, particularly given the large apparent difference in potency between animal and human studies.

It is recommended that multiple candidate cancer slope factors be developed, including ones based on other epidemiologic studies of sufficient quality (which is important given the exclusion of earlier human studies from consideration) as well as animal cancer bioassays. Each study’s strengths and limitations (e.g., exposure uncertainties, confounding [including possible reverse causation]) would need to be discussed and a judgment made as to whether to select one or more studies to represent the overall slope factor.

Toxicokinetic Models

1. Human model –

- A. For endpoints observed in adults, EPA used a steady-state approach to calculate the HED, which assumes a relatively constant exposure and clearance during adulthood. Please comment on this method of HED calculation. Are there alternative approaches that EPA should consider? If so, please describe the rationale for recommending this approach(es).

CHIU: I agree that for endpoints observed in (non-pregnant) adults where the AUC is the appropriate dose metric, the steady-state approach is adequate for calculating the HED. However, given that a model is available, comparison between lifetime exposure using the model and steady state could be useful.

One broader issue is the use of the model when deriving drinking water MCLG values. In particular, a constant drinking water concentration will not equal a constant dose rate in mg/kg-d due to age-dependent changes in drinking water consumption, so if the goal is to “not exceed” an “internal dose” POD based on AUC or C_{max}, then it seems that the model could be used (with exposure factor distributions) to derive equivalent drinking water concentration that would correspond to the “internal dose” POD. In the Goeden et al. (2019) study on PFOA, the “internal dose” POD was further adjusted for inter-species and intra-species uncertainty/variability so that the “RfD” was expressed on a dose metric equivalent. This approach could also be considered by EPA.

- B. Two key parameters are the half-life and volume of distribution, which were used to calculate clearance. Half-life and volume of distribution were assumed to be constant across sex and age groups because of a lack of strong quantitative data to parametrize changes across sex and age. Please comment on the strengths and weakness of the use of this assumption and the choice of these parameters by the EPA. Please describe the rationale for alternative recommended approaches. For endpoints observed in human neonates or children, EPA used a one-compartment TK model to simulate dosimetry during pregnancy and a two-compartment TK model (one-compartment models for the mother and the child) to simulate dosimetry during lactation, to calculate the HED for each POD. Please comment on the strengths and weaknesses of this choice of model structure for the task of predicting dosimetry in the human fetus and child compared to dosimetry in mice and rats in the similar lifestages. Please provide the rationale for any alternative recommended approaches.

CHIU: I would suggest that EPA further evaluate the Goeden et al. (2019) model, which was evaluated with respect to infant serum data (higher reported R² than Verner et al. (2016) model when compared to Fromme et al. data), and as well develops a parameterized age-dependent volume of distribution. Additionally, it seems that it would not be challenging to include a “growth dilution” factor into the Goeden et al. (2019), which was the main critique of this model in the PFOA document. Of course, another consideration may be that the Verner et al. (2016) model includes both PFOA and PFOS, whereas Goeden et al. (2019) is just for PFOS. Nonetheless, a direct comparison of performance and HED calculations between Goeden and Verner may be useful as a sensitivity analysis.

- C. The key chemical-specific parameters that describe the transfer of the chemical from the mother to the child during gestation and lactation are the maternal to fetal serum ratio and the ratio of maternal serum to milk PFOA/S concentration. These ratios were assumed to be constant during gestation and lactation, respectively. Another important parameter is the rate of milk ingestion, which is chemical-independent and varies throughout lactation. Please comment on the

strengths and weaknesses of the choice of parameters for fetal to maternal partitioning and partitioning into breastmilk, as well as the choice for lactation rate. Please also comment on the choice to assume that fetal to maternal partitioning and partitioning to breastmilk did not vary in time. Please describe whether there are other methods you would recommend to account for these changes over time and across development.

CHIU: It would be useful to characterize the uncertainty that results from these assumptions, for instance through sensitivity analyses or Monte Carlo simulation with a range or distribution of values. For instance, the Goeden et al. (2019) transgenerational TK model includes at least both central and upper bound estimates for different parameters.

2. Animal Model –

- A. After a review of the available toxicokinetic models for PFOA/S predictions in laboratory animals, EPA selected the Wambaugh et al. (2013) model because it was parametrized using all species of interest, demonstrated good agreement with training and test datasets, and used a single, biologically motivated, model structure across all species. Does the panel agree with selecting this model? If not, please describe the rationale for alternative recommended approaches for the calculation of the internal dose metrics in adult animals.

CHIU: I agree that the Wambaugh et al. (2013) model is adequate for deriving HEDs from experimental animal studies.

- B. The animal model parameters were obtained through a Bayesian inference parameterization which produced wide credible intervals for some parameter values, but relatively tight credible intervals for the predicted serum concentration. Does the panel agree with using the median values of the estimated animal parameter distributions for prediction of serum concentration and internal dose metrics?

CHIU: I agree that median values can be used for deriving the HED. However, the uncertainty in both the human and animal models predictions should be characterized, particularly on their impact on the HED calculation. This is particularly important if a probabilistic approach were to be used to calculate risk-specific doses instead of traditional deterministic RfDs.

- C. Based on visual inspection of model predictions to the calibration datasets, EPA utilized sex-independent parameters for PFOS. The male-specific parameters were used for all rat-specific PFOS predictions including predictions in pregnant and nursing dams and the female-specific parameters were used for all mouse-specific PFOS predictions because the parameter values obtained from fitting the female-specific rat data and male-specific mouse data were not consistent with the overall TK parameters for PFOS and produced poor fits to the training and test datasets. Does the panel agree with this approach and justification for this assumption for PFOS? If not, please describe other approaches that could be considered?

CHIU: Defer to other reviewers.

- D. EPA assumed a one compartment model for the developing infant based on the lack of infant-specific toxicokinetic data from rats and mice. This model utilizes averages of half-life and volume of distribution from the literature coupled with physiologically relevant lactational parameters for pup nursing. Does the panel agree with the decision to use this model structure for infant animals? If not, please provide data on infant-specific changes during the animal lactational-period that could be used to account for toxicokinetic differences between the adult and infant rats and mice.

CHIU: I agree that the one compartment model for the developing infant is adequate for deriving HEDs from experimental animal studies.

- E. Several parameters dictate the transfer of chemical from the mother to her pup. Does the panel agree with the selection of these parameters for the animal model? If not, please provide your justification and alternative parameters.

CHIU: Sensitivity analyses or uncertainty analyses would be helpful to better characterize the impact of uncertainty of these parameters.

- F. For neonatal animals, EPA assumed no sex differences in clearance in neonatal animals based on the lack of identification of sex-dependent differences in PFOA/S toxicokinetics from the available data. Does the panel agree with this assumption? If not, please provide your justification and available data on sex differences in neonatal rats.

CHIU: I agree that data are inadequate to parameterize sex differences in neonatal animals.

Epidemiological Study RfD Derivation

1. EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only 'medium' and 'high' quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?

CHIU: [MOVED TO BELOW] For birth weight, it is not clear why a meta-analysis approach was not used to derive the POD. For instance, the CVD document reviewed separately contains a meta-analysis for total cholesterol. It would seem straight forward to apply the same methodology to the beta coefficients ("re-expressed" as per ng/mL) for decreased birth weight. Then, the meta-beta coefficient could be used to deriving PODs. Additionally, a 5% extra risk in the incidence of low birth weight seems like quite a large BMR (from a baseline rate of 8.27% to 12.86%!), with a shift in the mean of almost 100g from 3,261 g to 3169 g! This does not seem to be adequately health protective. Moreover, the BMD values (in ng/mL) are often outside of the

range of serum concentrations in the study, meaning the method is essentially extrapolating “upwards” to *higher* exposures (this would be less of a problem for a smaller BMR). Nonetheless, it seems that an economic benefit-cost analysis could be performed either on the continuous endpoints of birth weight or the dichotomous endpoint of increased incidence of low birth weight babies.

One concern about these epidemiologic studies is whether other PFAS may be contributing to the effect because they are correlated with PFOA or PFOS, but not measured. If this were the case, then the actual potencies of PFOA or PFOS would be lower than implied by the studies. This possibility needs to be addressed more explicitly, particularly given the large apparent difference in potency between animal and human studies.

2. Studies of developmental immune health outcomes (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]) after PFOA/S exposure identified associations with very low doses of either PFOA or PFOS with developmental immune effects. The RfD for this outcome was selected as the critical effect because it was the lowest among the candidate RfDs for PFOA or PFOS and can result in severe illness. Does the panel agree with the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS?
 - A. If so, please explain your justification.
 - B. If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of chronic RfDs.
 - C. Are any additional analyses or rationales needed to increase the confidence in the chronic RfDs for PFOA and PFOS?

CHIU:

For PODs based on human data, several additional analyses/rationales would increase confidence:

- Consideration of multiple studies in a meta-analysis approach For instance, the CVD document reviewed separately contains a meta-analysis for total cholesterol. It would seem straight forward to apply the same methodology to the beta-coefficients (“re-expressed,” if necessary, in units of per ng/mL) for other endpoints. Then, the meta-beta coefficient could be used to deriving PODs.
- Better justification of BMR levels so they reflect similar degrees of “severity.” For instance, the impact of a 5% extra risk in the incidence of low birth weight seems like quite a large BMR (from a baseline rate of 8.27% to 12.86%!), with a shift in the mean of almost 100g from 3,261 g to 3169 g!
- Ensuring that BMR levels reflect BMD values that are within the range of observation, preferably at the low end. For instance, the BMD values (in serum concentration ng/mL) for low birth weight appear to often be outside (or at the high end) of the range of serum concentrations in the study, meaning the method is essentially extrapolating “upwards” to *higher* exposures.
- Expressing study results in terms of a “slope” (e.g., beta coefficients) from which a risk-specific dose could be derived. Additionally, the “slope” values would be more amenable to economic benefit-cost analysis.

Additionally, it would be useful to ensure that, to the extent possible, animal and human PODs have comparable units (e.g., preferably serum ng/mL, or human equivalent doses) and BMR values, so that they may be more directly compared.

Regarding selection of studies for POD, it is not clear why PODs based on experimental animal studies were not also used to derive candidate RfDs. It would be more transparent to derive candidate values from all eligible studies (adequate confidence, etc.) first, and then subsequently select health effect-specific and overall RfDs. Notably, it is not clear that health effect-specific RfDs were chosen, even among the existing candidate values. Such values are clearly needed in order to implement the draft mixture framework being reviewed concurrently.

Additional transparency is also needed to justify selecting among different candidate RfDs to select the health effect-specific RfD. The strengths and limitations for each study/POD in terms of the strength of evidence for that particular endpoint (including risk of bias), animal vs. human studies, dose-response uncertainties, etc. should be described to justify the selection of a health effect-specific RfD. Thereafter, selecting the overall RfD across health effects should also discuss the strengths and limitations, including strength of evidence for hazard and the uncertainties. Just being the lowest value is not enough justification.

Furthermore, as discussed previously with respect to TK modeling, consideration should be given to deriving candidate RfDs in internal dose units (e.g., serum ng/mL). This would mean applying appropriate uncertainty factors to the internal dose PODs. The advantage of this approach is that, for drinking water applications, an exposure at a constant drinking water concentration could be modeled using the TK model and appropriate exposure factors, with the MCLG derived as the dose for which the internal dose POD (over the appropriate exposure window or duration) is not exceeded. The distinction between drinking water concentration and oral dose is especially important because several health outcomes under consideration occur as a result of exposures during particular windows, such as pre-birth and during childhood, during which exposure factors differ substantially from during adulthood.

One particular endpoint where there appears to be high concordance of evidence between animal and human studies is decreased birth weight. However, the apparent lack of concordance in dose-response may be due in part to the different definitions of endpoint and benchmark response. In particular, the animal studies use a SD-based benchmark response for the continuous endpoint of birth weight. However, the human studies use a “hybrid approach” that calculates the percent of additional “low birth weight” infants. However, the animal and human studies could be directly compared based on % change in the continuous outcome per unit serum concentration. This would reveal the extent to which there is (or is not) concentration-response concordance between animal and human studies.

3. The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and

Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.

CHIU: [Comments made above instead] The main concern is that the different candidate PODs should reflect a similar degree of “severity.” As mentioned above, a 5% increase in incidence of low birth weight appears to be rather high level of severity; on the other hand, a 5% decrease in antibody response may not have clinical significance (akin to the ALT discussion above).

4. EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for PFOA and PFOS.
 - A. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.
 - B. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

CHIU: The recent NASEM review of the IRIS Handbook recommended application of the WHO/IPCS probabilistic framework (including UF distributions, rather than fixed values) to calculate risk-specific doses as a replacement for traditional RfDs, in line with the recommendations of the NASEM (2009) Science and Decisions report. A recent publication Chiu et al. (2018) [<https://doi.org/10.1289/EHP3368>] demonstrated broad application of this approach using experimental animal studies across many chemicals and endpoints, and included EPA authors from EPA’s National Center for Environmental Economics. This probabilistic framework not only includes default UF distributions based on reviews of the literature, but also enables derivation of dose-response functions (or risk-specific doses) that can be used for benefit-cost analysis. EPA should consider whether applying this approach here would be useful for MCLG derivation and the regulatory impact assessments that will be needed to set MCLs.

Relative Source Contribution

1. EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual’s total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the

general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources. Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at: <https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS.

- A. Are you aware of additional relevant exposure data that EPA should consider in developing the RSCs for PFOA and PFOS? If so, please provide citations.
- B. Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS is adequately supported and clearly described.

CHIU: Defer to assigned reviewers.

CHARGE QUESTIONS AND DRAFT ASSIGNMENTS

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Introduction

Per- and polyfluoroalkyl substances (PFAS) present many unique challenges from a risk management perspective due to the large number (1000's) and structural diversity of members in this chemical class, limitations in available human health and exposure information, and the spatial and temporal variability of their presence in drinking water and other environmental media. To inform various decision contexts in addressing PFAS contamination, EPA has developed a draft *Framework for Estimating Noncancer Health Risks Associated with Mixtures of PFAS* to illustrate the practical application of EPA chemical mixtures approaches and methods^{1,2} for two or more PFAS co-occurring in environmental media. Specifically, this document describes an approach for providing a tiered, flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS under an assumption of dose additivity. While this framework is being developed to inform the National Primary Drinking Water Regulation for PFAS, it is not intended to be media-specific in practical applications.

Overall charge: EPA is seeking SAB comment on whether the framework and illustrative examples provided in the document are scientifically supported, clearly described, and informative for assessing potential health risk(s) associated with exposure to mixtures of PFAS.

CHIU: Overall, the flowchart describing the different tiers is not always adequately justified as to why one approach or another would be selected. For instance, if component dose-response data are amenable to BMD modeling, why wouldn't this also support de novo derivation of toxicity values? Additionally, it is not clear that the "Tier 1" TOSHI approach is actually a

“screening” calculation. As described briefly below, TOSHI will give the same equivalent answers as RPFs and Mixture BMD approaches under certain conditions, and moreover, it is not clear that TOSHI is always “conservative” relative to the RPF and Mixture BMD approaches. In a Tiered approach, the lower Tiers are generally more “conservative” so as to emphasize sensitivity (lower false negatives) and only expend additional effort at “higher” tiers to provide more specificity (identifying false positives). However, it not clear this is the case for TOSHI vs. RPF and Mixture BMD.

Therefore, the “Tiered” framework may be better replaced by a “menu”-type framework, where the data requirements, advantages/disadvantages, and uncertainties/potential under- or over-estimation from the different approaches are described, so the user can select the most “fit for purpose” approach for their context.

Charge questions

1. **The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, an assumption of dose addition for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA’s supplementary guidance (EPA, 2000) states: “The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration).” This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which “similarity” can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.**

A. Please comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.

CHIU: I agree and endorse this approach for component-based mixture evaluation of PFAS under an assumption of dose additivity. Specifically, I agree that the most appropriate assumption is similarity at the level of toxicity endpoint/health effect rather than MOA

B. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).

CHIU: Not applicable.

2. **Section 4.3 (Hazard Index; HI) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and**

hypothetical exposure information. The example calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as “GenX chemicals”).

- A. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.
- B. Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment.

CHIU: I agree and endorse the HI approach, and the HI methodologies proposed, as reasonable screening-level approaches for indicating potential risk associated with mixture of PFAS. However, the TOSHI approach necessitates endpoint/health effect-specific reference values, and not just overall reference values. Therefore, the document should be clearer that that endpoints/health effect-specific reference values be developed for individual PFAS.

Furthermore, the TOSHI approach is quite analogous to the RPF and mixture BMD approaches, in that it consists of a weighted average for a specific outcome/health effect, and thus should not necessarily be considered a “lower tier” methodology.

Additionally, the NASEM review of the IRIS handbook recently endorsed the IRIS program’s development of probabilistic risk-specific doses to replace traditional deterministic reference values. EPA should consider the extent to which using the corresponding “probabilistic RfD” or “risk-specific doses” would change the proposed HI/TOSHI approach, or whether such probabilistic reference values can be used as direct replacements for the traditional RfD in HI/TOSHI calculations. It should be noted that the risk-specific doses derived from these methods provide actual estimates of risk in the form of population incidence (e.g., 1% of the population) for a particular magnitude of effect (e.g., 5% change in ALT) at a particular confidence level (e.g., 95% confidence), and thus provide more than “indicating potential risk” and are more akin to “estimating risk.”

3. Section 4.4 (Relative Potency Factor; RPF) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and HFPO dimer acid and GenX chemicals.

- A. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.

- B. Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.

CHIU: I agree and endorse the RPF approach as a reasonable methodology for estimating risk associated with mixtures from PFAS. However, the framework should also summarize when the TOSHI and RPF approach will give essentially the same answer (e.g., when the ratio of the POD values used to calculate the RPFs is equal to the ratio of the endpoint-specific RfD values used to calculate the HI), and the extent to which consistency is appropriate or inappropriate (e.g., if one is supposed to be more “conservative” for screening, rather than more “predictive.”).

4. Section 4.5 (Mixture BMD) of the framework document demonstrates the application of a component-based mixture approach using established EPA dose-response modeling (i.e., benchmark dose; BMD) of hypothetical PFAS dose-response data, and hypothetical exposure information.

- A. Please provide specific feedback on whether the Mixture BMD approach is a reasonable methodology for estimating what is in essence a mixture-based point-of-departure. If not, please provide an alternative.
- B. Please provide specific feedback on whether the proposed Mixture BMD methodology in the framework is scientifically supported for PFAS mixture risk assessment.

CHIU: I agree and endorse the Mixture BMD approach as a reasonable methodology for estimating a mixture-based POD. As with the RPF approach, the framework should also summarize when the TOSHI and Mixture BMD approach will give essentially the same answer (e.g., when the ratio of the BMD values used to calculate the mixture MBD is equal to the ratio of the endpoint-specific RfD values used to calculate the HI), and the extent to which consistency is appropriate or inappropriate (e.g., if one is supposed to be more “conservative” for screening, rather than more “predictive.”).

EPA’s draft Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water

Introduction

To fulfill the Health Risk Reduction and Cost Analysis (HRRCA) requirements under Safe Drinking Water Act, EPA is developing a benefit-cost analysis and other related rule analyses to inform consideration of regulatory alternatives. According to the *Proposed Approaches to the Derivation of Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water, currently under concurrent SAB review*, current epidemiologic literature supports positive associations between PFOA and PFOS exposure and total cholesterol. EPA is developing national-level benefits estimates for avoided cardiovascular disease risk as a result of PFOA and PFOS exposure reduction via drinking water. The draft document *Analysis of Avoided Cardiovascular Disease Risk from Reduced PFOA and PFOS Exposure* presents a methodology that could be used to determine the avoided cases of cardiovascular disease events (e.g., heart

attack, stroke, death from coronary heart disease). EPA intends to use this methodology to quantify the cardiovascular risk-reduction benefits for the population served by public water systems PWSs expected to take action to comply with a PFAS drinking water regulation. EPA is seeking input from SAB on the proposed methodology for estimating the avoided cases of cardiovascular disease (CVD) that result from reductions of PFOA and PFOS in drinking water. Evaluation of CVD impacts involves three main steps:

- 1) Estimate the changes in serum PFOA and PFOS levels that result from changes in drinking water concentrations with pharmacokinetic (PK) model¹;
- 2) Estimate the changes in total cholesterol that result from changes in PFOA and PFOS serum concentrations using dose-response functions for PFOA/PFOS; and
- 3) Estimate the change in probability of hard CVD events that result from changes in total cholesterol to estimate CVD event incidence in baseline and policy scenarios.

Overall charge: EPA is seeking SAB comment on the extent to which the approach to estimating reductions in CVD risk associated with reductions in exposure to PFOA and PFOS in drinking water is scientifically supported and clearly described.

CHIU: The main overarching concern is the apparent discrepancy between this document's focus on CVD risk, and the ~~hazard conclusions from the MCLG documents~~² ~~apparent conclusions that CVD did not have sufficient evidence to form the basis of a RfD~~. Additionally, this document raises the question as to what methodologies will be used for estimating health benefits for other endpoints (i.e., those with candidate RfD values in the MCLG document). For instance, it seems that decreased birth weight (as a continuous endpoints or as an increase in the percentage of low birth weight infants) would be a good candidate for estimates of risk reduction. Overall, more discussion is needed as to the rationale for selecting particular endpoints for risk reduction analysis (e.g., strength of hazard conclusion with respect to PFOA or PFOS, availability of dose-response data from which to derive a dose-response function or risk-specific dose estimates, strength of data connecting changes in biomarker to changes in morbidity or mortality, and availability of data for monetizing benefits).

Moreover, the NASEM review of the IRIS handbook recently endorsed the IRIS program's development of probabilistic risk-specific doses ~~from experimental animal data~~ to replace traditional deterministic reference values – and one of the main motivations that the NASEM Science and Decisions Report (2009) noted for using risk-specific doses was so as to inform benefit-cost analysis for non-cancer endpoints. Therefore, consideration should be given to use of experimental animal data to derive risk-specific doses that could be used for risk-reduction analysis. ~~Therefore, the question remains as to what methods will be applied for estimating health risk reduction if risk-specific doses are developed for PFAS.~~

Charge Questions

¹ EPA describes the human PK model within [OST's MCLG document] and requests SAB comments on the PK model to be provided in response to that document.

1. **Section 4.2 presents EPA’s meta-analysis for the total cholesterol dose-response function.**
 - A. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.
 - B. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.
2. **Section 5.1 presents EPA’s life table approach methodology.**
 - A. Please comment on the extent to which this analysis is scientifically supported and clearly described. To the extent improvements are suggested, please provide specific changes that are implementable in a U.S. national-level benefits analysis with readily available data.
3. **Section 5.2 presents EPA’s application of the atherosclerotic cardiovascular disease (ASCVD) risk model used to estimate the probability of hard CVD events corresponding to total cholesterol changes.**
 - A. Please comment on the scientific validity of the ASCVD model application for estimating the probability of first time CVD events in various sub-populations and the extent to which it is clearly described.
 - B. Please comment on whether EPA’s approach and assumption, of a uniform first CVD event hazard distribution over the 10-year period, is sufficiently robust given current data sources and literature. If additional distributional sources of information are suggested, please provide specific citations/sources for EPA’s consideration.
 - C. Please comment on the scientific validity of using the ASCVD risk model for estimating reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA and PFOS in drinking water.

CHIU: As a general principle, I endorse the use of chemical to biomarker followed by biomarker to risk approach to estimating health effect risk reduction. However, I am not an expert in this particular model.

4. **Section 7 and Appendix A describe the limitations and uncertainties of the CVD risk reduction analysis.**

- A. Has EPA clearly described the individual contributions of the sources of uncertainty?

CHIU: My main concern is that heterogeneity appears to be large and its sources are unclear, so should be considered a contribution to uncertainty. It is not clear if/how this is addressed. A secondary concern is the role of HDL—how much would including it affect the results, since increased HDL is associated with lower “hard outcome” risks—is the impact minimal or large?

PRELIMINARY COMMENTS

Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Study Identification and Inclusion

2. EPA used systematic review methods consistent with the current ORD systematic review practice to ensure transparency and completeness of literature identification, sorting, and study quality evaluation. Is the process clearly described? Please identify additional peer-reviewed studies that the panel is aware of that could inform toxicity value derivation.

CHIU: Defer to assigned reviewers.

Noncancer Hazard Identification

4. Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

CHIU: The hazard identification process is not fully transparent, either in the evaluation of different evidence “streams” (human, animal, mechanistic), or in their integration. For instance, although study quality/risk of bias is discussed, the “synthesis” within an evidence stream does not appear structured. Examples of how to structure “synthesis” include the Hill criteria (see IRIS Handbook and NASEM review of the IRIS Handbook), GRADE, NTP/OHAT, and the Navigation Guide. Similarly, there is no framework for integration of human, animal, and mechanistic data, which is also addressed in several of these examples. These frameworks include templates for tabular summaries of evidence both “within” and “across” evidence streams that could be adapted to provide more transparency.

A second issue is that the document is not clear as to the demarcation between the process of hazard identification and the process of dose-response assessment. For instance, the evidence integration sections discuss POD derivations as part of evidence synthesis. Separating the steps of hazard identification and identifying PODs would provide greater transparency. In particular, a conclusion as to hazard should not depend on whether the data can provide PODs – indeed, the dependency should be the other way around, where only health effects with a certain level of confidence in “hazard” should be considered for dose-response. This separation of hazard and dose-response is important because studies that provide strong evidence for hazard do not necessarily have to be amenable to POD derivations (e.g., epidemiologic studies with semi-quantitative exposure metrics), and conversely, the availability of studies amenable to POD derivations is not sufficient to provide strong evidence of hazard.

One approach would be to follow existing frameworks whereby for each health effect, a “synthesis” conclusion is made for each evidence stream (animal, human, mechanistic), and then

an overall “hazard” conclusion is made by integrating across evidence streams. This would provide additional transparency as to the hazard conclusions, as well as clarify the reasons why a particular health endpoint is chosen for candidate RfD derivations. Selecting studies for POD derivation would be a subsequent step, and consider both whether studies are amenable to POD derivation (especially BMD analysis) as well as study confidence/quality indicators.

Of particular importance is to make more transparent the strength of evidence conclusion with respect to serum lipids and CVD, given the prominence of that endpoint in the CVD document separately reviewed. It appears that the data on total cholesterol in humans, although showing an effect in the adverse direction, have high heterogeneity. Indeed, the meta-analysis from the CVD document could well be included to support the hazard identification conclusions. However, there does not seem to be strong evidence on “hard” outcomes; and moreover, the experimental animal studies show effects in the opposite direction – decreases in serum lipids. Additional discussion as to this lack of concordance would be helpful (e.g., are there known “positive” controls in humans that increase serum lipids in rodents?).

5. Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.

6. Does the SAB panel agree with EPA’s rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.

A. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.

B. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

CHIU: The main concern here is consistency across endpoints. The two reasons cited – “1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease” – could arguable also apply to some of the other endpoints.

I defer to subject matter experts as to interpreting whether the endpoints and BMR levels for specific health effects reflect a “minimally adverse” level of effect.

Cancer

3. Cancer classification for PFOA/PFOS

A. PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a ‘likely carcinogen’ categorization which is a change from ‘suggestive’ in the 2016 HA. Does the panel agree with the ‘likely’ designation based on the new evidence? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

B. PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a ‘suggestive’ categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

CHIU: Similar comments as above regarding Hazard Identification for carcinogenicity apply, except that the EPA Cancer Guidelines provide a structured approach (though not as formal as other more recent frameworks) that can enhance transparency. Included in this would be a discussion of epidemiologic data structured along the Hill criteria, an assessment of evidence from animal studies, and assessment of MOA, and a “weight of evidence narrative” that justifies why the evidence fits into one of the “descriptors” defined by the Cancer Guidelines.

4. Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends.

CHIU: One concern about these epidemiologic studies is whether other PFAS may be contributing to the effect because they are correlated with PFOA, but not measured. If this were the case, then the actual potencies of PFOA would be lower than implied by the studies. This possibility needs to be addressed more explicitly, particularly given the large apparent difference in potency between animal and human studies.

Toxicokinetic Models

3. Human model –

A. For endpoints observed in adults, EPA used a steady-state approach to calculate the HED, which assumes a relatively constant exposure and clearance during adulthood. Please comment on this method of HED calculation. Are there alternative approaches that EPA should consider? If so, please describe the rationale for recommending this approach(es).

CHIU: I agree that for endpoints observed in (non-pregnant) adults where the AUC is the appropriate dose metric, the steady-state approach is adequate for calculating the HED. However, given that a model is available, comparison between lifetime exposure using the model and steady state could be useful.

One broader issue is the use of the model when deriving drinking water MCLG values. In particular, a constant drinking water concentration will not equal a constant dose rate in mg/kg-d due to age-dependent changes in drinking water consumption, so if the goal is to “not exceed” an “internal dose” POD based on AUC or Cmax, then it seems that the model could be used (with exposure factor distributions) to derive equivalent drinking water concentration that would correspond to the “internal dose” POD. In the Goeden et al. (2019) study on PFOA, the “internal dose” POD was further adjusted for inter-species and intra-species uncertainty/variability so that the “RfD” was expressed on a dose metric equivalent. This approach could also be considered by EPA.

B. Two key parameters are the half-life and volume of distribution, which were used to calculate clearance. Half-life and volume of distribution were assumed to be constant across sex and age groups because of a lack of strong quantitative data to parametrize changes across sex and age. Please comment on the strengths and weakness of the use of this assumption and the choice of these parameters by the EPA. Please describe the rationale for alternative recommended approaches. For endpoints observed in human neonates or children, EPA used a one-compartment TK model to simulate dosimetry during pregnancy and a two-compartment TK model (one-compartment models for the mother and the child) to simulate dosimetry during lactation, to calculate the HED for each POD. Please comment on the strengths and weaknesses of this choice of model structure for the task of predicting dosimetry in the human fetus and child compared to dosimetry in mice and rats in the similar lifestages. Please provide the rationale for any alternative recommended approaches.

CHIU: I would suggest that EPA further evaluate the Goeden et al. (2019) model, which was evaluated with respect to infant serum data (higher reported R2 than Verner et al. (2016) model when compared to Fromme et al. data), and as well develops a parameterized age-dependent volume of distribution. Additionally, it seems that it would not be challenging to include a “growth dilution” factor into the Goeden et al. (2019), which was the main critique of this model in the PFOA document. Of course, another consideration may be that the Verner et al. (2016) model includes both PFOA and PFOS, whereas Goeden et al. (2019) is just for PFOS. Nonetheless, a direct comparison of performance and HED calculations between Goeden and Verner may be useful as a sensitivity analysis.

C. The key chemical-specific parameters that describe the transfer of the chemical from the mother to the child during gestation and lactation are the maternal to fetal serum ratio and the ratio of maternal serum to milk PFOA/S concentration. These ratios were assumed to be constant during gestation and lactation, respectively. Another important parameter is the rate of milk ingestion, which is chemical-independent and varies throughout lactation. Please comment on the strengths and weaknesses of the choice of parameters for fetal to maternal partitioning and partitioning into breastmilk, as well as the choice for lactation rate. Please also comment on the choice to assume that fetal to maternal partitioning and partitioning to breastmilk did not vary in time. Please describe

whether there are other methods you would recommend to account for these changes over time and across development.

CHIU: It would be useful to characterize the uncertainty that results from these assumptions, for instance through sensitivity analyses or Monte Carlo simulation with a range or distribution of values. For instance, the Goeden et al. (2019) transgenerational TK model includes at least both central and upper bound estimates for different parameters.

4. Animal Model –

G. After a review of the available toxicokinetic models for PFOA/S predictions in laboratory animals, EPA selected the Wambaugh et al. (2013) model because it was parametrized using all species of interest, demonstrated good agreement with training and test datasets, and used a single, biologically motivated, model structure across all species. Does the panel agree with selecting this model? If not, please describe the rationale for alternative recommended approaches for the calculation of the internal dose metrics in adult animals.

CHIU: I agree that the Wambaugh et al. (2013) model is adequate for deriving HEDs from experimental animal studies.

H. The animal model parameters were obtained through a Bayesian inference parameterization which produced wide credible intervals for some parameter values, but relatively tight credible intervals for the predicted serum concentration. Does the panel agree with using the median values of the estimated animal parameter distributions for prediction of serum concentration and internal dose metrics?

CHIU: I agree that median values can be used for deriving the HED. However, the uncertainty in both the human and animal models predictions should be characterized, particularly on their impact on the HED calculation. This is particularly important if a probabilistic approach were to be used to calculate risk-specific doses instead of traditional deterministic RfDs.

I. Based on visual inspection of model predictions to the calibration datasets, EPA utilized sex-independent parameters for PFOS. The male-specific parameters were used for all rat-specific PFOS predictions including predictions in pregnant and nursing dams and the female-specific parameters were used for all mouse-specific PFOS predictions because the parameter values obtained from fitting the female-specific rat data and male-specific mouse data were not consistent with the overall TK parameters for PFOS and produced poor fits to the training and test datasets. Does the panel agree with this approach and justification for this assumption for PFOS? If not, please describe other approaches that could be considered?

CHIU: Defer to other reviewers.

J. EPA assumed a one compartment model for the developing infant based on the lack of infant-specific toxicokinetic data from rats and mice. This model utilizes averages of half-life and volume of distribution from the literature coupled with physiologically relevant lactational parameters for pup nursing. Does the panel agree with the decision to use this model structure for infant animals? If not, please provide data on infant-specific changes during the animal lactational-period that could be used to account for toxicokinetic differences between the adult and infant rats and mice.

CHIU: I agree that the one compartment model for the developing infant is adequate for deriving HEDs from experimental animal studies.

K. Several parameters dictate the transfer of chemical from the mother to her pup. Does the panel agree with the selection of these parameters for the animal model? If not, please provide your justification and alternative parameters.

CHIU: Sensitivity analyses or uncertainty analyses would be helpful to better characterize the impact of uncertainty of these parameters.

L. For neonatal animals, EPA assumed no sex differences in clearance in neonatal animals based on the lack of identification of sex-dependent differences in PFOA/S toxicokinetics from the available data. Does the panel agree with this assumption? If not, please provide your justification and available data on sex differences in neonatal rats.

CHIU: I agree that data are inadequate to parameterize sex differences in neonatal animals.

Epidemiological Study RfD Derivation

5. EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only 'medium' and 'high' quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?

CHIU: For birth weight, it is not clear why a meta-analysis approach was not used to derive the POD. For instance, the CVD document reviewed separately contains a meta-analysis for total cholesterol. It would seem straight forward to apply the same methodology to the beta-coefficients ("re-expressed" as per ng/mL) for decreased birth weight. Then, the meta-beta coefficient could be used to deriving PODs. Additionally, a 5% extra risk in the incidence of low birth weight seems like quite a large BMR (from a baseline rate of 8.27% to 12.86%!), with a shift in the mean of almost 100g from 3,261 g to 3169 g! This does not seem to be adequately health protective. Moreover, the BMD values (in ng/mL) are often outside of the range of serum concentrations in the study, meaning the method is essentially extrapolating "upwards" to *higher* exposures (this would be less of a problem for a smaller BMR). Nonetheless, it seems that an

economic benefit-cost analysis could be performed either on the continuous endpoints of birth weight or the dichotomous endpoint of increased incidence of low-birth weight babies.

One concern about these epidemiologic studies is whether other PFAS may be contributing to the effect because they are correlated with PFOA or PFOS, but not measured. If this were the case, then the actual potencies of PFOA or PFOS would be lower than implied by the studies. This possibility needs to be addressed more explicitly, particularly given the large apparent difference in potency between animal and human studies.

6. Studies of developmental immune health outcomes (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]) after PFOA/S exposure identified associations with very low doses of either PFOA or PFOS with developmental immune effects. The RfD for this outcome was selected as the critical effect because it was the lowest among the candidate RfDs for PFOA or PFOS and can result in severe illness. Does the panel agree with the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS?

- A. If so, please explain your justification.
- B. If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of chronic RfDs.
- C. Are any additional analyses or rationales needed to increase the confidence in the chronic RfDs for PFOA and PFOS?

CHIU: Regarding selection of studies for POD, it is not clear why PODs based on experimental animal studies were not also used to derive candidate RfDs. It would be more transparent to derive candidate values from all eligible studies (adequate confidence, etc.) first, and then subsequently select health effect-specific and overall RfDs. Notably, it is not clear that health effect-specific RfDs were chosen, even among the existing candidate values. Such values are clearly needed in order to implement the draft mixture framework being reviewed concurrently.

Additional transparency is also needed to justify selecting among different candidate RfDs to select the health effect-specific RfD. The strengths and limitations for each study/POD in terms of the strength of evidence for that particular endpoint (including risk of bias), animal vs. human studies, dose-response uncertainties, etc. should be described to justify the selection of a health effect-specific RfD. Thereafter, selecting the overall RfD across health effects should also discuss the strengths and limitations, including strength of evidence for hazard and the uncertainties. Just being the lowest value is not enough justification.

One particular endpoint where there appears to be high concordance of evidence between animal and human studies is decreased birth weight. However, the apparent lack of concordance in dose-response may be due in part to the different definitions of endpoint and benchmark response. In particular, the animal studies use a SD-based benchmark response for the continuous endpoint of birth weight. However, the human studies use a “hybrid approach” that calculates the percent of additional “low birth weight” infants. However, the animal and human studies could be directly compared based on % change in the continuous outcome per unit serum concentration. This

would reveal the extent to which there is (or is not) concentration-response concordance between animal and human studies.

7. The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.

CHIU: The main concern is that the different candidate PODs should reflect a similar degree of “severity.” As mentioned above, a 5% increase in incidence of low birth weight appears to be rather high level of severity; on the other hand, a 5% decrease in antibody response may not have clinical significance (akin to the ALT discussion above).

8. EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for PFOA and PFOS.

A. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

B. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

CHIU: The recent NASEM review of the IRIS Handbook recommended application of the WHO/IPCS probabilistic framework (including UF distributions, rather than fixed values) to calculate risk-specific doses as a replacement for traditional RfDs, in line with the recommendations of the NASEM (2009) Science and Decisions report. A recent publication demonstrated broad application of this approach across many chemicals and endpoints, and included EPA authors from EPA’s National Center for Environmental Economics. This probabilistic framework not only includes default UF distributions based on reviews of the literature, but also enables derivation of dose-response functions that can be used for benefit-cost

analysis. EPA should consider whether applying this approach here would be useful for MCLG derivation and the regulatory impact assessments that will be needed to set MCLs.

Relative Source Contribution

2. EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources. Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at: <https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS.

A. Are you aware of additional relevant exposure data that EPA should consider in developing the RSCs for PFOA and PFOS? If so, please provide citations.

B. Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS is adequately supported and clearly described.

CHIU: Defer to assigned reviewers.

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Introduction

Per- and polyfluoroalkyl substances (PFAS) present many unique challenges from a risk management perspective due to the large number (1000's) and structural diversity of members in this chemical class, limitations in available human health and exposure information, and the spatial and temporal variability of their presence in drinking water and other environmental media. To inform various decision contexts in addressing PFAS contamination, EPA has developed a draft *Framework for Estimating Noncancer Health Risks Associated with Mixtures of PFAS* to illustrate the practical application of EPA chemical mixtures approaches and methods^{1,2} for two or more PFAS co-occurring in environmental media. Specifically, this document describes an approach for providing a tiered, flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS under an assumption of dose additivity. While this framework is being developed to inform the National Primary Drinking Water Regulation for PFAS, it is not intended to be media-specific in practical applications.

Overall charge: EPA is seeking SAB comment on whether the framework and illustrative examples provided in the document are scientifically supported, clearly described, and informative for assessing potential health risk(s) associated with exposure to mixtures of PFAS.

CHIU: Overall, the flowchart describing the different tiers is not always adequately justified as to why one approach or another would be selected. For instance, if component dose-response data are amenable to BMD modeling, why wouldn't this also support de novo derivation of toxicity values? Additionally, it is not clear that the "Tier 1" TOSHI approach is actually a "screening" calculation. As described briefly below, TOSHI will give the same equivalent answers as RPFs and Mixture BMD approaches under certain conditions, and moreover, it is not clear that TOSHI is always "conservative" relative to the RPF and Mixture BMD approaches. In a Tiered approach, the lower Tiers are generally more "conservative" so as to emphasize sensitivity (lower false negatives) and only expend additional effort at "higher" tiers to provide more specificity (identifying false positives). However, it not clear this is the case for TOSHI vs. RPF and Mixture BMD.

Charge questions

5. The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, an assumption of dose addition for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA's supplementary guidance (EPA, 2000) states: "The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which "similarity" can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.

C. Please comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.

CHIU: I agree and endorse this approach for component-based mixture evaluation of PFAS under an assumption of dose additivity. Specifically, I agree that the most appropriate assumption is similarity at the level of toxicity endpoint/health effect rather than MOA

D. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).

CHIU: Not applicable.

6. Section 4.3 (Hazard Index; HI) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and hypothetical exposure information. The example calculations presented are

primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as “GenX chemicals”).

- A. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.
- B. Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment.

CHIU: I agree and endorse the HI approach, and the HI methodologies proposed, as reasonable screening-level approaches for indicating potential risk associated with mixture of PFAS. However, the TOSHI approach necessitates endpoint/health effect-specific reference values, and not just overall reference values. Therefore, the document should be clearer that that endpoints/health effect-specific reference values be developed for individual PFAS. Additionally, the NASEM review of the IRIS handbook recently endorsed the IRIS program’s development of probabilistic risk-specific doses to replace traditional deterministic reference values. EPA should consider the extent to which using the corresponding “probabilistic RfD” would change the proposed HI approach, or whether such probabilistic reference values can be used as direct replacements for the traditional RfD in HI calculations.

7. Section 4.4 (Relative Potency Factor; RPF) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and HFPO dimer acid and GenX chemicals.

- A. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.
- B. Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.

CHIU: I agree and endorse the RPF approach as a reasonable methodology for estimating risk associated with mixtures from PFAS. However, the framework should also summarize when the TOSHI and RPF approach will give essentially the same answer (e.g., when the ratio of the POD values used to calculate the RPFs is equal to the ratio of the endpoint-specific RfD values used to calculate the HI), and the extent to which consistency is appropriate or inappropriate (e.g., if one is supposed to be more “conservative” for screening, rather than more “predictive.”).

8. Section 4.5 (Mixture BMD) of the framework document demonstrates the application of a component-based mixture approach using established EPA dose-response modeling (i.e., benchmark dose; BMD) of hypothetical PFAS dose-response data, and hypothetical exposure information.

A. Please provide specific feedback on whether the Mixture BMD approach is a reasonable methodology for estimating what is in essence a mixture-based point-of-departure. If not, please provide an alternative.

B. Please provide specific feedback on whether the proposed Mixture BMD methodology in the framework is scientifically supported for PFAS mixture risk assessment.

CHIU: I agree and endorse the Mixture BMD approach as a reasonable methodology for estimating a mixture-based POD. As with the RPF approach, the framework should also summarize when the TOSHI and Mixture BMD approach will give essentially the same answer (e.g., when the ratio of the BMD values used to calculate the mixture MBD is equal to the ratio of the endpoint-specific RfD values used to calculate the HI), and the extent to which consistency is appropriate or inappropriate (e.g., if one is supposed to be more “conservative” for screening, rather than more “predictive.”).

EPA’s draft Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water

Introduction

To fulfill the Health Risk Reduction and Cost Analysis (HRRCA) requirements under Safe Drinking Water Act, EPA is developing a benefit-cost analysis and other related rule analyses to inform consideration of regulatory alternatives. According to the *Proposed Approaches to the Derivation of Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water, currently under concurrent SAB review*, current epidemiologic literature supports positive associations between PFOA and PFOS exposure and total cholesterol. EPA is developing national-level benefits estimates for avoided cardiovascular disease risk as a result of PFOA and PFOS exposure reduction via drinking water. The draft document *Analysis of Avoided Cardiovascular Disease Risk from Reduced PFOA and PFOS Exposure* presents a methodology that could be used to determine the avoided cases of cardiovascular disease events (e.g., heart attack, stroke, death from coronary heart disease). EPA intends to use this methodology to quantify the cardiovascular risk-reduction benefits for the population served by public water systems PWSs expected to take action to comply with a PFAS drinking water regulation. EPA is seeking input from SAB on the proposed methodology for estimating the avoided cases of cardiovascular disease (CVD) that result from reductions of PFOA and PFOS in drinking water. Evaluation of CVD impacts involves three main steps:

1) Estimate the changes in serum PFOA and PFOS levels that result from changes in drinking water concentrations with pharmacokinetic (PK) model²;

² EPA describes the human PK model within [OST’s MCLG document] and requests SAB comments on the PK model to be provided in response to that document.

- 2) Estimate the changes in total cholesterol that result from changes in PFOA and PFOS serum concentrations using dose-response functions for PFOA/PFOS; and
- 3) Estimate the change in probability of hard CVD events that result from changes in total cholesterol to estimate CVD event incidence in baseline and policy scenarios.

Overall charge: EPA is seeking SAB comment on the extent to which the approach to estimating reductions in CVD risk associated with reductions in exposure to PFOA and PFOS in drinking water is scientifically supported and clearly described.

CHIU: The main overarching concern is the apparent discrepancy between this document's focus on CVD risk, and the MCLG documents' apparent conclusions that CVD did not have sufficient evidence to form the basis of a RfD. Additionally, this document raises the question as to what methodologies will be used for estimating health benefits for other endpoints (i.e., those with candidate RfD values in the MCLG document). For instance, it seems that decreased birth weight (as a continuous endpoints or as an increase in the percentage of low birth weight infants) would be a good candidate for estimates of risk reduction.

Moreover, the NASEM review of the IRIS handbook recently endorsed the IRIS program's development of probabilistic risk-specific doses to replace traditional deterministic reference values – and one of the main motivations that the NASEM Science and Decisions Report (2009) noted for using risk-specific doses was so as to inform benefit-cost analysis for non-cancer endpoints. Therefore, the question remains as to what methods will be applied for estimating health risk reduction if risk-specific doses are developed for PFAS.

Charge Questions

1. Section 4.2 presents EPA's meta-analysis for the total cholesterol dose-response function.

A. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

B. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.

2. Section 5.1 presents EPA's life table approach methodology.

A. Please comment on the extent to which this analysis is scientifically supported and clearly described. To the extent improvements are suggested, please provide specific changes that are implementable in a U.S. national-level benefits analysis with readily available data.

3. Section 5.2 presents EPA’s application of the atherosclerotic cardiovascular disease (ASCVD) risk model used to estimate the probability of hard CVD events corresponding to total cholesterol changes.

- A. Please comment on the scientific validity of the ASCVD model application for estimating the probability of first time CVD events in various sub-populations and the extent to which it is clearly described.
- B. Please comment on whether EPA’s approach and assumption, of a uniform first CVD event hazard distribution over the 10-year period, is sufficiently robust given current data sources and literature. If additional distributional sources of information are suggested, please provide specific citations/sources for EPA’s consideration.
- C. Please comment on the scientific validity of using the ASCVD risk model for estimating reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA and PFOS in drinking water.

CHIU: As a general principle, I endorse the use of chemical to biomarker followed by biomarker to risk approach to estimating health effect risk reduction. However, I am not an expert in this particular model.

4. Section 7 and Appendix A describe the limitations and uncertainties of the CVD risk reduction analysis.

- A. Has EPA clearly described the individual contributions of the sources of uncertainty?

CHIU: My main concern is that heterogeneity appears to be large and its sources are unclear, so should be considered a contribution to uncertainty. It is not clear if/how this is addressed. A secondary concern is the role of HDL – how much would including it affect the results? Since increased HDL is associated with lower “hard outcome” risks – is the impact minimal or large?

Kevin Boyle

NO RESPONSES RECEIVED

REVISED COMMENTS

2. Noncancer Hazard Identification

- b. Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease. **Burman, Cory-Slechta, Kamendulis and Slitt**
- i. Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.
 - ***EPA's rationale for not considering the ALT endpoint for derivation of a POD needs to be clarified. The principal reasons cited for the exclusion, i.e., the magnitude of the effect was not large compared to control levels; and concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease, do not appear to be unique to this endpoint. In addition to this, the EPA (2002) guideline for RfD development states that a Reference Dose should be based on an adverse effect. EPA does state in that increased ALT is indicative of liver damage. Therefore, a more detailed explanation should be provided as to why this endpoint was not selected for RfD development.***
 - ii. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.
 - iii. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Charge Question #2

Section 4.3 (**Hazard Index; HI**) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and hypothetical exposure information. The example calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as “GenX chemicals”). **Burman, Fisher, Kamendulis and Pullen-Fedinick**

- A. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.
- I agree that the HI approach, and the HI methodologies proposed, as reasonable approaches for indicating risk associated with mixture of PFAS. *
(*This added statement to clarify my general agreement is the only revision to the preliminary response. Section B remains unchanged.)*
- B. Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment.
- There are some limitations and potential complications in terms of the intended users such as states and public water systems applying this framework in context of implementing the Safe Drinking Water Act. Additional clarity and guidance from EPA will be helpful in mitigating any inadvertent uncertainties caused by the issuance of this framework in a final form. More details on the intent, purpose, and potential applications of this framework by stakeholders such as states, public water systems and others will be helpful. For instance, some states that have promulgated either regulatory or guidance values for PFAS, are using a mixtures-based approach for the specific combination of PFAS compounds prevalent in the state. Methods analogous to those classified by EPA as ‘Screening Level’ or ‘Tier 1’ in the framework are potentially being used by states in a decision-making capacity. Issuance of this framework without recognition of that fact may create confusion for public water supplies and risk communication challenges for the public. Additionally, should EPA promulgate NPDWRs for PFOS and PFOA as proposed, it should be clarified how those will factor into a mixtures approach for making decisions at public water systems*
 - The TOSHI approach presents additional robustness compared to the Screening Level HI given the identification of human health/toxicity values that are effect/endpoint specific. However, the framework appears to classify both the Screening Level HI and the TOSHI method as being equivalent Tier 1 methods that should lead to a more robust Tier 2 approach (i.e., RPF). The TOSHI approach may merit consideration to be classified as a higher tier method compared to the Screening – Level HI method for decision making purposes. This may also reflect current and future practices amongst states and others. Currently both Screening-Level HI and TOSHI are classified as Tier 1 methods that should be followed up by a Tier 2 method, such as RPF.*
 - Even for the Screening Level HI calculations, the number of PFAS with available toxicological assessments remains limited and many users will not have the ability to derive them using the methods outlined in the framework. Additional directions will likely be necessary for most users.*

- *The equivalency of HI calculations using the different categories of toxicity assessment information available as presented in Table 4-2 could be clarified more. It would also be beneficial to outline the validity of, and procedures for, calculating the HI should the mixture present include PFAS compounds with varying levels of information available, i.e., fall in different rows of Table 4-2.*

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Charge Question #3

Section 4.4 (**Relative Potency Factor; RPF**) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and GenX chemicals. **Burman, DeWitt, Fisher, and Slitt**

- C. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.
- *I agree that in appropriate situations the RPF approach is a reasonable methodology for indicating risk associated with mixture of PFAS.*
(*This added statement to clarify my general agreement is the only revision to the preliminary response. Section B remains unchanged.)*
- D. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.
- *In general, the RPF approach is a more data intensive approach as compared to the HI methods, which are likely to see greater applications for PFAS. The number of PFAS compounds with sufficient hazard and dose-response information necessary for the RPF approach is likely to remain limited. This coupled with the level of technical expertise needed may preclude the RPF method from becoming a practical approach for evaluating mixtures of PFAS. The RPF method has only been used for a small number of chemicals so far and based on a common mode of action. For PFAS, mode of action data is extremely limited. EPA is therefore proposing to use toxicological similarity as a surrogate. While EPA states that there is support for this flexibility from both the EPA guidance as well as NAS in evaluating chemicals that cause common adverse health outcomes through diverse biological pathways, there appears to be lesser certainty with this approach to mixtures assessment as compared to the HI approaches.*
 - *Additional clarity could be provided by EPA in general to the Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances to enhance its utility. Some of those general considerations take on added significance given the relative complexity and*

sophistication of the RPF method. There are some limitations and potential complications in terms of the intended users such as states and public water systems applying these overall mixtures framework in context of implementing the Safe Drinking Water Act. Additional clarity and guidance from EPA will be helpful in mitigating any inadvertent uncertainties caused by the issuance of this framework in a final form. More details on the intent, purpose, and potential applications of this framework by stakeholders such as states, public water systems and others will be helpful. For instance, some states that have promulgated either regulatory or guidance values for PFAS, are using a mixtures-based approach for the specific combination of PFAS compounds prevalent in the state. Methods analogous to those classified by EPA as ‘Screening Level’ or ‘Tier 1’ in the framework are potentially being used by states in a decision-making capacity. Issuance of this framework without recognition of that fact may create confusion for public water supplies and risk communication challenges for the public. This is likely to be compounded should the RPF approach for evaluating mixtures also be presented as an option, and especially if it is deemed needed for decision making as a Tier 2 evaluation based on a prior Tier 1 (i.e., HI) evaluation. Additionally, should EPA promulgate NPDWRs for PFOS and PFOA as proposed, it should be clarified how those will factor into a mixtures approach for making decisions at public water systems.

MCLG CQ#6 (RSC) revised response –

EPA’s recommendation of an RSC of 20% for both PFOA and PFOS in context of the proposed RfDs is valid as per the 2000 EPA Guidance. However, it is not clear from the RSC Section in the MCLG draft documents if EPA derived the recommended 20% RSC in accordance with the 2000 Guidance. Clarity is needed to demonstrate that the 2000 Guidance was followed.

EPA’s recommendation of an RSC of 20% for both PFOA and PFOS could also be clarified, and transparency added by providing additional details and discussions on a few key issues outlined below.

- 1) There is a significant national database of blood serum concentrations of several PFAS based on the work of the CDC NHANES. This is discussed in the MCLG draft documents, but EPA could better explain the relevancy of this data, and if and how this data was considered in the RSC determination.
- 2) The recommended RSC of 20% is for the general population. A better explanation of how the impacts on highly or disproportionately exposed populations are relevant to or have been considered in arriving at the 20% RSC recommendation is important to include in the recommendations for both PFOS and PFOA. EPA references this in the draft MCLG document but the application is not clear to RSC determination or to broader RfD determination, especially in the context of being consistent with the EPA 2000 Guidance.
- 3) The EPA 2000 guidance also identifies the directive to the Agency for explicitly and consistently considering environmental health risks to infants and children in all risk assessments, risk characterizations, and public health standards. EPA should clearly

detail if this is relevant to the development of the 20% RSC for PFOS and PFOA, or how it has been accounted for otherwise in the RfD development.

- 4) PFAS are now a well-studied group of chemicals, with multiple well documented sources/uses. In that context, EPA should better explain how the decision tree approach as per the 2000 guidance led to the 20% RSC recommendation.

PRELIMINARY COMMENTS

1. EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Charge Question # 2A, 2 B

In general, the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS and is likely to see increasing applications. The proposed HI methods in the draft framework appear scientifically supported and accepted by risk assessment practitioners. However, additional clarity could be provided by EPA in general to the *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances* to enhance its utility.

- 1) There are some limitations and potential complications in terms of the intended users such as states and public water systems applying this framework in context of implementing the Safe Drinking Water Act. Additional clarity and guidance from EPA will be helpful in mitigating any inadvertent uncertainties caused by the issuance of this framework in a final form. More details on the intent, purpose, and potential applications of this framework by stakeholders such as states, public water systems and others will be helpful. For instance, some states that have promulgated either regulatory or guidance values for PFAS, are using a mixtures-based approach for the specific combination of PFAS compounds prevalent in the state. Methods analogous to those classified by EPA as 'Screening Level' or 'Tier 1' in the framework are potentially being used by states in a decision-making capacity. Issuance of this framework without recognition of that fact may create confusion for public water supplies and risk communication challenges for the public. Additionally, should EPA promulgate NPDWRs for PFOS and PFOA as proposed, it should be clarified how those will factor into a mixtures approach for making decisions at public water systems
- 2) The TOSHI approach presents additional robustness compared to the Screening Level HI given the identification of human health/toxicity values that are effect/endpoint specific. However, the framework appears to classify both the Screening Level HI and the TOSHI method as being equivalent Tier 1 methods that should lead to a more robust Tier 2 approach (i.e., RPF). The TOSHI approach may merit consideration to be classified as a higher tier method compared to the Screening – Level HI method for decision making purposes. This may also reflect current and future practices

amongst states and others. Currently both Screening-Level HI and TOSHI are classified as Tier 1 methods that should be followed up by a Tier 2 method, such as RPF.

- 3) Even for the Screening Level HI calculations, the number of PFAS with available toxicological assessments remains limited and many users will not have the ability to derive them using the methods outlined in the framework. Additional directions will likely be necessary for most users.
- 4) The equivalency of HI calculations using the different categories of toxicity assessment information available as presented in Table 4-2 could be clarified more. It would also be beneficial to outline the validity of, and procedures for, calculating the HI should the mixture present include PFAS compounds with varying levels of information available, i.e., fall in different rows of Table 4-2.

Charge questions # 3A, 3B

In general, the RPF approach is a more data intensive approach as compared to the HI methods, which are likely to see greater applications for PFAS. The number of PFAS compounds with sufficient hazard and dose-response information necessary for the RPF approach is likely to remain limited. This coupled with the level of technical expertise needed may preclude the RPF method from becoming a reasonable practical approach for evaluating mixtures of PFAS. The RPF method has only been used for a small number of chemicals so far and based on a common mode of action. For PFAS, mode of action data is extremely limited. EPA is therefore proposing to use toxicological similarity as a surrogate. While EPA states that there is support for this flexibility from both the EPA guidance as well as NAS in evaluating chemicals that cause common adverse health outcomes through diverse biological pathways, there appears to be lesser certainty with this approach to mixtures assessment as compared to the HI approaches.

Additional clarity could be provided by EPA in general to the *Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances* to enhance its utility. Some of those general considerations take on added significance given the relative complexity and sophistication of the RPF method. There are some limitations and potential complications in terms of the intended users such as states and public water systems applying these overall mixtures framework in context of implementing the Safe Drinking Water Act. Additional clarity and guidance from EPA will be helpful in mitigating any inadvertent uncertainties caused by the issuance of this framework in a final form. More details on the intent, purpose, and potential applications of this framework by stakeholders such as states, public water systems and others will be helpful. For instance, some states that have promulgated either regulatory or guidance values for PFAS, are using a mixtures-based approach for the specific combination of PFAS compounds prevalent in the state. Methods analogous to those classified by EPA as ‘Screening Level’ or ‘Tier 1’ in the framework are potentially being used by states in a decision-making capacity. Issuance of this framework without recognition of that fact may create confusion for public water supplies and risk communication challenges for the

public. This is likely to be compounded should the RPF approach for evaluating mixtures also be presented as an option, and especially if it is deemed needed for decision making as a Tier 2 evaluation based on a prior Tier 1 (i.e., HI) evaluation. Additionally, should EPA promulgate NPDWRs for PFOS and PFOA as proposed, it should be clarified how those will factor into a mixtures approach for making decisions at public water systems.

1. Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Relative Source Contribution

Charge Question # 1

EPA's recommendation of an RSC of 20% for both PFOA and PFOS could be strengthened by providing additional details and discussions on a few key issues outlined below.

- 5) There is a significant national database of blood serum concentrations of several PFAS based on the work of the CDC NHANES. EPA could better explain how and to what extent this data was considered.
- 6) The recommended RSC of 20% is for the general population. A better explanation of how the impacts on highly or disproportionately exposed populations have been considered in arriving at the 20% RSC recommendation is important to include in the recommendations for both PFOS and PFOA. Such considerations are called for in the EPA 2000 guidance on deriving water quality criteria.
- 7) The EPA 2000 guidance also identifies the directive to the Agency for explicitly and consistently considering environmental health risks to infants and children in all risk assessments, risk characterizations, and public health standards. EPA should clearly detail how that has been done in the development of the 20% RSC for PFOS and PFOA.
- 8) PFAS are now a well-studied group of chemicals, with multiple well documented sources/uses. In that context, EPA should better explain how the decision tree approach as per the 2000 guidance led to the 20% RSC conclusion.

Noncancer Hazard Identification

Charge Question #3

In general EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects appears reasonable. The magnitude of the effects, clinical relevance of findings, and non-specify of the biomarkers associated with the ALT endpoint contrasted with the effects associated with the endpoints and studies that EPA is considering for dose-response modeling and POD derivation. For instance, the immune effects in human male and female children and infants and serum lipid effects in human males and females were larger in magnitude and more coherent with epidemiological evidence.

REVISED COMMENTS

EPA's draft Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water

Charge Questions

5. Section 4.2 presents EPA's meta-analysis for the total cholesterol dose-response function.

- C. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

Response: The meta-analysis selection criteria are well reasoned, and EPA identified 14 relevant studies for analysis. One interesting study below was not included probably due to its different study design of modeling prospective cholesterol level changes by PFAS level changes in the C8 Health Study, but it is really relevant to the topic of the meta-analysis. Even its design may not make it easy to be integrated into the meta-analysis, the interpretation of the meta-analysis findings may need to consider the context of this study.

Fitz-Simon N, et al. Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid. *Epidemiology* 2013;24:569-576.

<https://pubmed.ncbi.nlm.nih.gov/23685825/>

- D. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.

Response: Because the NHANES has been repeatedly analyzed for the association between PFAS and total cholesterol levels, using meta-analysis may not yield additional value compared with the approach Dong et al. used in the 2019 publication. If the results were from different study populations, meta-analysis results would be preferred.

2. Section 7 and Appendix A describe the limitations and uncertainties of the CVD risk reduction analysis.

- A. Has EPA clearly described the individual contributions of the sources of uncertainty?

Response: EPA has clearly described the individual contributions of the sources of uncertainty. The six studies used in meta-analysis may be subject to publication bias and the uncertainty was considered. Several studies are cross-sectional using NHANES, and no lag between exposure to PFAS and TC changes was considered as potential overestimate of the effect of PFAS reduction. Longitudinal study of PFAS and TC change over time needs to be considered. More research is developing in assessing cumulative effects of PFAS chemicals and that uncertainty was also discussed as the CVD analysis assumes independent effect but not potential interactions.

Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Study Identification and Inclusion

3. EPA used systematic review methods consistent with the current ORD systematic review practice to ensure transparency and completeness of literature identification, sorting, and study quality evaluation. Is the process clearly described? Please identify additional peer-reviewed studies that the panel is aware of that could inform toxicity value derivation.

Response: The process of systematic review is clearly described, including database search and “gray” literature identification. The use of SWIFT and DistillerSR to sort the literature and process for quality evaluation and confidence determination are reasonable steps. It is not clear if literature is added from the reference list of the primary literature, and if existing systematic review and meta-analysis are considered in the interpretation of the literature. The number of papers may be larger than the number of studies in epidemiology, and sometimes it is described in that way but not always. The number of cohorts could be mentioned if necessary to provide the context of study settings and different ages at follow-up visits. It is not clear how heterogeneity and weighting of the studies is handled to derive the BMD as in some cases BMD is given for each individual study.

The literature review was completed in September 2020, and newer studies are published later. As vaccine response is a key outcome used to derive BMD and POD, I checked the later publications to see if the findings are consistent in showing immunotoxicity after PFAS exposure. The newer papers and a review article support the association of PFAS with reduced vaccine response.

Shih YH, et al. Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substances: A birth cohort in the Faroe Islands. *Journal of Immunotoxicology* 2021;18(1):85-92 (Hepatitis A antibody)

<https://pubmed.ncbi.nlm.nih.gov/34143710/>

Timmermann CAG et al. Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7–12 years exposed to marine pollutants, a cross sectional study. *Environmental Research* 2022;203:111712 (Cross-sectional in Greenlandic children at ages 7-12 years)

<https://pubmed.ncbi.nlm.nih.gov/34343554/>

Von Holst H et al. Perfluoroalkyl substances exposure and immunity, allergic response, infection, and asthma in children: review of epidemiologic studies. *Heliyon* 2021;7:e08160 (review article)

<https://pubmed.ncbi.nlm.nih.gov/34712855/>

Epidemiological Study RfD Derivation

3. The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.

Response: The benchmark response (BMR) of 5% is supported by the developmental effect and potential severity of vaccine-preventable illnesses (i.e., tetanus and diphtheria). The clinical significant decrease in tetanus and diphtheria antibody concentrations is generally considered to have the antibody concentrations below 0.1 IU/mL. Considering large number of people (30-40%) having antibody concentrations close to 0.1 IU/mL, further 5% decrease in antibody concentrations could be problematic for disease protection. While mortality from tetanus in the U.S. from 2009-2017 was mainly in adults over the age of 55 years, it is a precaution to protect the most vulnerable neonates and young children from these vaccine-preventable diseases.

Relative Source Contribution

3. EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources.

Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at: <https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS.

- C. Are you aware of additional relevant exposure data that EPA should consider in developing the RSCs for PFOA and PFOS? If so, please provide citations.
- D. Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS is adequately supported and clearly described.

Response: The EPA 2000 guidance needs to be followed to derive the RSC as it seems the algorithm to derive RSC does not consider specific study population for the relation between drinking water and biomarker levels. The 20% RSC is the most conservative and health protective allowed by the EPA 2000 guidance. This RSC is adequately supported.

PRELIMINARY COMMENTS

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Not assigned

EPA's draft Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water

Charge Questions

6. Section 4.2 presents EPA's meta-analysis for the total cholesterol dose-response function.

- A. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

Response: The meta-analysis selection criteria are well reasoned, and EPA identified 14 relevant studies for analysis. One interesting study below was not included probably due to its different study design of modeling prospective cholesterol level changes by PFAS level changes in the C8 Health Study, but it is really relevant to the topic of the meta-analysis. Even its design may not make it easy to be integrated into the meta-analysis, the interpretation of the meta-analysis findings may need to consider the context of this study.

Fitz-Simon N, et al. Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid. *Epidemiology* 2013;24:569-576.

- B. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.

Response: Because the NHANES has been repeatedly analyzed for the association between PFAS and total cholesterol levels, using meta-analysis may not yield additional value compared with the approach Dong et al. used in the 2019 publication. If the results were from different study populations, meta-analysis results would be preferred.

4. Section 7 and Appendix A describe the limitations and uncertainties of the CVD risk reduction analysis.

- A. Has EPA clearly described the individual contributions of the sources of uncertainty?

Response: EPA has clearly described the individual contributions of the sources of uncertainty. The six studies used in meta-analysis may be subject to publication bias and the uncertainty was considered. Several studies are cross-sectional using NHANES, and no lag between exposure to PFAS and TC changes was considered as potential overestimate of the effect of PFAS reduction. Longitudinal study of PFAS and TC change over time needs to be considered. More research is developing in assessing cumulative effects of PFAS chemicals and that uncertainty was also discussed as the CVD analysis assumes independent effect but not potential interactions.

Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Study Identification and Inclusion

4. EPA used systematic review methods consistent with the current ORD systematic review practice to ensure transparency and completeness of literature identification, sorting, and study quality evaluation. Is the process clearly described? Please identify additional peer-reviewed studies that the panel is aware of that could inform toxicity value derivation.

Response: The process of systematic review is clearly described, including database search and “gray” literature identification. The use of SWIFT and DistillerSR to sort the literature and process for quality evaluation and confidence determination are reasonable steps. It is not clear if literature is added from the reference list of the primary literature, and if existing systematic

review and meta-analysis are considered in the interpretation of the literature. The number of papers may be larger than the number of studies in epidemiology, and sometimes it is described in that way but not always. The number of cohorts could be mentioned if necessary to provide the context of study settings and different ages at follow-up visits. It is not clear how heterogeneity and weighting of the studies is handled to derive the BMD as in some cases BMD is given for each individual study.

The literature review was completed in September 2020, and newer studies are published later. As vaccine response is a key outcome used to derive BMD and POD, I checked the later publications to see if the findings are consistent in showing immunotoxicity after PFAS exposure. The newer papers and a review article support the association of PFAS with reduced vaccine response.

Shih YH, et al. Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substances: A birth cohort in the Faroe Islands. *Journal of Immunotoxicology* 2021;18(1):85-92 (Hepatitis A antibody)

Timmermann CAG et al. Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7–12 years exposed to marine pollutants, a cross sectional study. *Environmental Research* 2022;203:111712 (Cross-sectional in Greenlandic children at ages 7-12 years)

Von Holst H et al. Perfluoroalkyl substances exposure and immunity, allergic response, infection, and asthma in children: review of epidemiologic studies. *Heliyon* 2021;7:e08160 (review article)

Epidemiological Study RfD Derivation

5. The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.

Response: The benchmark response (BMR) of 5% is supported by the developmental effect and potential severity of vaccine-preventable illnesses (i.e., tetanus and diphtheria). The clinical significant decrease in tetanus and diphtheria antibody concentrations is generally considered to have the antibody concentrations below 0.1 IU/mL. Considering large number of people (30-40%) having antibody concentrations close to 0.1 IU/mL, further 5% decrease in antibody concentrations could be problematic for disease protection. While mortality from tetanus in the U.S. from 2009-2017 was mainly in adults over the age of 55 years, it is a precaution to protect the most vulnerable neonates and young children from these vaccine-preventable diseases.

Relative Source Contribution

4. EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources. Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at: <https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS.
 - E. Are you aware of additional relevant exposure data that EPA should consider in developing the RSCs for PFOA and PFOS? If so, please provide citations.
 - F. Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS is adequately supported and clearly described.

Response: Although not in the U.S., the following publication from China reported RSC of 23% for PFOA and 12.7% for PFOS in drinking water samples across 28 cities. The range of the RSCs was quite large depending on the cities and the blood, not serum, PFAS concentrations were used, but this study involved both males and females, which is different from Hu et al. 2019 paper from the Nurses' Health Study in the U.S.

Zhang S, et al. Relationship between perfluorooctanoate and perfluorooctane sulfonate blood concentrations in the general population and routine drinking water exposure. *Environmental International* 2019;126:54-60

From the U.S. study by Hu et al. and the Chinese study by Zhang et al., it is reasonable to recommend an RSC of 20% for PFOA and PFOS from drinking water. The method to derive the RSC has been adequately supported and clearly described.

PRELIMINARY COMMENTS

9. The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, an assumption of dose addition for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA’s supplementary guidance (EPA, 2000) states: “The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration).” This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which “similarity” can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.

E. Please comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.

Evidence supporting a dose-addition approach to mixtures is strong and makes logical sense when one considers human physiology. In fact, an approach based on similarity at the level of toxicity endpoint makes most sense, indeed more sense than a common molecular mode of action. Human function is based on an integrated systems of systems and not on single molecular changes as the sole drivers of any health outcome. Rather than the common MOA as being the EPA position, common physiological outcome should be the defining position.

Consider a health outcome such as elevated blood pressure (not one for PFAS or PFOS but just a general example). It is known that there are many different physiological systems that contribute to regulation of blood pressure beyond the renin-angiotensin system (Joyner and Limberg, 2014).

The document might be assisted by specifically defining the difference between an MOA and a health endpoint. Again, in defining the consequences of a chemical, there may be multiple MOAs that can contribute to a health outcome and thus disparate MOAs should be considered if they can contribute to the health endpoint.

F. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).

It isn’t clear to this reviewer that any other approach is currently valid. While the document discusses the potential inclusion of NAMS, such approaches seem far from

ready for any prime time incorporation into human risk assessment. Obviously, this remains a topic of some contention, but significant limitations remain.

10. Section 4.5 (Mixture BMD) of the framework document demonstrates the application of a component-based mixture approach using established EPA dose-response modeling (i.e., benchmark dose; BMD) of hypothetical PFAS dose-response data, and hypothetical exposure information.

A. Please provide specific feedback on whether the Mixture BMD approach is a reasonable methodology for estimating what is in essence a mixture-based point-of-departure. If not, please provide an alternative.

The proposed method employs a dose-additive model-based calculation of a mixture BMD based on a defined benchmark response (e.g., ED10) for a PFAS mixture with a specific mixing-ratio of component chemicals, as dose additivity has been viewed as the most appropriate model for estimating combined effects of “toxicologically similar” compounds.

B. Please provide specific feedback on whether the proposed Mixture BMD methodology in the framework is scientifically supported for PFAS mixture risk assessment.

Yes, the mixture BMD methodology in the framework is supported for PFAS mixture risk assessment. Furthermore, both its criteria for application and its potential limitations are well described.

Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Noncancer Hazard Identification

7. Does the SAB panel agree with EPA’s rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.

A. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.

In the opinion of this reviewer, elimination of ALT as an endpoint does not seem appropriate. Elevations above normal indicate deviation from control, and the fact that it cannot necessarily be related to some disease outcome does not necessarily mean that it is not detrimental, but may only suggest that the appropriate screening has not been carried out. There may also be species differences in how it ultimately manifests. According to the ACG, “Multiple studies have demonstrated that the presence of an elevated ALT has

been associated with increased liver-related mortality.” (Kwo et al., 2017). In this report, the authors state the following:

Elevated aminotransferase levels and the effect on morbidity and mortality

There is an accumulating set of data demonstrating that AST and ALT elevations correlate with morbidity and mortality ([Table 3](#)). An initial report from Germany noted that those with AST>18 U/l had a 3X increased risk of all-cause mortality ⁽²⁷⁾. A Korean study found that, compared with men with AST or ALT<20 IU/l, the 30–39 IU/l group had an 8X (AST) or 9.5X (ALT) relative risk (RR) for liver-related death ⁽²⁸⁾. Similar results were demonstrated from a study comparing the standardized mortality ratios in subjects from Olmsted County where higher ALT levels correlated with higher mortality with the standardized mortality ratio being 0.95 for normal ALT (defined as ULN 45 IU/l for men, 29 IU/l for females), 1.32 for 1–2X ULN, and 1.78 for >2X ULN with a similar relationship for AST levels ⁽²⁹⁾.

ALT and AST levels and liver related mortality

Author /year	Proposed ALT/AST cutoff level	ALT/AST level for increased mortality	Comments
Arndt <i>et al.</i> (27)	AST 18	AST>18	3X increase in all cause mortality
Kim <i>et al.</i> (28)	ALT<20	ALT 30–39	RR of liver mortality 2.9 (2.4–3.5) and 9.5 (7.9–11.5) in men, 3.8 (1.9–7.7) and 6.6 (1.5–25.6) in women
Lee <i>et al.</i> (29)	ALT (ULN 45 IU/l for M, 29 for F)	ALT 45–90 M 29–58 for F	SMR risk 1.32 for 1–2X ULN, and 1.78 for >2X ULN
Ruhl and Everhart (30)	ALT 30 IU/l M, 19 IU/l for F	ALT>30 for M ALT >19 for F	Increased liver related mortality

ALT, alanine aminotransferase; AST, aspartate aminotransferase; F, female; RR, relative risk; M, male; ULN, upper limit of normal.

Studies have used the data from the NHANES databases to assess risk of morbidity and mortality in relationship to abnormal liver tests with one study demonstrating that elevated ALT (ULN defined as 30 U/l for men and 19 U/l for women) was associated with significant increases in liver-related mortality (11.2X) and diabetes-related mortality (3.3X) ⁽³⁰⁾. Another analysis demonstrated that ALT>43 IU/l for men and >30 IU/l for women was related to the presence of coronary heart disease, even when patients with obesity, chronic viral hepatitis, and excessive alcohol use were excluded ⁽³¹⁾.

The following studies appear to be relevant to this issue as well:

1. PMID: 34234521

[Application of a Novel Prediction Model for Predicting 2-Year Risk of Non-Alcoholic Fatty Liver Disease in the Non-Obese Population with Normal Blood Lipid Levels: A Large Prospective Cohort Study from China.](#)

Ji L, Cai X, Bai Y, Li T. *Int J Gen Med.* 2021 Jun 28;14:2909-2922. doi: 10.2147/IJGM.S319759. eCollection 2021. PMID: 34234521 **Free PMC article.**

2. PMID: 34127778

[Long-term mortality due to infection associated with elevated liver enzymes: a population-based cohort study.](#)

Oh TK, Jang ES, Song IA. *Sci Rep.* 2021 Jun 14;11(1):12490. doi: 10.1038/s41598-021-92033-1. PMID: 34127778 **Free PMC article.**

3. PMID: 33716920

[Associations of Serum Liver Function Markers With Brain Structure, Function, and Perfusion in Healthy Young Adults.](#)

Chen J, Liu S, Wang C, Zhang C, Cai H, Zhang M, Si L, Zhang S, Xu Y, Zhu J, Yu Y. *Front Neurol*. 2021 Feb 25;12:606094. doi: 10.3389/fneur.2021.606094. eCollection 2021. PMID: 33716920 **Free PMC article**.

4. PMID: 33072167

[Changes in alanine aminotransferase in adults with severe and complicated obesity during a milk-based meal replacement programme.](#)

Abdalgwad R, Rafey MF, Murphy C, Ioana I, O'Shea PM, Slattery E, Davenport C, O'Keeffe DT, Finucane FM. *Nutr Metab (Lond)*. 2020 Oct 16;17:87. doi: 10.1186/s12986-020-00512-5. eCollection 2020. PMID: 33072167 **Free PMC article**.

5. PMID: 32416645

[Alanine Aminotransferase and Gamma-Glutamyl Transpeptidase Predict Histologic Improvement in Pediatric Nonalcoholic Steatohepatitis.](#)

Newton KP, Lavine JE, Wilson L, Behling C, Vos MB, Molleston JP, Rosenthal P, Miloh T, Fishbein MH, Jain AK, Murray KF, Schwimmer JB; Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN). *Hepatology*. 2021 Mar;73(3):937-951. doi:

6. PMID: 32267017

[Revision of serum ALT upper limits of normal facilitates assessment of mild liver injury in obese children with non-alcoholic fatty liver disease.](#)

Lu Y, Wang Q, Yu L, Yin X, Yang H, Xu X, Xia Y, Luo Y, Peng Y, Yu Q, Chen Z, Yu J, Lai M, Wu N, Pan XB, Zheng X. *J Clin Lab Anal*. 2020 Jul;34(7):e23285. doi: 10.1002/jcla.23285. Epub 2020 Apr 8. PMID: 32267017 **Free PMC article**.

7. PMID: 31873887

[Insecticide and metal exposures are associated with a surrogate biomarker for non-alcoholic fatty liver disease in the National Health and Nutrition Examination Survey 2003-2004.](#)

Wahlang B, Appana S, Falkner KC, McClain CJ, Brock G, Cave MC. *Environ Sci Pollut Res Int*. 2020 Feb;27(6):6476-6487. doi: 10.1007/s11356-019-07066-x. Epub 2019 Dec 23. PMID: 31873887 **Free PMC article**.

8. PMID: 31514449

[Low Alanine Aminotransferase Cut-Off for Predicting Liver Outcomes; A Nationwide Population-Based Longitudinal Cohort Study.](#)

Park JH, Choi J, Jun DW, Han SW, Yeo YH, Nguyen MH. *J Clin Med*. 2019 Sep 11;8(9):1445. doi: 10.3390/jcm8091445. PMID: 31514449 **Free PMC article**.

9. PMID: 30973940

[Lowering the upper limit of serum alanine aminotransferase levels may reveal significant liver disease in the elderly.](#)

Schmilovitz-Weiss H, Gingold-Belfer R, Grossman A, Issa N, Boltin D, Beloosesky Y, Morag Koren N, Meyerovitch J, Weiss A. *PLoS One*. 2019 Apr 11;14(4):e0212737. doi: 10.1371/journal.pone.0212737. eCollection 2019. PMID: 30973940 **Free PMC article**.

10. PMID: 30081587

[Association between Serum Liver Enzymes and Metabolic Syndrome in Korean Adults.](#)

Kim HR, Han MA. *Int J Environ Res Public Health*. 2018 Aug 5;15(8):1658. doi: 10.3390/ijerph15081658. PMID: 30081587 **Free PMC article**.

11. PMID: 29897928

[Nonalcoholic fatty liver disease with elevated alanine aminotransferase levels is negatively associated with bone mineral density: Cross-sectional study in U.S. adults.](#)

B. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

In the opinion of this reviewer, it would be useful to reconsider the liver story; new studies seem to have been published in the past 3-5 years that include analyses of markers of liver function and disease.

Toxicokinetic Models

G. Human model –

For endpoints observed in adults, EPA used a steady-state approach to calculate the HED, which assumes a relatively constant exposure and clearance during adulthood. Please comment on this method of HED calculation. Are there alternative approaches that EPA should consider? If so, please describe the rationale for recommending this approach(es).

Of the various options, the assumption of a relatively constant exposure and clearance during adulthood would seem to make the most sense. In addition, this assumption would be the most conservative in terms of modeling, i.e., the most all-encompassing.

5. Epidemiological Study RfD Derivation

EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for PFOA and PFOS.

A. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

In this reviewer's opinion, uncertainty has been adequately accounted for in the derivation of the RfDs. As EPA moves forward, it is essential to consider cumulative risk, not just from multiplicity of chemical exposures, but from other environmental factors that enhance susceptibility. One potential uncertainty that comes to mind would be potentially higher exposures in low SES communities, i.e., by race/SES. However, there is scant data related to this particular issue in the context of PFOA/PFOS, and, if anything, evidence to suggest that higher SES communities actually have higher levels of exposures. Another would be co-morbidities, i.e., the potential for individuals with co-morbidities related to the health outcomes associated with PFOA/PFOS however, e.g., individuals with immune suppression via other sources, or with extant liver disease. However, many of the epidemiological studies essentially examine effects in populations with co-morbidities.

B. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

Yes.

REVISED COMMENTS

Question not assigned. Response modified from preliminary response (slightly).

1. Study Identification and Inclusion

EPA used systematic review methods consistent with the current ORD systematic review practice to ensure transparency and completeness of literature identification, sorting, and study quality evaluation. Is the process clearly described? Please identify additional peer-reviewed studies that the panel is aware of that could inform toxicity value derivation.

Response:

The overall systematic review process itself is clearly described in the document. However, specific parts of it are not entirely clear. For example, decision points behind the inclusion or exclusion of individual studies/articles are not clear. For example, a study by Vriens et al. (Vriens A, et al. 2019. Exposure to environmental pollutants and their association with biomarkers of aging: A multipollutant approach. Environ Sci Technol. 53:5966-5976) is not listed in the PFOA or PFOS documents; this study could inform mechanisms. While this is a single specific example, it is challenging to determine if studies were missed in the search strategy or not included for a specific reason such as if they were deemed uninformative or low confidence. For example, the HERO PFOA and PFOS database lists nearly 5,000 references and without comparing references in the PFOA/PFOS documents to every HERO PFOA and PFOS database entry, it is extremely challenging to determine the included and excluded studies. It is recommended that this aspect of the systematic review methods be clarified.

Question assigned. Response modified from preliminary response.

5. Noncancer Hazard Identification

Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

Response:

It appears as if the Agency has considered the available evidence for health systems and endpoints for POD derivation. The current documents cover a wide range of health systems/endpoints, and it does not appear as if any category for which there were multiple publications/studies has been ignored or inadequately addressed. In the 2016 health effects documents for PFOA and PFOS ("2016 documents"), fewer than 10 individual studies for each chemical were used to derive human equivalent doses (HEDs) and these were limited to toxicological studies. In the current documents, over 20 individual studies, including both toxicological and epidemiological studies, were used to derive HEDs. This reflects a significant expansion of the health effects considered for POD derivation.

However, it does not appear as if the Agency were completely clear and transparent in how they determine strong vs. suggestive evidence designations for the various outcome categories. While

the current documents for PFOA and PFOS are significantly improved from the 2016 documents for each chemical in that levels of confidence are included in the evidence synthesis, how this level of confidence was derived is not clear. The 2016 documents contained in-depth descriptions of studies as well as subjective evaluations of strengths and weaknesses of specific studies, but did not include detailed descriptions of the Agency's assessment of the strength of the evidence for specific studies or for specific health outcome categories. The current documents do include these assessments and it is much clearer why the Agency made specific choices and/or recommendations for selection of health systems/endpoints for consideration of POD derivation, it is not clear why one health endpoint was considered stronger over another.

As a result, the process by which the health outcome(s) ultimately selected for POD derivation lacks depth and transparency, making it challenging for readers of the document to understand the decision-making process for outcome(s) selection. It is recommended that the strength of evidence classifications used to guide decisions throughout the document be clearly stated. It is recommended that the strength of evidence classification for each health outcome being considered in the document be clearly stated and adequately supported with examples of evidence considered strong, suggestive, weak, etc. It is recommended that this evidence synthesis be applied to health outcomes that were not considered for POD derivation as well.

Question not assigned. Response modified from preliminary response (slightly).

6. Noncancer Hazard Identification

Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.

8. Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.

Response:

The approach appears to be reasonable considering the relatively small magnitude of effect and perceived concerns about clinical relevance and non-specificity of ALT to adverse liver injury and disease. However, like suppression of vaccine responses, which represent immunotoxicological risk even in the absence of clinical indicators of increases in infection or disease, it appears as if alterations to ALT also can represent

hepatotoxicological risk. I defer to other board members with more extensive experience in hepatotoxicology to better address this point.

9. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.

Response:

I defer to other board members with more extensive experience in hepatotoxicology to better address this point.

10. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

Response:

Neither the PFOA nor PFOS documents appear to address non-alcoholic fatty liver disease/steatosis as an adverse liver endpoint. Studies addressing this adverse outcome include:

Bassler J et al. 2019. Environmental perfluoroalkyl acid exposures are associated with liver disease characterized by apoptosis and altered serum adipocytokines. Environ Pollut. 247:1055-1063.

Jin R, et al. 2020. Perfluoroalkyl substances and severity of nonalcoholic fatty liver in children: An untargeted metabolomics approach. Environ Int. 134:105220.

Wahlang B, et al. 2019. Mechanisms of environmental contributions to fatty liver disease. Curr Environ Health. Rep 6:80–94 (Table 1 of this manuscript contains additional citations exploring fatty liver disease and associations with PFOA/PFOS).

It is recommended that studies of this liver outcome be considered.

Question assigned. Response not modified from preliminary response (except to correct minor typos).

1. Cancer

Cancer classification for PFOA/PFOS

PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a ‘likely carcinogen’ categorization which is a change from ‘suggestive’ in the 2016 HA. Does the panel agree with the ‘likely’ designation based on the new evidence? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

Response:

EPA's *Guidelines for Carcinogen Risk Assessment* (2005) describes five categories for the weight of evidence regarding carcinogenic potential of an agent: carcinogenic to humans, likely to be carcinogenic to humans, suggestive evidence of carcinogenic potential, inadequate information to assess carcinogenic potential, and not likely to be carcinogenic to humans. Existing cancer data for PFOA from epidemiological studies and from studies of experimental animal models indicate that cancers/tumors have been observed, thus the latter category is not appropriate. Additionally, at least 10 epidemiological studies and two chronic cancer bioassays in rodents for PFOA exist, thus a designation of inadequate information is not appropriate. Since the publication of the 2016 document for PFOA, at least eight epidemiological studies considering links between PFOA exposure and cancer have been published and the Agency determined that these were medium confidence studies. At least one new nested case-control study in the general population (Shearer et al., 2021) supports previous evidence of kidney cancer in highly exposed populations (Barry et al., 2013 and Vieira et al, 2013) and at least one new chronic cancer bioassay in rats (NTP, 2020) supports previous evidence of tumorigenesis in multiple sites (Butenhoff et al, 2012). The 2020 NTP study identified clear evidence of carcinogenic activity in rats exposed over a lifetime, including the pre-natal, pre-weaning, and post-weaning stages. Evidence of cancer was observed in the liver and pancreas. "Clear evidence" is the strongest line of evidence used by the NTP in evaluating data from studies of this kind.

These data also appear to exceed the descriptor for suggestive evidence, which may include a positive cancer result from only a single animal or human study with additional studies of mixed results. However, these data also appear to exceed the descriptor for likely evidence. Supporting data for the likely descriptor may include (from the EPA's *Guidelines for Carcinogen Risk Assessment*, 2005):

- Plausible associations between human exposure and cancer with some supporting experimental evidence (not necessarily carcinogenicity data from animal experiments);
- Positive tests in animal experiments in more than one species, strain, sex, site, or exposure routes, with *or without* (emphasis added) evidence of carcinogenicity in humans;
- A positive tumor study that raises additional biological concerns such as early age at onset or a high degree of malignancy;
- A rare animal tumor response in a single experiment assumed to be relevant to humans;
- A positive tumor study strengthened by other links of evidence such as a plausible association between human exposure and cancer or evidence that agent or metabolite causes events generally known to be associated with tumor formation

The descriptor for carcinogenic to humans described in the EPA's *Guidelines for Carcinogen Risk Assessment* (2005) includes:

- Convincing epidemiologic evidence of a causal association between human exposure and cancer.
- In the absence of the latter, the following conditions must be met:
 - Strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association.

- Extensive evidence of carcinogenicity in animals.
- Mode(s) of carcinogenic action and associated precursor events have been identified in animals; and
- There is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information.

The question is, therefore, whether the current dataset for PFOA meet the criteria for carcinogenic to humans according to these Guidelines. Bartell and Vieira (2021), in a critical review and meta-analysis of epidemiological literature concluded that associations between PFOA and kidney and testicular cancer were likely causal, which would seem to satisfy the first descriptor of “convincing epidemiologic evidence of a causal association between human exposure and cancer.” Considering additional evidence of carcinogenicity in animals, the Agency authors are encouraged to reconsider the carcinogenicity assessment of “likely to be carcinogenic to humans” in favor of “carcinogenic to humans.”

PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a ‘suggestive’ categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

Response:

As described in the response concerning the cancer classification for PFOA, the 2005 EPA *Guidelines for Carcinogen Risk Assessment* contains descriptors for five categories for the weight of evidence regarding carcinogenic potential of an agent. Suggestive evidence of carcinogenic potential may include a positive cancer result from only a single animal or human study with additional studies of mixed results. As described in the PFOS document, the epidemiological evidence for the carcinogenicity of PFOS appear to be mixed and/or the studies were designed in such a way to constrain firm conclusions. A single chronic cancer bioassay in rodents showed increases in tumors in the liver, thyroid gland, and mammary gland; these tumors did not appear in a dose-responsive pattern. These data however, appear to meet the descriptor for likely to be carcinogenic to humans rather than as suggestive evidence of carcinogenic potential. Data supporting the likely to be carcinogenic descriptor is fairly broad, for example, the first description of supporting data reads “an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments.” The available dataset for PFOS appears to be supportive of PFOS being likely to be carcinogenic to humans.

Additionally, the state of California (CA) has proposed (effective December 24, 2021) listing PFOS and its salts and transformation and degradation precursors on the list of chemicals known to the state to cause cancer for purposes of the Safe Water Drinking and Toxic Enforcement Act of 1986 (Proposition 65) (OEHHA, 2021a). The human, animal, and mechanistic evidence presented by the Reproductive and Cancer Hazard Assessment Branch

of the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency appear to be consistent with the evidence presented in the PFOS document (OEHHA, 2021b). The OEHHA summary of human evidence indicates that the results were mixed and summary of animal evidence highlights a chronic carcinogenicity study in rodents supported by a tumor promotion study in rainbow trout (OEHHA, 2021b), which is mostly consistent with evidence provided in the PFOS document (The tumor promotion study by Benninghoff et al., 2012 is not included in the PFOS document; this study demonstrated that PFOS acted as a tumor promoter in rainbow trout.). Similarly, the mechanistic considerations in the OEHHA summary (OEHHA, 2021b) appear to be consistent with evidence provided in the PFOS document. However, it appears as if the OEHHA conclusion to propose listing PFOS on the list of chemicals known to the state to cause cancer differs from the PFOS document. It is therefore recommended that authors reconsider the “suggestive evidence of carcinogenic potential” classification for PFOS to the “likely to be carcinogenic to humans” classification.

OEHHA, 2021a. Notice to interested parties chemicals listed effective December 24, 2021 as known to the State of California to cause cancer: Perfluorooctane sulfonic acid (PFOS) and its salts and transformation and degradation precursors. <https://oehha.ca.gov/proposition-65/crn/notice-interested-parties-chemicals-listed-effective-december-24-2021-known>. Accessed December 2021.

OEHHA, 2021b. Evidence of the carcinogenicity of perfluorooctane sulfonic acid (PFOS) and its salts and transformation and degradation precursors. <https://oehha.ca.gov/media/downloads/crn/pfoshid092421.pdf>. d

2. **Cancer**

Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends. Does SAB support the selection of this CSF in the derivation of a risk specific dose for PFOA (i.e., risk)? If not, please provide input on the strengths and weaknesses of the other candidate CSFs that the EPA derived.

Response:

No response provided.

Question not assigned. Response not modified from preliminary response.

6. **Human Toxicokinetic Model**

A. For endpoints observed in adults, EPA used a steady-state approach to calculate the HED, which assumes a relatively constant exposure and clearance during adulthood. Please comment on this method of HED calculation. Are there alternative approaches that EPA should consider? If so, please describe the rationale for recommending this approach(es).

Response:

No response provided.

- B. Two key parameters are the half-life and volume of distribution, which were used to calculate clearance. Half-life and volume of distribution were assumed to be constant across sex and age groups because of a lack of strong quantitative data to parametrize changes across sex and age. Please comment on the strengths and weakness of the use of this assumption and the choice of these parameters by the EPA. Please describe the rationale for alternative recommended approaches. For endpoints observed in human neonates or children, EPA used a one-compartment TK model to simulate dosimetry during pregnancy and a two-compartment TK model (one-compartment models for the mother and the child) to simulate dosimetry during lactation, to calculate the HED for each POD. Please comment on the strengths and weaknesses of this choice of model structure for the task of predicting dosimetry in the human fetus and child compared to dosimetry in mice and rats in the similar lifestages. Please provide the rationale for any alternative recommended approaches.

Response:

No response provided.

- C. The key chemical-specific parameters that describe the transfer of the chemical from the mother to the child during gestation and lactation are the maternal to fetal serum ratio and the ratio of maternal serum to milk PFOA/S concentration. These ratios were assumed to be constant during gestation and lactation, respectively. Another important parameter is the rate of milk ingestion, which is chemical-independent and varies throughout lactation. Please comment on the strengths and weaknesses of the choice of parameters for fetal to maternal partitioning and partitioning into breastmilk, as well as the choice for lactation rate. Please also comment on the choice to assume that fetal to maternal partitioning and partitioning to breastmilk did not vary in time. Please describe whether there are other methods you would recommend to account for these changes over time and across development.

Response:

No response provided

Question not assigned. Response not modified from preliminary response.

7. Animal Toxicokinetic Model

- M. After a review of the available toxicokinetic models for PFOA/S predictions in laboratory animals, EPA selected the Wambaugh et al. (2013) model because it was parametrized using all species of interest, demonstrated good agreement with training and test datasets, and used a single, biologically motivated, model structure across all species. Does the panel agree with selecting this model? If not, please describe the rationale for alternative recommended approaches for the calculation of the internal dose metrics in adult animals.

Response:

The model by Wambaugh et al. (2013) appears to be an appropriate model for calculation of internal dose metrics in adult animals and the rationale for the choice of this model is clearly indicated in the documents.

- N. The animal model parameters were obtained through a Bayesian inference parameterization which produced wide credible intervals for some parameter values, but relatively tight credible intervals for the predicted serum concentration. Does the panel agree with using the median values of the estimated animal parameter distributions for prediction of serum concentration and internal dose metrics?

Response:

No response provided.

- O. Based on visual inspection of model predictions to the calibration datasets, EPA utilized sex-independent parameters for PFOS. The male-specific parameters were used for all rat-specific PFOS predictions including predictions in pregnant and nursing dams and the female-specific parameters were used for all mouse-specific PFOS predictions because the parameter values obtained from fitting the female-specific rat data and male-specific mouse data were not consistent with the overall TK parameters for PFOS and produced poor fits to the training and test datasets. Does the panel agree with this approach and justification for this assumption for PFOS? If not, please describe other approaches that could be considered?

Response:

No response provided.

- P. EPA assumed a one compartment model for the developing infant based on the lack of infant-specific toxicokinetic data from rats and mice. This model utilizes averages of half-life and volume of distribution from the literature coupled with physiologically relevant lactational parameters for pup nursing. Does the panel agree with the decision to use this model structure for infant animals? If not, please provide data on infant-specific changes during the animal lactational-period that could be used to account for toxicokinetic differences between the adult and infant rats and mice.

Response:

No response provided.

- Q. Several parameters dictate the transfer of chemical from the mother to her pup. Does the panel agree with the selection of these parameters for the animal model? If not, please provide your justification and alternative parameters.

Response:

No response provided.

- R. For neonatal animals, EPA assumed no sex differences in clearance in neonatal animals based on the lack of identification of sex-dependent differences in PFOA/S toxicokinetics from the available data. Does the panel agree with this assumption? If not, please provide your justification and available data on sex differences in neonatal rats.

Response:

No response provided.

Question not assigned. Response not modified from preliminary response.

1. Epidemiological Study RfD Derivation

1. EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only ‘medium’ and ‘high’ quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?

Response:

No response provided.

Question assigned. Response modified from preliminary response (slightly).

2. Epidemiological Study RfD Derivation

Studies of developmental immune health outcomes (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]) after PFOA/S exposure identified associations with very low doses of either PFOA or PFOS with developmental immune effects. The RfD for this outcome was selected as the critical effect because it was the lowest among the candidate RfDs for PFOA or PFOS and can result in severe illness. Does the panel agree with the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS?

If so, please explain your justification.

Response:

The choice of immunotoxicological effects of PFOA and PFOS as the critical effect for derivation of chronic RfDs is appropriate and the selection of the critical study is representative of this effect in a human population exposed to PFOA/PFOS. First, the endpoint identified as the critical effect is a functional immune response. In other words, the measurement of antigen-specific antibodies following vaccinations is an overall measure of the ability of the immune system to respond to a challenge. The antigen-specific antibody response is extremely useful for evaluating the entire cycle of adaptive immunity and is a sweeping approach to detect immunosuppression across a range of cells and signals (Myers, 2018). This response also is translatable across multiple species, including rodents and

humans, and historical data indicating that suppression of antigen-specific antibody responses by exogenous agents is predictive of immunotoxicity is extensive (Myers, 2018). Second, when immunosuppression occurs in the developing immune system, such as observed in the critical studies used to derive the chronic RfDs, the risks of developing infectious diseases and other immunosuppression-linked diseases increases, often across a lifetime (Dietert et al., 2010). Third, immunosuppression linked with chemical stressors is *not* the same as an immunodeficiency associated with, for example, genetic-based diseases, but is still an endpoint associated with increased health risks. As pointed out Selgrade (2007), the lack of an obvious AIDS-like epidemic from exposure to chemical immunosuppressants does not mean that the human population is not at serious risk from exposure to immunosuppressants. Evidence from specific populations experiencing mild to moderate immunosuppression indicates that the risk of infections with pathogens commonly encountered in the general population is real (Selgrade, 2007). Finally, immunotoxicity that occurs in the developing organism generally occurs at doses lower than required to affect the adult immune system, thus providing a more sensitive endpoint upon which protective measures, such as RfD, can be based (vonderEmbse and DeWitt, 2018). Therefore, the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS is appropriate and will be protective of the general population, and likely including sensitive subpopulations.

Dietert RR, DeWitt JC, Germolec DR, and Zelikoff JT. 2010. Breaking patterns of environmentally influenced disease for health risk reduction: Immune perspectives. *Environmental Health Perspectives* 118:1091-1099.

Myers LP. 2018. Clinical immunotoxicology. In: *Immunotoxicity Testing: Methods and Protocols* (DeWitt JC, Rockwell CE, and Bowman CC, eds), Methods in Molecular Biology Series. Springer Science + Business Media, LLC.

Selgrade MK. 2007. Immunotoxicity – The risk is real. *Toxicological Sciences*. 100:328-332.

vonderEmbse AN and DeWitt JC. 2018. Developmental immunotoxicity (DIT) testing: Current recommendations and the future of DIT testing. In: *Immunotoxicity Testing: Methods and Protocols* (DeWitt JC, Rockwell CE, and Bowman CC, eds), Methods in Molecular Biology Series. Springer Science + Business Media, LLC.

If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of chronic RfDs.

Response:

Not applicable.

Are any additional analyses or rationales needed to increase the confidence in the chronic RfDs for PFOA and PFOS?

Response:

Yes. The critical study, which is an epidemiological study, is supported by several studies in rodent models showing that exposure to PFOA or PFOS alone dose-responsively suppresses

the antigen-specific antibody response. These studies are described in section 3.3.4.2.4 of the PFOA document and in section 3.3.4.2.7 of the PFOS document but not the sections of RfD selection (4.1.6 for both documents). Confidence in the chronic RfDs for PFOA and PFOS could be increased if the supportive evidence from animal studies is at least mentioned in section 4.1.6 for both documents.

Question assigned. Response modified from preliminary response.

3. Epidemiological Study RfD Derivation

The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.

Response:

Yes, the 5% BMR selection for modeling to identify the POD is supported. The developing immune system (i.e., the immune system of children) is generally regarded as more sensitive to exogenous perturbations such as those from chemical stressors than the adult immune system. Therefore, changes observed in the developing immune system reflect developmental effects. The immune response to childhood vaccines may be “an excellent indicator for developmental immunotoxicity when conducted under appropriate conditions” (Luster et al., 2005). Responses to childhood vaccines are thought to be sensitive enough to detect changes in populations with moderate degrees of immunosuppression, such as those exposed to an immunotoxic agent (Luster et al., 2005). However, the degree to which mild to moderate immunosuppression from exposure to chemical agents produces measurable clinical outcomes is challenging to determine (DeWitt et al., 2017). Evidence from specific populations experiencing mild to moderate immunosuppression indicates that the risk of infections with pathogens commonly encountered in the general population is real (Selgrade, 2007). Reductions in antibody titers to a specific vaccine below a level that is considered protective does increase *the risk* of susceptibility to the disease against which the vaccine was intended. Additionally, “a compromised immune system should be considered more prone to escape homeostasis, enhancing risk for disease development” (Hessel et al., 2015).

Hessel et al. (2015) also evaluated data from experimental animal studies of nine different chemicals known or suspected to be immunotoxicants and that had been evaluated for immunotoxicity across a range of tests, including guideline tests for regulatory toxicology testing. With respect to the antigen-specific antibody response, which when performed in experimental animals is analogous to the vaccine response in humans, Hessel et al. concluded that any compound-related effect is indicative of adversity. Therefore, a BMR of 5% would likely be small enough to be protective against shifts in the vaccine response that are considered to be small, mild, or moderate, but that are still differentiated in some way from no or low exposure groups.

DeWitt JC, Germolec DR, Luebke RW, and Johnson VJ. 2017. Associating changes in the immune system with clinical diseases for interpretation in risk assessment. *Current Protocols in Toxicology*. 67:18.1.1-18.1.22.

Hessel EVS, Tonk ECM, Bos PMJ, van Loveren H, and Piersma AH. 2015. Developmental immunotoxicity of chemicals in rodents and its possible regulatory impact. *Critical Reviews in Toxicology*. 45:68-82.

Luster MI, Johnson VJ, Yucesoy B, and Simeonova PP. 2005. Biomarkers to assess potential developmental immunotoxicity in children. *Toxicology and Applied Pharmacology*. 206:229-236.

Selgrade MK. 2007. Immunotoxicity – The risk is real. *Toxicological Sciences*. 100:328-332.

Question not assigned. Response not modified from preliminary response.

4. Epidemiological Study RfD Derivation

EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UF_H), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for PFOA and PFOS.

Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

Response:

It appears as all recommended sources of uncertainty have been adequately accounted for in the derivation of the RfDs.

Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

Response:

Only one UF has a value greater than one for both the PFOA and PFOS RfDs and it is the UF_H, which reflects the intraspecies UF and has a value of 10 for both chemicals. This UF is used to account for variability in the responses within the human population due to various factors. Such variability includes genetics, life stage, health status, lifestyle, and other factors that may produce variability in how specific human subpopulations may respond to the chemical(s) under evaluation. The rationale for a value of 10 for the UF_H is clearly described

in the documents. The other UFs are all equal to one and the rationale for this value is clearly described in the documents.

Question not assigned. Response modified from preliminary response (slightly).

6. Relative Source Contribution

EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources. Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at: <https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS.

Are you aware of additional relevant exposure data that EPA should consider in developing the RSCs for PFOA and PFOS? If so, please provide citations.

Response:

It appears as if the relevant exposure data has been considered in developing the RSCs for PFOA and PFOS.

Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS is adequately supported and clearly described.

Response:

It appears as if the recommended RSC of 20% for PFOA and PFOS is adequately supported and clearly described. However, there may be instances where a more conservative approach is warranted to protect those in highly exposed populations such as those in occupational settings or in communities with heightened drinking water exposures and who receive a greater proportion of their RfD from these exposures. While those with community-level exposures via drinking water experience a higher burden of their exposure to PFOA and PFOS through their drinking water, the potential for additional pathways of exposure also exists in these high exposure communities, which means that they have higher total exposures. For example, contaminated biosolids from water treatment facilities may be applied to lands in and around those communities with heightened drinking water exposures, potentially increasing exposures via food grown in the presence of PFAS. Similarly, such communities may also be at increased risk of exposure to PFAS in ambient air and indoor dust, in recreational waters, and through food obtained from the aquatic environment, especially if a known source of PFAS water contamination exists. It therefore may be more protective, especially for high exposure communities, to consider a more conservative RSC to account for sources of exposure that may

adjust the proportion of total exposure that comes from ingestion of water and/or fish and to ensure that total exposures do not exceed an acceptable level.

PRELIMINARY COMMENTS

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

1. The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, an assumption of dose addition for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA's supplementary guidance (EPA, 2000) states: "The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which "similarity" can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.

- A. Please comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.

Response:

The PFAS included in the mixtures document, PFOA, PFOS, PFBS, and GenX, all have evidence of dose-responsive effects on developing organisms, the liver, and the thyroid gland. These toxicities also have been observed for PFAS other than these four, suggesting that developing organisms, the liver, and the thyroid gland are targets of multiple PFAS. Therefore, the assumption of similarity at the level of toxicity endpoint/health effect appears to be an appropriate approach for a component-based mixture evaluation of PFAS.

- B. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).

Response:

Common toxicity endpoint/health effect is considered an optimal similarity domain for those PFAS with limited or no available MOA-type data. EPA's *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000) notes that "in some cases the class may be linked by common effect with only suggestive or indirect information concerning the underlying mode of action." It is therefore appropriate to

move forward with a component-based mixture evaluation in the absence of direct information concerning the underlying mode of action.

2. Section 4.3 (Hazard Index; HI) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and hypothetical exposure information. The example calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as “GenX chemicals”).
 - a) Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.

Response:

EPA’s *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000) indicates that dose addition can be interpreted as simple similar action “where the component chemicals act as if they were dilutions of each other differing only in relative toxicity.” To account for differences in relative toxic potency, different types of toxicity, and/or different routes, the hazard index (HI) can be developed for each exposure route of interest, for a single toxicity effect, or for toxicity to a single target organ (EPA, 2000). Therefore, a mixture may be assessed by several HIs with each HI representing one route and one toxic effect or target organ (EPA, 2000). The EPA’s *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000) also indicates that “in practice, because of the common lack of information on mode of action and pharmacokinetics, the requirement of toxicologic similarity is usually relaxed to that of similarity of target organs.” The HI approach appears to be a reasonable methodology for indicating potential risk associated with mixtures of PFAS.

- b) Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment.

Response:

The EPA’s *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000) provides a variety of formulas/alternative formulas that can be used to determine the HI based on the available data and interpretation of risks desired. The Guidance also emphasizes throughout the document that scientific judgment plays a vital role in the HI approach as well as other approaches for evaluating potential risks of exposure to chemical mixtures (EPA, 2000). Throughout the mixtures document for PFAS, the authors clearly explain steps, procedures, and rationale for decisions, which is recommended by *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000).

3. Section 4.4 (Relative Potency Factor; RPF) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and HFPO dimer acid and GenX chemicals.
- a) Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.

Response:

The mixtures document very clearly explains the concept of relative potency factors (RPFs) and how they are used in dose addition (DA) models. Briefly summarized from the mixtures document, RPFs are assigned numerical values to individual chemicals within a class of chemicals compared to an index or benchmark chemical, typically for a specific mechanism of action or adverse health outcome. The index chemical (IC) can be a member of the class that is the most toxicologically studied or most representative with respect to toxicity and as indicated in the mixtures document is an “anchor against which all other components are compared” (p 14). Cumulative toxicity of the mixture can then be calculated using the RPFs of individual chemicals in a mixture, which is one approach in DA models.

EPA’s *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000) indicates that the RPF “approach relies on both the existence of toxicologic dose-response data for at least one component of the mixture (referred to as the index compound) and scientific judgment as to the toxicity of the other individual compounds in the mixture and of the mixture as a whole.” Additionally, EPA’s *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000) also indicates that the RPF approach relies heavily on the judgment of scientific data and often requires a cross-disciplinary team for the analysis. It appears as if these guidelines set out in the EPA’s *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000) have been satisfied by the mixtures document.

Section 3.4 of the mixtures document briefly summarizes evidence supporting dose additivity of PFOA, PFOS, and other PFAS with linear or branched alkyl or alkyl ether chains and sulfonic or carboxylic acid functional groups. Although these PFAS lack detailed molecular mechanisms, they share common key events and/or adverse outcomes that support the RPF approach.

The mixtures document clearly defines the RPF approach, including data requirements for the approach. The mixtures document also provides several examples of the RPF approach using a mix of PFOA, PFOS, PFBS, and/or GenX and with different toxicological endpoints and provides a summary of pertinent positives and negatives of

the RPF approach. Therefore, as explained in the mixtures document, it appears as if the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS.

- b) Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.

Response:

Goodrum et al. (2020), which was not included in the mixtures document and contains a RPF analysis for subsets of PFAS, indicated that concentration addition and an RPF approach for PFAS was inappropriate due to differences among sensitive effects and target organ potencies, and noncongruent dose-response curves for the same effect endpoints. However, Goodrum et al. (2020) appears to be narrow in their interpretation of EPA's various guidance documents for performing mixtures risk assessments. Goodrum et al. (2020) suggest that if dose-response curves for PFAS being assessed for the RPF approach are not geometrically congruent, the non-congruent PFAS cannot be grouped for the RPF approach. However, EPA's *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000) provides adequate room for the use of scientific judgment in the RPF approach. For example, the EPA indicates that "the biological basis for dose addition is the similarity of chemical components regarding toxicologic behavior, such as toxic mechanism, mode of action, or endpoint and includes room for scientific judgment on similarity (EPA, 2000). Additionally, Dinse and Umbach (2011) indicate that when chemicals do not have similar dose-response curves, they can still be used in the RPF approach provided relative potency functions, i.e., mathematical functions, are applied to calculate the RPF. It is therefore recommended that as the Agency moves forward with the RPF approach for PFAS, that the development of appropriate relative potency functions be derived for PFAS that appear to have dissimilar dose-response curves.

Dinse GE and Umbach DM. 2011. Characterizing non-constant relative potency. *Regulatory Toxicology and Pharmacology*. 60:342-353.

Goodrum PE, Anderson JK, Luz AL, and Ansell GK. 2020. Application of a framework for grouping and mixtures toxicity assessment of PFAS: A closer examination of dose-additivity approaches. *Toxicological Sciences*. 179:262-278.

4. Section 4.5 (Mixture BMD) of the framework document demonstrates the application of a component-based mixture approach using established EPA dose-response modeling (i.e., benchmark dose; BMD) of hypothetical PFAS dose-response data, and hypothetical exposure information.
- a) Please provide specific feedback on whether the Mixture BMD approach is a reasonable methodology for estimating what is in essence a mixture-based point-of-departure. If not, please provide an alternative.

Response:

EPA's *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000) indicates that another approach for determining relative toxicity of components in a mixture is to calculate a benchmark dose for the target organ of interest. The Mixture BMD approach appears to be a reasonable methodology for estimating a mixture-based point-of-departure.

- b) Please provide specific feedback on whether the proposed Mixture BMD methodology in the framework is scientifically supported for PFAS mixture risk assessment.

Response:

Throughout the mixtures document for PFAS, the authors clearly explain the BMD process and approach and appears to follow basic recommendations in the EPA's *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (2000). Therefore, the Mixture BMD methodology in the framework appears to be scientifically supported for PFAS mixture risk assessment.

EPA's draft Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water

DeWitt did not provide any responses to charge questions for this document.

Overall

EPA is seeking SAB evaluation on the extent to which the approach to estimating reductions in CVD risk associated with reductions in exposure to PFOA and PFOS in drinking water is scientifically supported and clearly described.

- 7. Section 4.2 presents EPA's meta-analysis for the total cholesterol dose-response function.
 - a) Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.
 - b) To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.
- 8. Section 5.1 presents EPA's life table approach methodology.

- a) Please comment on the extent to which this analysis is scientifically supported and clearly described. To the extent improvements are suggested, please provide specific changes that are implementable in a U.S. national-level benefits analysis with readily available data.
9. Section 5.2 presents EPA's application of the atherosclerotic cardiovascular disease (ASCVD) risk model used to estimate the probability of hard CVD events corresponding to total cholesterol changes.
 - a) Please comment on the scientific validity of the ASCVD model application for estimating the probability of first time CVD events in various sub-populations and the extent to which it is clearly described.
 - b) Please comment on whether EPA's approach and assumption, of a uniform first CVD event hazard distribution over the 10-year period, is sufficiently robust given current data sources and literature. If additional distributional sources of information are suggested, please provide specific citations/sources for EPA's consideration.
 - c) Please comment on the scientific validity of using the ASCVD risk model for estimating reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA and PFOS in drinking water.
 10. Section 7 and Appendix A describe the limitations and uncertainties of the CVD risk reduction analysis.

Has EPA clearly described the individual contributions of the sources of uncertainty?

Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

1. Study Identification and Inclusion

EPA used systematic review methods consistent with the current ORD systematic review practice to ensure transparency and completeness of literature identification, sorting, and study quality evaluation. Is the process clearly described? Please identify additional peer-reviewed studies that the panel is aware of that could inform toxicity value derivation.

Response:

The overall systematic review process is clearly described in the document. However, it is not entirely clear how individual studies/articles were excluded. For example, a study by Vriens et al. (Vriens A, et al. 2019. Exposure to environmental pollutants and their association with biomarkers of aging: A multipollutant approach. *Environ Sci Technol.* 53:5966-5976) is not listed in the PFOA or PFOS documents; this study could inform mechanisms. While this is a single specific example, it is unclear why individual studies may have been excluded for the analyses. It is unclear if which studies were excluded if they were deemed uninformative or low confidence. For example, the HERO PFOA and PFOS database for lists nearly 5,000 references and without comparing references in the PFOA/PFOS documents to every HERO PFOA and

PFOS database entry, it is extremely challenging to determine the included and excluded studies. Is there a way for readers of the documents to know which studies were excluded and why?

2. Noncancer Hazard Identification

Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

Response:

It appears as if the Agency has considered the available evidence for health systems and endpoints for POD derivation. The current documents cover a wide range of health systems/endpoints, and it does not appear as if any category for which there were multiple publications/studies has been ignored or inadequately addressed. In the 2016 health effects documents for PFOA and PFOS (“2016 documents”), fewer than 10 individual studies for each chemical were used to derive human equivalent doses (HEDs) and these were limited to toxicological studies. In the current documents, over 20 individual studies, including both toxicological and epidemiological studies, were used to derive HEDs. This reflects a significant expansion of the health effects considered for POD derivation.

Similarly, it appears as if the various health outcome categories have been given appropriate designations regarding strength of the evidence. The current documents for PFOA and PFOS are significantly improved from the 2016 documents for each chemical in that levels of confidence are included in the evidence synthesis. The 2016 documents contained in-depth descriptions of studies as well as subjective evaluations of strengths and weaknesses of specific studies, but did not include detailed descriptions of the Agency’s assessment of the strength of the evidence for specific studies or for specific health outcome categories. The current documents do include these assessments and as a result, it is much clearer why the Agency made specific choices and/or recommendations for selection of health systems/endpoints for consideration of POD derivation.

3. Noncancer Hazard Identification

Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance

of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.

1. Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.

Response:

The approach appears to be reasonable considering the relatively small magnitude of effect and concerns about clinical relevance and non-specificity of ALT to adverse liver injury and disease.

2. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.

Response:

I am not aware of additional studies that support the ALT levels as markers of adverse liver effects.

3. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

Response:

Neither the PFOA or PFOS documents appear to address non-alcoholic fatty liver disease/steatosis as an adverse liver endpoint. Studies addressing this adverse outcome include:

Bassler J et al. 2019. Environmental perfluoroalkyl acid exposures are associated with liver disease characterized by apoptosis and altered serum adipocytokines. Environ Pollut. 247:1055-1063.

Jin R, et al. 2020. Perfluoroalkyl substances and severity of nonalcoholic fatty liver in children: An untargeted metabolomics approach. Environ Int. 134:105220.

Wahlang B, et al. 2019. Mechanisms of environmental contributions to fatty liver disease. Curr Environ Health. Rep 6:80–94 (Table 1 of this manuscript contains additional citations exploring fatty liver disease and associations with PFOA/PFOS).

4. Cancer

1. Cancer classification for PFOA/PFOS

- i. PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a 'likely carcinogen' categorization which is a change from 'suggestive' in the 2016 HA. Does the panel agree with the 'likely' designation based on the new evidence? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

Response:

EPA's *Guidelines for Carcinogen Risk Assessment* (2005) describes five categories for the weight of evidence regarding carcinogenic potential of an agent: carcinogenic to humans, likely to be carcinogenic to humans, suggestive evidence of carcinogenic potential, inadequate information to assess carcinogenic potential, and not likely to be carcinogenic to humans. Existing cancer data for PFOA from epidemiological studies and from studies of experimental animal models indicate that cancers/tumors have been observed, thus the latter category is not appropriate. Additionally, at least 10 epidemiological studies and two chronic cancer bioassays in rodents for PFOA exist, thus a designation of inadequate information is not appropriate. Since the publication of the 2016 document for PFOA, at least eight epidemiological studies considering links between PFOA exposure and cancer have been published and the Agency determined that these were medium confidence studies. At least one new nested case-control study in the general population (Shearer et al., 2021) supports previous evidence of kidney cancer in highly exposed populations (Barry et al., 2013 and Vieira et al, 2013) and at least one new chronic cancer bioassay in rats (NTP, 2020) supports previous evidence of tumorigenesis in multiple sites (Butenhoff et al, 2012). The 2020 NTP study identified clear evidence of carcinogenic activity in rats exposed over a lifetime, including the pre-natal, pre-weaning, and post-weaning stages. Evidence of cancer was observed in the liver and pancreas. "Clear evidence" is the strongest line of evidence used by the NTP in evaluating data from studies of this kind.

These data also appear to exceed the descriptor for suggestive evidence, which may include a positive cancer result from only a single animal or human study with additional studies of mixed results. However, these data also appear to exceed the descriptor for likely evidence. Supporting data for the likely descriptor may include (from the EPA's *Guidelines for Carcinogen Risk Assessment*, 2005):

- Plausible associations between human exposure and cancer with some supporting experimental evidence (not necessarily carcinogenicity data from animal experiments);
- Positive tests in animal experiments in more than on species, strain, sex, site, or exposure routes, with *or without* (emphasis added) evidence of carcinogenicity in humans;
- A positive tumor study that raises additional biological concerns such as early age at onset or a high degree of malignancy;
- A rare animal tumor response in a single experiment assumed to be relevant to humans;
- A positive tumor study strengthened by other links of evidence such as a plausible association between human exposure and cancer or evidence that agent or metabolite causes events generally known to be associated with tumor formation

The descriptor for carcinogenic to humans described in the EPA's *Guidelines for Carcinogen Risk Assessment* (2005) includes:

- Convincing epidemiologic evidence of a causal association between human exposure and cancer.
- In the absence of the latter, the following conditions must be met:
 - Strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association.
 - Extensive evidence of carcinogenicity in animals.
 - Mode(s) of carcinogenic action and associated precursor events have been identified in animals; and
 - There is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information.

The question is, therefore, whether the current dataset for PFOA meet the criteria for carcinogenic to humans according to these Guidelines. Bartell and Vieira (2021), in a critical review and meta-analysis of epidemiological literature concluded that associations between PFOA and kidney and testicular cancer were likely causal, which would seem to satisfy the first descriptor of “convincing epidemiologic evidence of a causal association between human exposure and cancer.” Considering additional evidence of carcinogenicity in animals, the Agency authors are encouraged to reconsider the carcinogenicity assessment of “likely to be carcinogenic to humans” in favor of “carcinogenic to humans.”

ii. PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a ‘suggestive’ categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

Response:

As described in the response for 4.a.i, the 2005 EPA *Guidelines for Carcinogen Risk Assessment* contains descriptors for five categories for the weight of evidence regarding carcinogenic potential of an agent. Suggestive evidence of carcinogenic potential may include a positive cancer result from only a single animal or human study with additional studies of mixed results. As described in the PFOS document, the epidemiological evidence for the carcinogenicity of PFOS appear to be mixed and/or the studies were designed in such a way to constrain firm conclusions. A single chronic cancer bioassay in rodents showed increases in tumors in the liver, thyroid gland, and mammary gland; these tumors did not appear in a dose-responsive pattern. These data however, appear to meet the descriptor for likely to be carcinogenic to humans rather than as suggestive evidence of carcinogenic potential. Data supporting the likely to be carcinogenic descriptor is fairly broad, for example, the first description of supporting data reads “an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most

cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments.” The available dataset for PFOS appears to be supportive of PFOS being likely to be carcinogenic to humans. Additionally, the state of California (CA) has proposed (effective December 24, 2021) listing PFOS and its salts and transformation and degradation precursors on the list of chemicals known to the state to cause cancer for purposes of the Safe Water Drinking and Toxic Enforcement Act of 1986 (Proposition 65) (OEHHA, 2021a). The human, animal, and mechanistic evidence presented by the Reproductive and Cancer Hazard Assessment Branch of the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency appears to be consistent with the evidence presented in the PFOS document (OEHHA, 2021b). The OEHHA summary of human evidence indicates that the results were mixed and summary of animal evidence highlights a chronic carcinogenicity study in rodents supported by a tumor promotion study in rainbow trout (OEHHA, 2021b), which is mostly consistent with evidence provided in the PFOS document (The tumor promotion study by Benninghoff et al., 2012 is not included in the PFOS document; this study demonstrated that PFOS acted as a tumor promoter in rainbow trout.). Similarly, the mechanistic considerations in the OEHHA summary (OEHHA, 2021b) appear to be consistent with evidence provided in the PFOS document. However, it appears as if the OEHHA conclusion to propose listing PFOS on the list of chemicals known to the state to cause cancer differs from the PFOS document. It is therefore recommended that authors reconsider the “suggestive evidence of carcinogenic potential” classification for PFOS to the “likely to be carcinogenic to humans” classification.

OEHHA, 2021a. Notice to interested parties chemicals listed effective December 24, 2021 as known to the State of California to cause cancer: Perfluorooctane sulfonic acid (PFOS) and its salts and transformation and degradation precursors.

<https://oehha.ca.gov/proposition-65/crnrr/notice-interested-parties-chemicals-listed-effective-december-24-2021-known>. Accessed December 2021.

OEHHA, 2021b. Evidence of the carcinogenicity of perfluorooctane sulfonic acid (PFOS) and its salts and transformation and degradation precursors.

<https://oehha.ca.gov/media/downloads/crnrr/pfoshid092421.pdf>. d

2. Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends. Does SAB support the selection of this CSF in the derivation of a risk specific dose for PFOA (i.e., risk)? If not, please provide input on the strengths and weaknesses of the other candidate CSFs that the EPA derived.

Response:

No response provided.

5. **Human Toxicokinetic Model**

- A. For endpoints observed in adults, EPA used a steady-state approach to calculate the HED, which assumes a relatively constant exposure and clearance during adulthood. Please

comment on this method of HED calculation. Are there alternative approaches that EPA should consider? If so, please describe the rationale for recommending this approach(es).

Response:

No response provided.

- B. Two key parameters are the half-life and volume of distribution, which were used to calculate clearance. Half-life and volume of distribution were assumed to be constant across sex and age groups because of a lack of strong quantitative data to parametrize changes across sex and age. Please comment on the strengths and weakness of the use of this assumption and the choice of these parameters by the EPA. Please describe the rationale for alternative recommended approaches. For endpoints observed in human neonates or children, EPA used a one-compartment TK model to simulate dosimetry during pregnancy and a two-compartment TK model (one-compartment models for the mother and the child) to simulate dosimetry during lactation, to calculate the HED for each POD. Please comment on the strengths and weaknesses of this choice of model structure for the task of predicting dosimetry in the human fetus and child compared to dosimetry in mice and rats in the similar lifestages. Please provide the rationale for any alternative recommended approaches.

Response:

No response provided.

- C. The key chemical-specific parameters that describe the transfer of the chemical from the mother to the child during gestation and lactation are the maternal to fetal serum ratio and the ratio of maternal serum to milk PFOA/S concentration. These ratios were assumed to be constant during gestation and lactation, respectively. Another important parameter is the rate of milk ingestion, which is chemical-independent and varies throughout lactation. Please comment on the strengths and weaknesses of the choice of parameters for fetal to maternal partitioning and partitioning into breastmilk, as well as the choice for lactation rate. Please also comment on the choice to assume that fetal to maternal partitioning and partitioning to breastmilk did not vary in time. Please describe whether there are other methods you would recommend to account for these changes over time and across development.

Response:

No response provided.

6. Animal Toxicokinetic Model

- A. After a review of the available toxicokinetic models for PFOA/S predictions in laboratory animals, EPA selected the Wambaugh et al. (2013) model because it was parametrized using all species of interest, demonstrated good agreement with training and test datasets, and used a single, biologically motivated, model structure across all species. Does the panel agree with selecting this model? If not, please describe the rationale for alternative recommended approaches for the calculation of the internal dose metrics in adult animals.

Response:

The model by Wambaugh et al. (2013) appears to be an appropriate model for calculation of internal dose metrics in adult animals and the rationale for the choice of this model is clearly indicated in the documents.

- B. The animal model parameters were obtained through a Bayesian inference parameterization which produced wide credible intervals for some parameter values, but relatively tight credible intervals for the predicted serum concentration. Does the panel agree with using the median values of the estimated animal parameter distributions for prediction of serum concentration and internal dose metrics?

Response:

No response provided.

- C. Based on visual inspection of model predictions to the calibration datasets, EPA utilized sex-independent parameters for PFOS. The male-specific parameters were used for all rat-specific PFOS predictions including predictions in pregnant and nursing dams and the female-specific parameters were used for all mouse-specific PFOS predictions because the parameter values obtained from fitting the female-specific rat data and male-specific mouse data were not consistent with the overall TK parameters for PFOS and produced poor fits to the training and test datasets. Does the panel agree with this approach and justification for this assumption for PFOS? If not, please describe other approaches that could be considered?

Response:

No response provided.

- D. EPA assumed a one compartment model for the developing infant based on the lack of infant-specific toxicokinetic data from rats and mice. This model utilizes averages of half-life and volume of distribution from the literature coupled with physiologically relevant lactational parameters for pup nursing. Does the panel agree with the decision to use this model structure for infant animals? If not, please provide data on infant-specific changes during the animal lactational-period that could be used to account for toxicokinetic differences between the adult and infant rats and mice.

Response:

No response provided.

- E. Several parameters dictate the transfer of chemical from the mother to her pup. Does the panel agree with the selection of these parameters for the animal model? If not, please provide your justification and alternative parameters.

Response:

No response provided.

- F. For neonatal animals, EPA assumed no sex differences in clearance in neonatal animals based on the lack of identification of sex-dependent differences in PFOA/S toxicokinetics from the available data. Does the panel agree with this assumption? If not, please provide your justification and available data on sex differences in neonatal rats.

Response:

No response provided.

7. Epidemiological Study RfD Derivation

1. EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only ‘medium’ and ‘high’ quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?

Response:

No response provided.

2. Studies of developmental immune health outcomes (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]) after PFOA/S exposure identified associations with very low doses of either PFOA or PFOS with developmental immune effects. The RfD for this outcome was selected as the critical effect because it was the lowest among the candidate RfDs for PFOA or PFOS and can result in severe illness. Does the panel agree with the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS?

- i. If so, please explain your justification.

Response:

The choice of immunotoxicological effects of PFOA and PFOS as the critical effect for derivation of chronic RfDs is appropriate. First, the endpoint identified as the critical effect is a functional immune response. In other words, the measurement of antigen-specific antibodies following vaccinations is an overall measure of the ability of the immune system to respond to a challenge. The antigen-specific antibody response is considered to be extremely useful for evaluating the entire cycle of adaptive immunity and is a sweeping approach to detect immunosuppression across a range of cells and signals (Myers, 2018). This response also translatable across multiple species, including rodents and humans, and historical data associated with suppression of antigen-specific antibody responses by exogenous agents is extensive (Myers, 2018). Second, when immunosuppression occurs in the developing immune system, such as observed in the critical studies used to derive the chronic RfDs, the risks of developing infectious diseases and other immunosuppression-linked diseases increases (Dietert et al., 2010). Third, immunosuppression linked with chemical stressors is *not* the same as an immunodeficiency associated with, for example, genetic-based diseases. As pointed out Selgrade (2007), the lack of an obvious AIDS-like epidemic from exposure to chemical immunosuppressants does not mean that the human population is not at serious risk from exposure to immunosuppressants. Evidence from specific populations experiencing mild to moderate immunosuppression indicates that the risk of infections with pathogens commonly encountered in the general population is real (Selgrade, 2007). Finally, immunotoxicity that occurs in the developing organism generally occurs at doses lower than required to affect the adult immune system, thus providing a more

sensitive endpoint upon which protective measures, such as RfD, can be based (vonderEmbse and DeWitt, 2018). Therefore, the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS is appropriate and will be protective of the general population, and likely including sensitive subpopulations.

Dietert RR, DeWitt JC, Germolec DR, and Zelikoff JT. 2010. Breaking patterns of environmentally influenced disease for health risk reduction: Immune perspectives. *Environmental Health Perspectives* 118:1091-1099.

Myers LP. 2018. Clinical immunotoxicology. In: *Immunotoxicity Testing: Methods and Protocols* (DeWitt JC, Rockwell CE, and Bowman CC, eds), Methods in Molecular Biology Series. Springer Science + Business Media, LLC.

Selgrade MK. 2007. Immunotoxicity – The risk is real. *Toxicological Sciences*. 100:328-332.

vonderEmbse AN and DeWitt JC. 2018. Developmental immunotoxicity (DIT) testing: Current recommendations and the future of DIT testing. In: *Immunotoxicity Testing: Methods and Protocols* (DeWitt JC, Rockwell CE, and Bowman CC, eds), Methods in Molecular Biology Series. Springer Science + Business Media, LLC.

- ii. If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of chronic RfDs.

Response:

Not applicable.

- iii. Are any additional analyses or rationales needed to increase the confidence in the chronic RfDs for PFOA and PFOS?

Response:

Yes. The critical study, which is an epidemiological study, is supported by several studies in rodent models showing that exposure to PFOA or PFOS alone dose-responsively suppresses the antigen-specific antibody response. These studies are described in section 3.3.4.2.4 of the PFOA document and in section 3.3.4.2.7 of the PFOS document but the sections of RfD selection (4.1.6 for both documents). Confidence in the chronic RfDs for PFOA and PFOS could be increased if the supportive evidence from animal studies is at least mentioned in section 4.1.6 for both documents.

3. The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID:

3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.

Response:

Yes, the 5% BMR selection for modeling to identify the POD is supported. As explained in 7.b.i, the developing immune system (i.e., the immune system of children) is generally regarded as more sensitive to exogenous perturbations such as those from chemical stressors than the adult immune system. Therefore, changes observed in the developing immune system reflect developmental effects, which supports a 5% BMR. The immune response to childhood vaccines may be “an excellent indicator for developmental immunotoxicity when conducted under appropriate conditions” (Luster et al., 2005). Responses to childhood vaccines are thought to be sensitive enough to detect changes in populations with moderate degrees of immunosuppression, such as those exposed to an immunotoxic agent (Luster et al., 2005). However, the degree to which mild to moderate immunosuppression from exposure to chemical agents produces measurable clinical outcomes is challenging to determine (DeWitt et al., 2017). Evidence from specific populations experiencing mild to moderate immunosuppression indicates that the risk of infections with pathogens commonly encountered in the general population is real (Selgrade, 2007). Reductions in antibody titers to a specific vaccine below a level that is considered protective does increase *the risk* of susceptibility to the disease against which the vaccine was intended. However, as “risk” can sometimes be challenging to quantify, a 5% BMR selection appears to be appropriately protective.

DeWitt JC, Germolec DR, Luebke RW, and Johnson VJ. 2017. Associating changes in the immune system with clinical diseases for interpretation in risk assessment. *Current Protocols in Toxicology*. 67:18.1.1-18.1.22.

Luster MI, Johnson VJ, Yucesoy B, and Simeonova PP. 2005. Biomarkers to assess potential developmental immunotoxicity in children. *Toxicology and Applied Pharmacology*. 206:229-236.

Selgrade MK. 2007. Immunotoxicity – The risk is real. *Toxicological Sciences*. 100:328-332.

4. EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations

(UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for PFOA and PFOS.

- i. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

Response:

It appears as all recommended sources of uncertainty have been adequately accounted for in the derivation of the RfDs.

- ii. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

Response:

Only one UF has a value greater than one for both the PFOA and PFOS RfDs and it is the UF_H, which reflects the intraspecies UF and has a value of 10 for both chemicals. This UF is used to account for variability in the responses within the human population due to various factors. Such variability includes genetics, life stage, health status, lifestyle, and other factors that may produce variability in how specific human subpopulations may respond to the chemical(s) under evaluation. The rationale for a value of 10 for the UF_H is clearly described in the documents. The other UFs are all equal to one and the rationale for this value is clearly described in the documents.

8. Relative Source Contribution

- a. EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources. Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at: <https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS.

- i. Are you aware of additional relevant exposure data that EPA should consider in developing the RSCs for PFOA and PFOS? If so, please provide citations.

Response:

It appears as if the relevant exposure data has been considered in developing the RSCs for PFOA and PFOS.

- ii. Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS is adequately supported and clearly described.

Response:

It appears as if the recommended RSC of 20% for PFOA and PFOS is adequately supported and clearly described. However, there may be instances where a more conservative approach is warranted to protect those in highly exposed populations

such as those in occupational settings or in communities with heightened drinking water exposures. While those with community-level exposures via drinking water experience a higher burden of their exposure to PFOA and PFOS through their drinking water, the potential for additional pathways of exposure also exists in these high exposure communities. For example, contaminated biosolids from water treatment facilities may be applied to lands in and around those communities with heightened drinking water exposures, potentially increasing exposures via food grown in the presence of PFAS. Similarly, such communities may also be at increased risk of exposure to PFAS in ambient air and indoor dust, in recreational waters, and through food obtained from the aquatic environment, especially if a known source of PFAS water contamination exists. It therefore may be more protective, especially for high exposure communities, to consider a more conservative RSC.

Comments for Mixtures document

REVISED COMMENTS

Topic 3 Cancer, Charge question #2

The details for how serum concentrations are used in dose-response (the modeling) are not shown. This is an important deficiency for these analyses because the external dose is not used. It appears that all MOA results across PFOA and PFOS are deferred until after the SAB meetings per multiple statements in the documents. It would be helpful to include the final MOA for kidneys if this endpoint is used by EPA. To use a human-based slope factor the confidence in the assessment should be high, not medium confidence. I am in favor of using the animal cancer bioassay data instead of the human epidemiology data. The doses and the responses are known in a controlled setting.

3. Cancer b.

The details for how the use serum concentrations are used in dose-response (the modeling) are not shown. This is an important deficiency for these analyses because estimated external dose is not used. It appears that all MOA results across PFOA and PFOS are deferred until after the SAB meetings per multiple statements in the documents. It would be helpful to include the final MOA for kidney if this endpoint is used by EPA. To use a human based slope factor the confidence in risk estimates should be high, not medium confidence. I am in favor of using the animal cancer bioassay data instead of the human epidemiology data.

Charge Question #3

Section 4.4 (Relative Potency Factor; RPF) FISHER

- A. The mixtures assessment methodology (RPFs) appears to be adequate if there is enough target organ information available to lump chemicals based on a common effect (or effects) and methods can be created to adjust for length of exposure if using animal data. Continued future efforts to calculate a HED is recommended. The strength of the animal to human extrapolation for target organ toxicity would be improved if the toxicity observed in animals was also observed in humans (exposed to the same molecule).
- B. The examples for the PFAS mixtures were referred to as illustrative examples. The methodology does have value for PFAS mixtures if there is enough information to lump the chemicals for common apical endpoints. If the shape of the dose-response curves were similar this approach would have value. An a priori test for dose-response adequacy for RPF inclusion would be helpful for screening large data sets.

Topic 4 -Toxicokinetic Models

Charge question #1-Human Models. This is where I placed suggestions beyond the scope of individual charge questions.

Documenting the models and their applications in humans and animals to increase transparency: I found a lack of information when trying to piece together how the models were used. Much more information is needed beyond citations and tables of model parameters. As an example, the BMD calculations in the Appendix apparently use model generated blood concentrations for dose-response analyses. For every human or animal simulation there should be information stating which model parameter values were changed to simulate the specific study. Place code in the document so someone can reproduce the work.

I suggest a workflow schematic (big picture) for the use of the models, how they fit in. Model performance for every model (including life stages) should be shown, if there is no data, state this for a particular model life stage. State what are acceptable performance metrics. This sets the stage for application. When a model is used in dose-response analyses provide details and assumptions, so someone can reproduce the simulations.

- A. The PFOA and PFOS compartmental models for adults (not pregnant or lactating females) are adequate for use in HED determinations. This method is a first approximation, highly empirical, and limited when looking to the future and using this model to ask questions about mixtures. The assumptions of steady state are reasonable, given the long half-life of each molecule. To better visualize the data and evaluate performance, I strongly recommend showing plots of model predicted plasma concentrations versus measured, with a line showing unity with upper and lower bounds of 2 and 0.5. This graph would provide an immediate portrayal of how the model did. Bias can be viewed if the distribution about unity is skewed. The portion of samples outside the bounds gives some indication of the acceptability of the model.
- B. Strength of using a simple, fixed two- compartment model: It provides a data driven first approximation of the pharmacokinetics. I am sure it avoids complexities and is less time consuming. I have never simplified life stage modeling to this degree. This methodology can answer general questions, with probable error, about POD dosimetry (using steady state assumptions). The use of fixed compartmental variables to describe pregnancy and lactation is incorrect, technically, but the question, is it sufficient? Can a simple linear approximation of fetal and lactational transfer be assumed? For me, a major weakness of the methodology is that it is probably a dead-end approach, that is, the methodology works for a few molecules. This methodology does not allow for the future use of mechanistic information and scaling methods, addressing mixtures, or PFAS with different pharmacokinetic (PK) behaviors and limited or no PK data. The PK behavior of PFOS and PFOA should be straight forward because these chemicals are cleared by urinary and fecal clearance. There is no metabolism to deal with. But the mechanisms governing the PK behavior are not simple. Protein binding and active transport of PFOS and PFOA into and out tissues by protein transporters occur, to such a degree that the pharmacokinetics is altered. What this looks like in a growing fetus and neonate/infant is not well understood based on PFOA or PFOS longitudinal data. The Verner et al. 2016

human pregnancy/lactation compartmental model for PFOA and PFAS provides simulations for two lactation studies. My view is that the nursing child serum levels were skewed to overpredict PFOA at 3 months and underpredict at 3 years. I view this as a red flag signaling something is going on that is not described in the model. The use of Monte Carlo captures the variability adequately. It is amazing that this simple of an approach works as well as it does.

Recommendation for the future: There is a wealth of information now for pediatric drugs and PBPK model that provides a foundational approach for addressing life-stage PBPK modeling in humans. There are literally hundreds of publications available in the literature. The strategy is to construct a modeling framework that can be used for many drugs, not one or two. This includes providing PK predictions when there are no data, such as first in human studies. I strongly recommend that EPA consider evaluating these principles for modeling life stages. The methodologies involve physical chemistry, QSAR, allometry, and empirical data on processes that govern clearance of drugs, mainly urinary or fecal excretion and metabolism. Unbound fraction is a huge factor throughout the life stages (and what physiological processes control the unbound fraction). I believe this methodology provides useful tools, based on physiology, for extrapolations across age groups in humans. Now pregnant and lactating women are not automatically excluded from clinical trials, so efforts are ongoing in the development of PBPK models for pregnancy, including fetus and for the lactating women, including the nursing infant. The value in exploring this modeling space is that human data are collected in some cases, which is usually not the case for chemicals. I am unsure of the databases available for laboratory animals to implement this comprehensive approach.

Below I give a brief example of some of the principles. This information can be entered into software or calculated with software tools. I am not recommending this course of action now for PFOA and PFOS.

To use PFOA as an example, its pK_a and the pH of plasma (and other tissue groups) would suggest that a small portion of the circulating PFPA is unionized, and the rest is ionized, bound and unbound to serum proteins. The unionized fraction is available for passive transport across membranes and the ionized fraction, by protein transporters. Urinary clearance (CL_u) would be described with GFR (passive), secretion, and reabsorption. With PFOA if CL_u in mice, rat, or human was less than GFR, this would signal further evaluation and consider, reabsorption by kidney protein transporters. The ontogeny of protein transporters (e.g., kidney, liver) is an active field of research and can be used for extrapolation or interpolation based on protein abundance or activity to provide simulations without the benefit of data. Ontogenies of important serum proteins are known and would be useful for describing PFOA binding. This approach is very different from the current methods used by EPA for PFOA and PFOS to address life stages. Ultimately this methodology provides a biologically based anchoring of key parameters that can be used for understanding the pharmacokinetics of chemicals across life stages and reproductive states. Of course, there can still be gaps in data or knowledge. I believe if time is invested in exploring the modeling work completed over the last 11 years for pediatric drugs, and over the last 3-4 years for pregnancy and lactation, many ideas will emerge.

Examples of References.

Mahmood 1999, “Prediction of Clearance, Volume of Distribution and Half-life by Allometric Scaling and by use of Plasma Concentrations Predicted from Pharmacokinetic Constants.

Johnson and Ke 2021, Physiologically Based Pharmacokinetic Modeling and Allometric Scaling in Pediatric Drug Development: Where Do We Draw the Line?

Huh et al. 2011, Interspecies scaling and prediction of human clearance: comparison of small- and macro-molecule drugs

Paine et al. 2011, Prediction of Human Renal Clearance from Preclinical Species for a Diverse Set of Drugs That Exhibit Both Active Secretion and Net Reabsorption

Van Groen et al. 2021, Ontogeny of Hepatic Transporters and Drug Metabolizing Enzymes in Humans and in Nonclinical Species.

Cristea et al. 2020, The Influence of Drug Properties and Ontogeny of Transporters on Pediatric Renal Clearance through Glomerular Filtration and Active Secretion: A Simulation-Based Study.

- C. The movement of PFOA or PFOS from blood, across the mammary tissue into milk and the movement back into the blood supply (not considered), is not at a steady state condition each time nursing occurs. The compartment empties and is replenished with fresh milk. For long lived chemicals, the influence of this dynamic compartment on steady-state assumptions is assumed to be minimal when evaluated over days/months. The ratio (milk/plasma) would vary to some degree with milk composition. Ingestion rates of breastmilk and composition of breastmilk as a function of postnatal age are known. The diffusion or active transport of PFOA/PFOS from milk to the mother blood should be considered. The assumptions of a fixed ratio for the fetus and breast milk are not correct, but are probably adequate for the long-lived chemicals, PFOA and PFOS. There are several publications for estimating the milk/plasma ratio for drugs, including acidic drugs. I doubt if these approaches would improve on the estimates.

PRELIMINARY COMMENTS

Charge question #2.

- A. The use of HI for risk assessment purposes must be carefully articulated, if used. In the charge question, “Is HI a reasonable methodology for indicating potential risks for mixtures of PFAS?” is probably an overstatement in my view. Summing HQ values for individual PFAS for a common target organ, using HI methodology, probably has validity, but not summing a mixture of chemicals with divergent or unknown MOA or target organs. I do I understand the historical use of HI at Superfund sites. I would call the latter situation, a screening method based on individual toxicity information to assist in decision making, which could include advancing to tier two risk assessment methodology. Per sec, it is not a

risk assessment methodology, but more of a decision support tool. The health risks for a mixture are not predicted based on this analysis methodology. As an aside, if mixture assessments are needed with several analytes there are examples of component-based risk methods to compare to the PFAS mixture issue. Complex mixtures of fuel: total organic hydrocarbon verses individual marker hydrocarbons and PBPK modeling approaches for handling gasoline and jet fuels. The range of analytes covers branched and straight chain carbon lengths of C3 or 4 to over C20 plus aromatics.

B. A rationale for binning PFAS based on a common target organ or MOA would work for the HI approach. This is a very high bar (apparently). If agreement could be reached on nuclear receptor(s) activation as a primary early event, this could be one approach for binning PFAS. NAMs methods for toxicity evaluation and for MOA/Pathway analyses could help advance the HI methodology because it can potentially include well designed mixture studies, which are limited when designing animal studies. Perhaps the NAM results can guide the design of a limited number of animal studies. A HI approach for a PFAS without binning criteria to lump them together, offers little to improve health risk methods, but it does provide information for decision-making in the 'real world'.

Charge question #3.

C. The mixtures assessment methodology (RPFs) appears to be adequate if there is enough target organ information available to lump chemicals based on a common effect (or effects) and methods can be created to adjust for length of exposure if using animal data. Adjustment of the dose (and route of exposure) for an animal study is recommended to calculate a human equivalent dose, assuming that there are adequate pharmacokinetic tools available to do this adjustment. The strength of the animal to human extrapolation for target organ toxicity would be improved if the toxicity observed in animals was also observed in humans (exposed to the same molecule).

D. The examples for the PFAS mixtures were referred to as illustrative examples. The methodology does have value for PFAS mixtures if there is enough information to lump the chemicals for common apical endpoints. If the shape of the dose-response curves were similar this approach would have value. An a priori test for dose-response adequacy for RPF inclusion would be helpful for screening large data sets.

Preliminary Comments for PFOA and PFOS documents

Cancer- charge question #2. I am waiting to see how the charge question is rewritten.

Toxicokinetic Models

I had the ask the question, "Is this modeling approach adequate, not is this the best modeling approach". I struggled because I have never used compartmental models in this way for life

stages and reproductive states. It would be very helpful to provide model performance evaluations for human and animal models, per comments below.

1. Human

A. The PFOA and PFOS compartmental models for adults are ok. This method is a first approximation, highly empirical, and limited when looking to the future and using this model to ask questions about mixtures. The assumptions of steady state are reasonable, given the long half-life of each molecule. To better visualize the data and evaluate performance, I strongly recommend showing plots of model predicted plasma concentrations versus measured, with a line showing unity with upper and lower bounds of 2 and 0.5. This graph would provide an immediate portrayal of how the model did. Bias can be viewed if the distribution about unity is skewed. The portion of samples outside the bounds gives some indication of the acceptability of the model.

B. Strength of using a simple, fixed two- compartment model: It provides a data driven first approximation of the pharmacokinetics. I am sure it avoids complexities and is less time consuming. I have never simplified life stage modeling to this degree. This methodology can answer general questions, with probable error, about POD dosimetry (using steady state assumptions). The use of fixed compartmental variables to describe pregnancy and lactation is incorrect, technically, but the question, is it sufficient? Can a simple linear approximation of fetal and lactational transfer be assumed? For me, a major weakness of the methodology is that it is probably a dead-end approach, that is, the methodology works for a few molecules. This methodology does not allow for the future use of mechanistic information and scaling methods, addressing mixtures, or PFAS with different pharmacokinetic (PK) behaviors and limited or no PK data. The PK behavior of PFOS and PFOA should be straight forward because these chemicals are cleared by urinary and fecal clearance. There is no metabolism to deal with. But the mechanisms governing the PK behavior are not simple. Protein binding and active transport of PFOS and PFOA into and out tissues by protein transporters occur, to such a degree that the pharmacokinetics is altered.

The Verner et al. 2016 human pregnancy/lactation compartmental model for PFOA and PFAS provides simulations for two lactation studies. My view is that the nursing child serum levels were skewed to overpredict PFOA at 3 months and underpredict at 3 years. I view this as a red flag signaling something is going on that is not described in the model. The use of Monte Carlo captures the variability adequately. It is amazing that this simple of an approach works as well as it does.

Alternative recommendation: This recommendation is for future consideration. There is a wealth of information now with pediatric drugs that provides a foundational approach for addressing life-stage PBPK modeling. Now pregnancy and lactation PBPK models are slowly being addressed with some of the same approaches. Hundreds of publications are available in the literature. A strategy is to construct a modeling framework that can be used for many drugs, not one or two. This includes providing PK predictions when there are no data, such as first in human studies. I strongly recommend that EPA consider using principles developed by 100s of people

involved in models for pediatrics, drug-drug interactions, and pregnancy and lactation modeling. Below I give a brief example of some of the principles that would be applied. I am not recommending this course of action now for PFOA and PFOS. To use PFOA as an example, its pK_a and the pH of plasma (and other tissue groups) would suggest that a small portion of the circulating PFPA is unionized, and the rest is ionized. The unionized fraction is available for passive transport across membranes and the ionized fraction, by protein transporters. Urinary clearance (CL_u) would be described with GFR (passive), secretion, and reabsorption. With PFOA if CL_u in mice, rat, or human was less than GFR, this would signal further evaluation and consider, reabsorption by kidney protein transporters. The ontogeny of protein transporters is an active field of research and can be used for extrapolation or interpolation based on protein abundance or activity. Ontogenies of important serum proteins are known, and this would be useful for describing PFOA binding. These are example of two important biological parameters. This approach is very different from the current methods used by EPA for PFOA and PFOS. Ultimately this methodology provides a biologically based anchoring of key parameters that can be used for chemicals across life stages and reproductive states. Of course, there can still be gaps in data or knowledge.

C. The movement of PFOA or PFOS from blood, across the mammary tissue into milk and the movement back into the blood supply (not considered), is not at a steady state condition each time nursing occurs. The compartment empties and is replenished with fresh milk. For long lived chemicals, the influence of this dynamic compartment on steady-state assumptions is assumed to be minimal when evaluated over days/months. The ratio (milk/plasma) would vary to some degree with milk composition. Ingestion rates of breastmilk and composition of breastmilk as a function of postnatal age are known. The diffusion or active transport of PFOA/PFOS from milk to the mother should be considered. The assumptions of a fixed ratio for the fetus and breast milk are not correct, but are probably adequate for the long-lived chemicals, PFOA and PFOS.

Animal Model

A. For PFOA and PFOS the Wambaugh et al model is ok. I do not find this model biologically motivated as suggested in the text.

B. It seems foolish to me to retain model parameter values that are outside the range of biological plausibility by orders of magnitude. While it may have little influence on a median parameter value, just placing such a value in a table detracts from the general acceptance of this method.

C. Calibration of the model, versus use of one sex over another, would be preferred. Even with doing this, the model predictions do not seem adequate. There is disagreement between model prediction and observation. Please plot model predicted plasma concentrations versus the observed or measured plasma concentrations with a unity line and lines representing 0.5 and 2x around the unity line, per a comment earlier. This provides an easy way to visualize the data and judge performance.

D. The use of a single V_d and half-life seems extreme. A mechanistic approach would be to tie both V_d and half-life to the ontogeny of serum protein binding, and urinary and fecal clearance (transporters). These would be called covariates for compartmental probability-based models.

E. Lactational transfer involves movement into the milk and from milk back into the mother's blood supply. You assume the movement is unidirectional into milk. The unbound fraction of PFOS or PFOA is considered available for movement into milk (and out of the milk). Consider the use of the unbound fraction in the model.

F. A better modeling approach would need to include the mechanism that is involved in sex dependent P_k for adults and then understand the ontogeny of this mechanism during development. As it stands now you have little choice for addressing this issue except by extrapolation from adults to neonates. I am not sure how this would be done.

REVISED COMMENTS

CVD Risk Reduction Analysis

Charge question 1. Meta-analysis for total cholesterol dose-response

The range of results is very wide. The linear untransformed models yield by far the highest slopes, especially for PFOA. Is this credible? Perhaps meta-analytic models should be estimated using other functional forms, at least as sensitivity analysis.

Charge question 2. Life table approach methodology

Overall, the methodology is appropriate and is reasonably well described. I do have some questions about the description, identified by the document (main analysis or Appendix B) and page number.

Analysis of CVD risk reduction

p. 11. EPA assumed drinking water accounts for 20% of total daily PFOA/PFOS dose under the baseline scenario, and that the other 80% is independent of drinking water PFOA/PFOS concentration. It tested the effect of alternatively assuming that drinking water accounts for 80% of the total and found “the assumption about drinking water source contribution does not affect the estimated changes in serum PFOA/PFOS.” It seems to me that the effect of a change in drinking-water PFOA/PFOS concentration on serum PFOA/PFOS should be four times as large in the alternative case. Please explain.

Appendix B

p. 28. How is integer age (a) defined? There are multiple statements like “at the beginning of integer age a and calendar year t .” Is integer age the age an individual reaches in the calendar year? Obviously, age and year do not change on the same date, unless everyone is born new year’s day.

The definition of $l_{b,a,s,r,t,p}$ should presumably say “we assume that people who have just been born do NOT have CVD history by definition” (the word “not” is missing).

p. 41. Table B-8 Post-Acute All-Cause Mortality After the First Myocardial Infarction shows all-cause death risk is lower for men than women who are non-

Hispanic Blacks aged 45-64, but higher for men than women in all other age/race groups. Is this correct?

p. 46. In “The uncalibrated number of persons experiencing their first hard CVD event in year t , $i_{b,a,s,r,t}$ ” I believe the $i_{b,a,s,r,t}$ should be $\tilde{n}_{b,a,s,r,t}$.

“EPA applies a constant baseline annual probability of first hard CVD event estimated at age 80 to those currently aged 81–89 years.” What is known about baseline probability for ages 81-89? If the probability rises with age, this assumption will lead to an underestimate.

p. 47. I do not understand the first term in brackets in eqn. (B-12), $[1 + \sum_{f \in F} (\mu_{a,s,r,f,0} - 1) \cdot \gamma_{a,s,r,f}]$. I think the term should be $\sum_{f \in F} (\mu_{a,s,r,f,0} \cdot \gamma_{a,s,r,f})$, i.e., the sum over types of nonfatal CVD events of the product of the mortality rate and share by type. It would be useful to explain the logic of the term as written. The same question applies to eqn. (B-23) on p. 48.

p. 48. How is the term $\rho_{b,a,s,r}$ calculated? Is it a rate of post-acute CVD mortality?

p. 49. I think there is a sign error in equations B-34 and B-36. As written, avoided deaths $\Delta m_{b,a,s,r,t}$ and ΔM_t are both less than zero (when treatment is associated with lower incidence of first CVD events, as stated).

Charge question 3.i. Scientific validity of the ASCVD model application for estimating the probability of first time CVD events

The ASCVD model appears to be a scientifically valid approach to estimating the probability of first CVD events. I note that the developers (Goff et al.) describe the model as intended for use by practicing physicians and that it is “based on the types of data that primary care providers could easily collect.” In principle, EPA need not limit its modeling to such data. I do not know if there are more accurate and reliable models that use other data that EPA could substitute.

A limitation of the ASCVD model is that it is estimated only for non-Hispanic white and Black populations. Goff et al. state the “sex-specific Pooled Cohort Equations for non-Hispanic whites may be considered for estimation of risk in patients from populations other than African Americans and non-Hispanic whites.” This is clearly a weak recommendation. EPA compared the use of the coefficients for non-Hispanic whites and for Blacks and determined the latter provided a better fit to population data for non-Hispanic Black and non-Hispanic other populations, so EPA appropriately uses the non-Hispanic Black coefficients for these populations.

The accuracy of the ASCVD model does not seem very good, even for the populations for which it is estimated (especially for females). Table B-12: Summary of ASCVD Model Validation reports the population-weighted average (over sex and race/ethnicity) of the absolute deviation of (actual - model)/model, where actual and model represent the actual and modeled numbers of CVD events. For females, the

value of this statistic is 2.00 for non-Hispanic whites and 1.37 for non-Hispanic Blacks.

Taking the 2.00 figure for whites implies suggests $\text{actual} = 3 \cdot \text{model}$ (for an age/race/sex category). The reported accuracy for men is much better, and interestingly the accuracy for the groups for which the model was not estimated are also generally better. The accuracy of the model predictions deserves more discussion.

Charge question 3.ii. Assumption of a uniform first CVD event hazard distribution over the 10-year period

The ASCVD model predicts the probability of a first CVD event in the following 10 years. EPA estimates the annual risk as the constant yielding the same 10-year risk (i.e., $(1 - x)^{10} = (1 - y)$ where x is the annual risk and y is the 10-year risk). This approach seems adequate. It might be possible to improve on it by recognizing that the risk at age a can be estimated by calculating the 10-year risks at ages $a - 9$, $a - 8$, $a - 7$, etc. and averaging these. This alternative is not recommended because it seems unlikely to affect the model results by much and does not seem to justify the complication.

Charge question 3.iii. Validity of using the ASCVD model to estimate reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA/PFOS in drinking water

The ASCVD model is calibrated to data from epidemiological studies that establish a relationship between total cholesterol and CVD risk. Such studies do not by themselves provide evidence that a change in total cholesterol will change CVD risk, nor do they provide information about whether the effect of a change in total cholesterol on CVD risk depends on the source of the change. There are presumably intervention studies that provide evidence about the causal effect of a change in total cholesterol on CVD risk that could be reviewed. However, such studies are unlikely to provide evidence about the causal effect of a change in total cholesterol due to reducing exposure to PFOA/PFOS in drinking water. It appears the validity of using the model in this context can be assessed only by scientific judgment about the plausibility that a change in total cholesterol due to a change in drinking water exposure has the same effect as a change due to sources that have been evaluated by intervention studies.

Charge question 4. Uncertainty analysis

Table E-1 reports that uncertainty about the slope of the relationships between TC and either PFOA or PFAS is represented by the confidence intervals from the meta-regressions. It is not clear if these confidence intervals are based on the variance of the error term for individual observations or include some contribution for between-model variation (e.g., model-specific random error τ^2). Moreover, uncertainty about the

functional form of the relationship seems likely to be important. The meta-regressions use only linear models, but the central estimates from using all studies or only linear-log models for TC both fall below the lower-end of the confidence interval for the linear models for PFOA (Table A-2), though not for PFAS (Table A-3).

Derivation of MCLG

Charge question 1. Study identification & inclusion

Section 2.6 (Evidence synthesis) is vague. Who synthesized the evidence? Did multiple individuals/teamssynthesize evidence independently then compare and resolve any disagreements? How were conflictingresults of different studies accommodated? Were syntheses independently reviewed by people knowledgeable about the studies?

Charge question 4. Cancer

The proposed CSF is 0.015 or 0.035 (ng/kg-day)⁻¹ using respectively the central tendency or upper 95% CI from the Shearer et al. renal cancer epidemiological study. If used to create a risk-specific dose for PFOA that would produce an incremental cancer risk of 10⁻⁶, the dose would be 1e-6 / [0.035 (ng/kg-day)⁻¹] = 3e-5 ng/kg-day = 3e-11 mg/kg-day using the upper 95% CI (and 7e-11 mg/kg-day using the central tendency). These values are 50 (and 20) times smaller than the RfD for PFOA based on decreased serum anti-tetanus antibodies in children (equal to 1.5e-9 mg/kg-day). This suggests the RfD should be decreased accordingly.

To evaluate the decrease in lifetime cancer risk associated with water treatment, consider the hypothetical decrease in PFOA drinking water concentration in the CVD risk reduction analysis. Assumed baseline concentration is 0.1 µg/L which is decreased to 0.028 µg/L, a reduction of 0.072 µg/L. Drinking water intake is 0.013 L/kg-day. The decrease in lifetime cancer risk is calculated as:

$$\begin{aligned}\text{cancer risk reduction} &= (\text{decrease in concentration}) \cdot (\text{drinking water consumption}) \cdot \text{CSF} \\ &= 0.072 \text{ } \mu\text{g/L} \cdot 0.013 \text{ L/kg-day} \cdot 0.035 \text{ (ng/kg-day)}^{-1} \cdot\end{aligned}$$

1e3 ng/µg ≈ 0.03. (using the upper 95% CI for the CSF).

Is this analysis correct? It does not seem to provide a plausible estimate of the effect of decreasing PFOA concentration in drinking water, since the calculated decrease exceeds the lifetime risk of (diagnosed) kidney and renal pelvis cancer of 0.017 suggested by SEER (<https://seer.cancer.gov/statfacts/html/kidrp.html>).

The CSFs derived from animal data are roughly 10 (mg/kg-day)⁻¹, a factor of 10⁹ smaller than the CSF from Shearer et al. This huge difference requires discussion and a strong rationale for using the slope from Shearer et al.

Charge question 7. Epidemiological study RfD derivation

The proposed RfD is based on studies of decreased serum antibody concentrations that are of medium quality. For PFOA, the candidate RfDs developed from high-quality studies of decreased birthweight are 3, 60, 70, 70, and 300 times larger (ranked by magnitude). For PFAS, the difference is smaller; the candidate RfDs for decreased birthweight are 20, 20, 20, and 100 time larger. The choice between a smaller RfD from lower-quality studies (all from the same research group and study population) and a larger RfD from higher-quality studies (from multiple groups and populations) requires a strong rationale and perhaps discussion of how much difference it makes to the result.

For PFOS, it might be worth mentioning that the supporting evidence from Timmerman et al. (2020) comes from Guinea-Bissau (I believe); this suggests the antibody effect is not unique to the Faroe Island population.

Mixtures

Charge questions 3. RPF and 4. Mixture BMD

The RPF and mixture BMD approaches appear to be very similar or even equivalent; differences between them should not be exaggerated. As I understand them, both approaches produce a summary measure of the toxicity of a mixture, $ICEC_{MIX}$ for the RPF approach and t_{add} for the mixture BMD approach. Both summaries are weighted sums of the component concentrations, with weights proportional to some measure of toxicity (e.g., inverse of BMD or of ED10).

Combining eqns. (4.2) and (4.3) implies

$$ICEC_{MIX} = \sum_j d_j \frac{ED10_{IC}}{ED10_j} = ED10_{IC} \sum_j \frac{d_j}{ED10_j}. \quad (1)$$

Taking reciprocals in eqn. (4.5) yields

$$\frac{1}{t_{add}} = \sum_i \frac{a_i}{BMD_i}. \quad (2)$$

Comparing these results shows that $ICEC_{MIX}$ and $(t_{add})^{-1}$ differ only in inessential details and are essentially proportional to one another. First, note that the RPF approach can use any common toxicity metric, e.g., one can replace ED10 with BMD in eqn. (1). Second, d_i (the “component chemical’s concentration”) and a_i (“the fixed proportions of the component PFAS in the mixture”) are either identical or strictly proportional to each other. Third, $ICEC_{MIX}$ includes a constant proportionality factor ($ED10_{IC}$).

Having constructed $ICEC_{MIX}$ and t_{add} , one can presumably use them to evaluate a risk in analogous ways. The summary t_{add} can be used as a BMD, from which one can calculate a hazard index or use it as a POD from which to extrapolate a dose-response function; similarly, one can divide $ICEC_{MIX}$ by ED10IC to calculate a hazard index or use it in a dose-response function for the index chemical as in eqn. (4.4).

If this analysis is roughly correct, it would be advisable to revise the discussion of these two approaches to present them as essentially the same (or highlighting any essential differences).

PRELIMINARY COMMENTS

CVD Risk Reduction Analysis

Charge question 1. Meta-analysis for total cholesterol dose-response

The range of results is very wide. The linear untransformed models yields by far the highest slopes, especially for PFOA. Is this credible?

Charge question 2. Life table approach methodology

Overall, the methodology is appropriate and is reasonably well described. I do have some questions about the description, identified by the document (main analysis or Appendix B) and page number.

Analysis of CVD risk reduction

p. 11. EPA assumed drinking water accounts for 20% of total daily PFOA/PFOS dose under the baseline scenario, and that the other 80% is independent of drinking water PFOA/PFOS concentration. It tested the effect of alternatively assuming that drinking water accounts for 80% of the total and found “the assumption about drinking water source contribution does not affect the estimated changes in serum PFOA/PFOS.” It seems to me that the effect of a change in drinking-water PFOA/PFOS concentration on serum PFOA/PFOS should be four times as large in the alternative case. Please explain.

Appendix B

p. 28. How is integer age (a) defined? There are multiple statements like “at the beginning of integer age a and calendar year t .” Is integer age the age an individual reaches in the calendar year? Obviously age and year do not change on the same date, unless everyone is born new year’s day.

The definition of $l_{b,a,s,r,t,p}$ should presumably say “we assume that people who have just been born do NOT have CVD history by definition” (the word “not” is missing).

p. 41. Table B-8 Post-Acute All-Cause Mortality After the First Myocardial Infarction shows all-cause death risk is lower for men than women who are non-Hispanic Blacks aged 45-64, but higher for men than women in all other age/race groups. Is this correct?

p. 46. In “The uncalibrated number of persons experiencing their first hard CVD event in year t , $i_{b,a,s,r,t}$ ” I believe the $i_{b,a,s,r,t}$ should be $\tilde{n}_{b,a,s,r,t}$.

“EPA applies a constant baseline annual probability of first hard CVD event estimated at age 80 to those currently aged 81–89 years.” What is known about baseline probability for ages 81–89? If the probability rises with age, this assumption will lead to an underestimate.

p. 47. I do not understand the first term in brackets in eqn. (B-12), $[1 + \sum_{f \in F} (\mu_{a,s,r,f,0} - 1) \cdot \gamma_{a,s,r,f}]$. I think the term should be $\sum_{f \in F} (\mu_{a,s,r,f,0} \cdot \gamma_{a,s,r,f})$, i.e., the sum over types of nonfatal CVD events of the product of the mortality rate and share by type. It would be useful to explain the logic of the term as written. The same question applies to eqn. (B-23) on p. 48.

p. 48. How is the term $rb_{a,s,r}$ calculated? Is it a rate of post-acute CVD mortality?

p. 49. I think there is a sign error in equations B-34 and B-36. As written, avoided deaths $Dm_{b,a,s,r,t}$ and DM_t are both less than zero (when treatment is associated with lower incidence of first CVD events, as stated).

Charge question 3.i. Scientific validity of the ASCVD model application for estimating the probability of first time CVD events

The ASCVD model appears to be a scientifically valid approach to estimating the probability of first CVD events. I note that the developers (Goff et al.) describe the model as intended for use by practicing physicians and that it is “based on the types of data that primary care providers could easily collect.” In principle, EPA need not limit its modeling to such data. I do not know if there are more accurate and reliable models that use other data that EPA could substitute.

A limitation of the ASCVD model is that it is estimated only for non-Hispanic white and Black populations. Goff et al. state the “sex-specific Pooled Cohort Equations for non-Hispanic whites may be considered for estimation of risk in patients from populations other than African Americans and non-Hispanic whites.” This is clearly a weak recommendation. EPA compared the use of the coefficients for non-Hispanic whites and for Blacks and determined the latter provided a better fit to population data for non-Hispanic Black and non-Hispanic other populations, so EPA appropriately uses the non-Hispanic Black coefficients for these populations.

The accuracy of the ASCVD model does not seem very good, even for the populations for which it is estimated (especially for females). Table B-12: Summary of ASCVD Model Validation reports the population-weighted average (over sex and race/ethnicity) of the absolute deviation of (actual - model)/model, where actual and model represent the actual and modeled numbers of CVD events. For females, the value of this statistic is 2.00 for non-Hispanic whites and 1.37 for non-Hispanic Blacks. Taking the 2.00 figure for whites implies suggests actual = 3 · model (for an age/race/sex category). The reported accuracy for men is much better, and interestingly the accuracy for the groups for which the model was not estimated are also generally better. The accuracy of the model predictions deserves more discussion.

Charge question 3.ii. Assumption of a uniform first CVD event hazard distribution over the 10-year period

The ASCVD model predicts the probability of a first CVD event in the following 10 years. EPA estimates the annual risk as the constant yielding the same 10-year risk (i.e., $(1 - x)^{10} = (1 - y)$ where x is the annual risk and y is the 10-year risk). This approach seems adequate. It might be possible to improve on it by recognizing that the risk at age a can be estimated by calculating the 10-year risks at ages $a - 9$, $a - 8$, $a - 7$, etc. and averaging these. This alternative is not recommended because it seems unlikely to affect the model results by much and does not seem to justify the complication.

Charge question 3.iii. Validity of using the ASCVD model to estimate reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA/PFOS in drinking water

The ASCVD model is calibrated to data from epidemiological studies that establish a relationship between total cholesterol and CVD risk. Such studies do not by themselves provide evidence that a change in total cholesterol will change CVD risk, nor do they provide information about whether the effect of a change in total cholesterol on CVD risk depends on the source of the change. There are presumably intervention studies that provide evidence about the causal effect of a change in total cholesterol on CVD risk that could be reviewed. However, such studies are unlikely to provide evidence about the causal effect of a change in total cholesterol due to reducing exposure to PFOA/PFOS in drinking water. It appears the validity of using the model in this context can be assessed only by scientific judgment about the plausibility that a change in total cholesterol due to a change in drinking water exposure has the same effect as a change due to sources that have been evaluated by intervention studies.

Derivation of MCLG

Charge question 4. Cancer

The proposed CSF is 0.015 or 0.035 (ng/kg-day)⁻¹ using respectively the central tendency or upper 95% CI from the Shearer et al. renal cancer epidemiological study. If used to create a risk-specific dose for PFOA that would produce an incremental cancer risk of 10⁻⁶, the dose would be 1e-6 / [0.035 (ng/kg-day)⁻¹] = 3e-5 ng/kg-day = 3e-11 mg/kg-day using the upper 95% CI (and 7e-11 mg/kg-day using the central tendency). These values are 50 (and 20) times smaller than the RfD for PFOA based on decreased serum anti-tetanus antibodies in children (equal to 1.5e-9 mg/kg-day). This suggests the RfD should be decreased accordingly.

To evaluate the decrease in lifetime cancer risk associated with water treatment, consider the hypothetical decrease in PFOA drinking water concentration in the CVD risk reduction analysis. Assumed baseline concentration is 0.1 mg/L which is decreased to 0.28 mg/L, a reduction of 0.072 mg/L. Drinking water intake is 0.013 L/kg-day. The decrease in lifetime cancer risk is calculated as:

cancer risk reduction = (decrease in concentration) · (drinking water consumption) · CSF
= 0.072 mg/L · 0.013 L/kg-day · 0.035 (ng/kg-day)⁻¹ · 1e3 ng/mg ≈ 0.03.
(using the upper 95% CI for the CSF).

Is this analysis correct? It does not seem to provide a plausible estimate of the effect of decreasing PFOA concentration in drinking water, since the calculated decrease exceeds the lifetime risk of (diagnosed) kidney and renal pelvis cancer of 0.017 suggested by SEER (<https://seer.cancer.gov/statfacts/html/kidrp.html>).

Mixtures

Charge questions 3. RPF and 4. Mixture BMD

The RPF and mixture BMD approaches appear to be very similar or even equivalent; differences between them should not be exaggerated. As I understand them, both approaches produce a summary measure of the toxicity of a mixture, ICECMIX for the RPF approach and t_{add} for the mixture BMD approach. Both summaries are weighted sums of the component concentrations, with weights proportional to some measure of toxicity (e.g., inverse of BMD or of ED10).

Combining eqns. (4.2) and (4.3) implies

$$ICEC_{MIX} = \sum_j d_j \frac{ED10_{IC}}{ED10_j} = ED10_{IC} \sum_j \frac{d_j}{ED10_j}. \quad (1)$$

Taking reciprocals in eqn. (4.5) yields

$$\frac{1}{t_{add}} = \sum_i \frac{a_i}{BMD_i}. \quad (2)$$

Comparing these results shows that $ICEC_{MIX}$ and $(t_{add})^{-1}$ differ only in inessential details and are essentially proportional to one another. First, note that the RPF approach can use any common toxicity metric; e.g., one can replace ED10 with BMD in eqn. (1). Second, d_i (the “component chemical’s concentration”) and a_i (“the fixed proportions of the component PFAS in the mixture”) are either identical or strictly proportional to each other. Third, $ICEC_{MIX}$ includes a constant proportionality factor ($ED10_{IC}$).

Having constructed $ICEC_{MIX}$ and t_{add} , one can presumably use them to evaluate a risk in analogous ways. The summary t_{add} can be used as a BMD, from which one can calculate a hazard index or use it as a POD from which to extrapolate a dose-response function; similarly, one can divide $ICEC_{MIX}$ by $ED10_{IC}$ to calculate a hazard index or use it in a dose-response function for the index chemical as in eqn. (4.4).

If this analysis is roughly correct, it would be advisable to revise the discussion of these two approaches to present them as essentially the same (or highlighting any essential differences).

REVISED COMMENTS

MCGLs PFOA/PFOS

Topic 2 – Noncancer Hazard Identification

2. Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.

- A. Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.

I do not agree with EPA's decision to not use ALT measurements from epidemiological studies for the derivation of a POD for adverse liver effects. The rationale provided in the draft document for not considering elevated ALT levels in epidemiological studies as a critical endpoint for deriving PODs for PFAS exposures centered on 1) the overall effect (fold-changes) were not large compared to control levels; 2) small changes in ALT levels, such as seen in the epidemiological studies, would not be expected to result in significant clinical outcomes in exposed populations. However, significant associations were consistently observed between PFAS levels and elevated ALT in several epidemiological studies, and upon review of additional reference provided, I agree that elevated ALT is indicative of potential adverse outcomes, and elevated ALT levels should be given additional consideration as an endpoint for POD derivation. One question remains as to the specificity of elevated serum ALT to PFAS exposure. As ALT release is observed following exposure to a number of chemicals, elevated ALT could be viewed as a biomarker of effect rather than a biomarker of exposure. The EPA document should clearly and transparently describe the strength of associations in the epidemiological studies and how well the studies controlled for other PFAS and other hepatotoxic chemical exposures.

- B. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.

Many of the supporting studies provided by SAB members and in the public comments should be included in the draft document.

- C. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

I am not aware of any other adverse liver endpoints that were identified in the epidemiological literature that are specific to PFAS exposure and would be appropriate to use for POD derivations.

Topic 3

2. Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor ~~using peak exposure~~ for PFOA. Has EPA adequately justified the use of this study ~~and peak exposure~~ for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends. Does SAB support the selection of this CSF in the derivation of a risk specific dose for PFOA (i.e. the concentration of PFOA in drinking water that would have a one-in-a-million chance of an increased cancer risk)? If not please provide input on the strengths and weaknesses of the other candidate CSFs that EPA derived.

The Shearer et al., 2021, is a large epidemiologic study that investigated PFOA exposure and RCC risk, and also RCC risk in relation to other 7 additional PFAS. The systematic review conducted by EPA categorized the overall confidence in this study as “medium”, apparently driven by a deficiency in controlling for confounding, and adequate confidence in selectivity and sensitivity of the study (Figure 123, though not specifically described in the text). This study was unique as it was the first to prospectively examine the association of serum PFAS (collected pre-cancer diagnosis) with RCC risk. The study adjusted for diminished kidney function through the measurement of serum cystatin C and creatinine in all cases and controls and calculated the estimated glomerular filtration rate (eGFR), that although kidney cancer was not the focus of PLCO study when the blood samples were collected, these endpoints were measured at the time of blood draw. This prospective study design therefore controlled for reverse causation due to reduced kidney function in their observed associations. Positive trends in RCC risk with increasing prediagnostic concentrations of PFOA (highest quartile vs lowest, OR = 2.63, 95% CI = 1.33 to 5.20). The association lost significance when PFOA was analyzed by quartiles then adjusted for other PFAS, however, when PFOA was modeled continuously, the association remained significant after adjusting for other PFAS. The study also revealed associations with RCC for PFOS (OR = 2.51, 95% CI = 1.28 to 4.92), and PFHxS (OR = 2.07, 95% CI = 1.06 to 4.04), in models unadjusted for other

PFAS. However, after adjusting for PFAS, only PFOA remained statistically associated with RCC, suggesting that PFOA is driving the increased risk.

Overall, the epidemiological studies have not consistently identified associations between PFOA and RCC; some epidemiological studies support that RCC as a critical finding associated with PFOA exposure while others (with several limitations noted) have failed to detect an association between PFOA and RCC. Overall, available epidemiological studies examining an association of PFOA with of RCC is inconsistent and often not dose-response.

The systematic review identified and classified 8 epidemiologic studies, including the Shearer 2021 only of “medium” overall confidence. In contrast, the systematic review classified the overall confidence of the NTP 2020 chronic bioassay in rats as “high”. This study demonstrated increased incidences of hepatocellular adenomas (or carcinomas) and PACTs (NTP, 2020). Of the three chronic bioassays that have been conducted in rodents, none have observed an increased incidence in RCC (NTP 202, Biegel et al., 2001, Butenhoff et al., 2012). While concordance of tumor sites between animal and human studies is not always observed, the lack of “high” confidence epidemiological studies would support that “high” confidence animal studies be selected for CSF derivation. The “medium” confidence studies in rodents and human epidemiological studies would provide supporting evidence for PFOA carcinogenicity. Therefore, chronic bioassay in rats (NTP, 2020) would be the preferred study for the development of CFSs.

Mixtures

Question 1

1. The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, an assumption of dose addition for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA’s supplementary guidance (EPA, 2000) states: “The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration).” This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which “similarity” can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.

- A. Please comment on the appropriateness of EPA’s approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.

The PFAS chemical class includes 1000’s of unique chemicals that exist in environmental media often as PFAS mixtures. Toxicological studies to inform

human health risk assessment are largely lacking, both for individual and PFAS mixtures. Further, MOA data is limited if not lacking for the majority of PFAS. This draft framework proposes a component-based approach to estimate the probability or magnitude of adverse non-cancer health outcomes for PFAS mixtures. EPA has developed this framework based on the concept of dose additivity (DA). The framework proposes that DA can be applied for cases in which the given adverse outcome (toxicity endpoint/adverse outcome/effect) associated with exposure to 2 or more PFAS chemical is similar. The underlying assumption is that similarity in an outcome measure can arise when component mixtures elicit effects on similar pathways, or if they function on independent pathways that converge on a common outcome/effect without requiring detailed mechanistic data for a chemical.

This document provides several examples of chemical mixtures (e.g Dioxin- like chemicals, organophosphate chemicals, etc.) that alter shared pathways that typically produce dose additive responses. The appropriateness of this approach for its applicability to PFAS mixtures is supported by 1) existing data demonstrates that many PFAS elicit effects on multiple biological pathways that have common adverse outcomes in several biological systems (e.g. hepatic, thyroid, lipid synthesis and metabolism, developmental and immune toxicities); and 2) EPA ORD has provided proof-of-concept data using developmental toxicity mixture studies with a combination of PFOA and PFOS and demonstrate that these PFAS behave in a DA manner.

A utility of the proposed framework is that it can provide an estimate of the composite effects (DA) of PFAS mixtures when individual chemical exposures are at or below their individual NOAELs. Unless data demonstrating that chemicals within a PFAS mixture interact in a manner other than additivity exist, I agree with the assumption that mixtures of PFAS exhibiting similar effects would act in a DA manner and would be health protective.

- B. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).

I agree, for PFAS with limited MOA (or similar) data, using similarities in a common endpoint/adverse health outcome is a rational and health protective approach for estimating the probability or magnitude of adverse non-cancer health outcomes for PFAS mixtures. NAMs evaluating specific endpoints, pathways, and other toxicity measurements can readily be incorporated into this framework to assist in estimating PFAS mixture toxicity, relative potency and potential risk.

Question 2

2. Section 4.3 (Hazard Index; HI) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and hypothetical exposure information. The example calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as “GenX chemicals”).

- A. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.

Given that toxicological studies to inform human health risk assessment are largely lacking for the large class of PFAS, and mixtures of PFAS commonly occur in environmental media, overall, the HI methodology is a reasonable approach for estimating the potential aggregate health hazards associated with the occurrence of chemical mixtures in environmental media. The HI is an approach based on DA that has been validated and used by EPA. The HI does not provide quantitative risk estimates (probability) for mixtures, nor does it provide an estimate of the magnitude of a specific toxicity. This approach is mathematically straightforward and may readily identify mixtures of potential toxicological concern as well as identify chemicals that drive the toxicity within a given mixture. As described in the framework document, this approach has advantages and limitations that were adequately described.

- B. Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment

HI's can be divided into “screening level” HI's, in which the most sensitive health effects for each chemical in the mixture are used in the model, or “target-organ specific” HI (TOSHI), in which the derivation of HQs for each chemical in the mixture is based on a “similar” effect. Of the two approaches, “screening level” HIs would be considered conservative estimates as they are based on the most sensitive endpoints for derivation of individual HQs. “Screening level” HI's can be useful as they may be able to categorize chemicals based on their potential to elicit joint toxicities of mixtures ($HI's \leq 1$ would be of little concern, whereas $HI's \geq 1$ would be suggestive of possible joint toxicity) and identify a need for further analysis of the potential for health effects associated with the individual chemicals in the mixture as well as their potential joint toxicity. As both screening level and TOSHI methodologies assume DA for a common effect, the document should clearly state that for either methodology, an HI approach, specifically “screening level” HI's can only be applied when all mixture components elicit adverse effects on the same endpoint, albeit likely of different magnitudes of responses.

Additional limitations of these approaches have been identified and were adequately described in the framework document. As noted in the response to 2A above, an HI does not provide quantitative estimates of risks associated with PFAS mixtures in a given exposure, yet could be useful for categorizing a specific mixture as to their potential hazard. Additionally, HI estimates need to be interpreted with caution in that different mixture exposure scenarios that containing the same chemicals may result in the derivation identical HI's, however, due to factors specific to each exposure scenario, may not necessarily exhibit the same potential for causing adverse health effects. Another disadvantage of the HI approach to specific exposure scenarios (and environmental media) is that it requires derivation of a health-based, media-specific concentrations (e.g. drinking water Health Advisory or MCLG), in addition to reference values. In addition, a TOSHI approach can only be utilized for PFAS that have derived target organ toxicity doses (TTD's or RfD's specific to a health effect) or can be calculated. As the available toxicity database for the large PFAS class is generally lacking, the ability to derive TTD's for PFAS is limited. Nonetheless, the approaches outlined have been used and validated, and are scientifically based.

3. Section 4.4 (Relative Potency Factor; RPF) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and HFPO dimer acid and GenX chemicals.

- A. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.
- B. Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.

A consideration for the RPF approach that should be addressed is centered around the selection of the index chemical (IC) for each mixture. EPA should provide guidance on how IC's are selected for a given mixture/data set. As the IC is always assigned a value of 1, the derived values for the RPFs for other components in the mixture and resulting ICEC's and mixture total ICEC's would be different depending on what constituent is selected as the IC. Setting the IC as the highest POD in the mixture would provide the highest total mixture ICEC, however, selection of the lowest POD as the reference chemical (IC) might offer the most biologically relevant approach. Clarification on criteria used for selecting the IC in RPF methodology is needed.

PRELIMINARY COMMENTS

MCGLs PFOA/PFOS

Topic 2 – Noncancer Hazard Identification

2. Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.

A. Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.

I agree with EPA's decision to not use ALT measurements from epidemiological studies for the derivation of a POD for adverse liver effects. The rationale provided in the draft document provides clear rationale for why elevated ALT levels in epidemiological studies may not be a critical endpoint for deriving PODs for PFAS exposures. 1) while significant associations were observed between PFAS levels and elevated ALT in several epidemiological studies, the overall effect (fold-changes) were not large compared to control levels; 2) small changes in ALT levels, such as seen in the epidemiological studies, would not be expected to result in significant clinical outcomes in exposed populations; 3) ALT release from damaged hepatocytes is observed following exposure to a number of chemicals (hepatotoxic chemicals), while some epidemiological studies have accounted for confounding related to PFAS co-exposures, co-exposure to the vast number of additional chemicals that also elicit hepatotoxicity cannot fully be controlled for in these studies. Therefore, the reliance on a non-(chemical)-specific endpoint (biomarker of effect rather than a biomarker of exposure) for establishing a POD for adverse liver effects attributed to PFAS exposure in human epidemiologic studies cannot be supported.

B. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.

Many toxicological (animal) studies that have identified elevated ALT as a critical endpoint related to their toxicities. Opposed to epidemiological studies, by their experimental design, controlled animal studies reduce, if not eliminate, co-exposures to other chemicals. Thus, the observation of increased serum markers of hepatotoxicity can be informative in animal studies. However, based on the limitations provided in Part A (above), the lack of specificity for this endpoint in epidemiological studies largely precludes its use as an endpoint for POD derivations for PFAS chemicals.

C. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

I am not aware of any other adverse liver endpoints that were identified in the epidemiological literature that are specific to PFAS exposure and would be appropriate to use for POD derivations. Perhaps a closer surrogate for determining an association between PFAS exposure and elevated serum ALT might be quantitative measures of PFAS within the target organ, in this case, the liver. To my knowledge, these type of data do not exist, but could be reasonable to propose and achievable in animal studies, however, this approach would pose significant challenges for human epidemiological studies.

Topic 3

2. Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends. Does SAB support the selection of this CSF in the derivation of a risk specific dose for PFOA (i.e. the concentration of PFOA in drinking water that would have a one-in-a-million chance of an increased cancer risk)? If not please provide input on the strengths and weaknesses of the other candidate CSFs that EPA derived.

The Shearer et al., 2021, is a large epidemiologic study that investigated PFOA exposure and RCC risk, and also RCC risk in relation to other 7 additional PFAS. The systematic review conducted by EPA categorized the overall confidence in this study as “medium”, apparently driven by a deficiency in controlling for confounding, and adequate confidence in selectivity and sensitivity of the study (Figure 123, though not specifically described in the text). This study was unique as it was the first to prospectively examine the association of serum PFAS (collected pre-cancer diagnosis) with RCC risk. The study adjusted for diminished kidney function through the measurement of serum cystatin C and creatinine in all cases and controls and calculated the estimated glomerular filtration rate (eGFR), that although kidney cancer was not the focus of PLCO study when the blood samples were collected, these endpoints were measured at the time of blood draw. This prospective study design therefore controlled for

reverse causation due to reduced kidney function in their observed associations. Positive trends in RCC risk with increasing prediagnostic concentrations of PFOA (highest quartile vs lowest, OR = 2.63, 95% CI = 1.33 to 5.20). The association lost significance when PFOA was analyzed by quartiles then adjusted for other PFAS, however, when PFOA was modeled continuously, the association remained significant after adjusting for other PFAS. The study also revealed associations with RCC for PFOS (OR = 2.51, 95% CI = 1.28 to 4.92), and PFHxS (OR = 2.07, 95% CI = 1.06 to 4.04), in models unadjusted for other PFAS. However, after adjusting for PFAS, only PFOA remained statistically associated with RCC, suggesting that PFOA is driving the increased risk. While diminished kidney function was adjusted using eGFR, a higher yet not significant number of cases had diminished kidney function (eGFR < 60 mL/min/1.73 m²) compared with controls (9.0% vs 5.6%). The association between RCC and PFOA persisted in analyses restricted to participants without evidence of diminished kidney function and was stronger for cases with histopathologically confirmed renal clear cell carcinoma. Notably, serum PFAS concentrations in the control group were similar to those observed in adults in the NHANES study during the same time period (highest quartile of PFOA serum concentrations in study participants (>7.3 ug/L), highest quartile of PFOA in NHANES in 1999-2000 (>7.0ug/L).

Other epidemiological studies support that RCC as a critical finding associated with PFOA exposure. Positive associations with RCC were observed among individuals highly exposed to PFOA who were working or living near a PFAS-producing facility in the mid-Ohio Valley, and mortality from kidney cancer was elevated among those with high estimated cumulative serum PFOA concentrations (Steenland et al., 2012; Vieira et al., 2013; Barry et al., 2013). Additional epidemiological studies, with several limitations noted have failed to detect an association between PFOA and RCC. Overall, available epidemiological studies examining an association of PFOA with of RCC is inconsistent and often not dose-response.

As detailed above, there are clear merits to the data described for PFOA-associated RCC risk in the Shearer et al., 2021 study, however, several factors should be considered prior to its selection for CSF derivation. The systematic review identified and classified 8 epidemiologic studies, including the Shearer 2021 only of “medium” overall confidence. In contrast, the systematic review classified the overall confidence of the NTP 2020 chronic bioassay in rats as “high”. This study demonstrated increased incidences of hepatocellular adenomas (or carcinomas) and PACTs (NTP, 2020). Of the three chronic bioassays that have been conducted in rodents, none have observed an increased incidence in RCC (NTP 202, Biegel et al., 2001, Butenhoff et al., 2012). While concordance of tumor sites between animal and human studies is not always observed, the lack of “high” confidence epidemiological studies would support that “high” confidence animal studies be selected for CSF derivation. The “medium” confidence studies in rodents and human epidemiological studies

would provide supporting evidence for PFOA carcinogenicity. Therefore, chronic bioassay in rats (NTP, 2020) would be the preferred study for the development of CFSs.

Mixtures

Question 1

1. The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, an assumption of dose addition for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA's supplementary guidance (EPA, 2000) states: "The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which "similarity" can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.

A. Please comment on the appropriateness of EPA's approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.

The PFAS chemical class includes 1000's of unique chemicals that exist in environmental media often as PFAS mixtures. Toxicological studies to inform human health risk assessment are largely lacking, both for individual and PFAS mixtures. Further, MOA data is limited if not lacking for the majority of PFAS. This draft framework proposes a component-based approach to estimate the probability or magnitude of adverse non-cancer health outcomes for PFAS mixtures. EPA has developed this framework based on the concept of dose additivity (DA). The framework proposes that DA can be applied for cases in which the given adverse outcome (toxicity endpoint/adverse outcome/effect) associated with exposure to 2 or more PFAS chemical is similar. The underlying assumption is that similarity in an outcome measure can arise when component mixtures elicit effects on similar pathways, or if they function on independent pathways that converge on a common outcome/effect without requiring detailed mechanistic data for a chemical.

This document provides several examples of chemical mixtures (e.g Dioxin- like chemicals, organophosphate chemicals, etc.) that alter shared pathways that typically produce *at least* dose additive responses. The appropriateness of this approach for its applicability to PFAS mixtures is supported by 1) existing data demonstrates that many PFAS elicit effects on multiple biological pathways that have common adverse outcomes in several biological systems (e.g. hepatic, thyroid, lipid synthesis and metabolism, developmental and immune toxicities);

and 2) EPA ORD has provided proof-of-concept data using developmental toxicity mixture studies with a combination of PFOA and PFOS and demonstrate that these PFAS behave in a DA manner.

A utility of the proposed framework is that it can provide an estimate of the composite effects (DA) of PFAS mixtures when individual chemical exposures are at or below their individual NOAELs. Unless data demonstrating that chemicals within a PFAS mixture interact in a manner other than additivity exist, I agree with the assumption that mixtures of PFAS exhibiting similar effects would act in a DA manner and would be health protective.

B. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).

I agree, for PFAS with limited MOA (or similar) data, using similarities in a common endpoint/adverse health outcome is a rational and health protective approach for estimating the probability or magnitude of adverse non-cancer health outcomes for PFAS mixtures. NAMs evaluating specific endpoints, pathways, and other toxicity measurements can readily be incorporated into this framework to assist in estimating PFAS mixture toxicity, relative potency and potential risk.

Question 2

2. Section 4.3 (Hazard Index; HI) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and hypothetical exposure information. The example calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as “GenX chemicals”).

A. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.

Given that toxicological studies to inform human health risk assessment are largely lacking for the large class of PFAS, and mixtures of PFAS commonly occur in environmental media, overall, the HI methodology is a reasonable approach for estimating the potential aggregate health hazard associated with the occurrence of chemical mixtures in environmental media. The HI is an approach based on DA that has

been validated and used by EPA. The HI does not provide quantitative risk estimates (probability) for mixtures, nor does it provide an estimate of the magnitude of a specific toxicity. In the HI approach, an HQ for each chemical in the mixture is calculated as the ratio of human exposure (E) to an adverse health hazard (e.g. RfV, MCLG, or other value), then individual HQ's are summed to yield an HI. As described in the framework document, this approach has advantages and limitation that were adequately described. This approach is mathematically straightforward and may readily identify mixtures of potential toxicological concern as well as identify chemicals that drive the toxicity within a given mixture. Another advantage of the HI approach is that it may be able to leverage data obtained from NAM's to support human health risk assessment for individual and PFAS mixtures. In theory, it is possible that NAM data could be used to identify PODs and corresponding non-cancer toxicity values that then could be converted into HBWCs and used to calculate HQs for understudied PFAS. This latter point could be considered disadvantage in that not quantifying HQ's for PFAS with limited data present in mixtures and excluding them in HI derivations would potentially underestimate the overall potential risk of the mixture.

B. Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment

HI's can be divided into "screening level" HI's, in which the most sensitive health effects for each chemical in the mixture are used in the model, or "target-organ specific" HI (TOSHI), in which the derivation of HQs for each chemical in the mixture is based on a "similar" effect. Of the two approaches, "screening level" HIs would be considered conservative estimates as they are based on the most sensitive endpoints for derivation of individual HQs. "Screening level" HI's can be useful as they may be able to categorize chemicals based on their potential to elicit joint toxicities of mixtures ($HI's \leq 1$ would be of little concern, whereas $HI's \geq 1$ would be suggestive of possible joint toxicity and identify a need for further analysis of the potential for health effects associated with the individual chemicals in the mixture as well as their potential joint toxicity. As both screening level and TOSHI methodologies assume DA for a common effect, the document should clearly state that for either methodology, an HI approach, specifically "screening level" HI's can only be applied when all mixture components elicit adverse effects on the same endpoint, albeit likely of different magnitudes of responses.

Additional limitations of these approaches have been identified and adequately described in the framework document. As noted in the response to 2A above, an HI does not provide quantitative estimates of risks associated with PFAS mixtures in a given exposure, yet they could be useful for categorizing a specific mixture as to their potential hazard. Additionally, HI estimates need to be interpreted with caution in that different mixture exposure scenarios that containing the same chemicals may result in the derivation identical HI's, however, due to factors specific to each exposure scenario, may not necessarily exhibit the same potential for causing adverse health effects. Another disadvantage of the HI approach to specific exposure scenarios (and environmental media) is that it requires derivation of a health-based, media-specific concentrations (e.g. drinking water Health Advisory or MCLG), in addition to reference values. In addition, a

TOSHI approach can only be utilized for PFAS that have derived target organ toxicity doses (TTD's or RfD's specific to a health effect) or can be calculated. As the available toxicity database for the large PFAS class is generally lacking, the ability to derive TTD's for PFAS is limited. Nonetheless, the approaches outlined have been used and validated, and are scientifically based.

3. Section 4.4 (Relative Potency Factor; RPF) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and HFPO dimer acid and GenX chemicals.

A. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.

B. Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.

A consideration for the RPF approach that should be addressed is centered around the selection of the index chemical (IC) for each mixture. EPA should provide guidance on how IC's are selected for a given mixture/data set. As the IC is always assigned a value of 1, the derived values for the RPFs for other components in the mixture and resulting ICEC's and mixture total ICEC's would be different depending on what constituent is selected as the IC. As an example, in the example provided in Table 4.7, PFOS was selected as the IC and the mixture total ICEC was calculated as 30 ppt.

Mixture Component	POD_{HED} (mg/kg/d); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L)^a	PFOS ICEC (ng/L)
PFOA	0.001 (NOAEL _{HED}) ^{b, c} (EPA, 2016a)	0.5	20	10
PFOS (IC)	0.00051 (NOAEL _{HED}) ^b (EPA, 2016b)	1	20	20
Mixture Total PFOS ICEC (ppt)				30

However, if PFOA was selected as the IC, then the resulting mixture total ICEC would be significantly higher (60 ppt).

Mixture Component	POD_{HED} (mg/kg/d); Decreased Body Weight in Offspring	Example RPF	Hypothetical Exposure Estimate (ng/L)^a	PFOS ICEC (ng/L)
PFOA (IC)	0.001 (NOAEL _{HED}) ^{b, c} (EPA, 2016a)	1	20	20
PFOS	0.00051 (NOAEL _{HED}) ^b (EPA, 2016b)	2	20	40
Mixture Total PFOS ICEC (ppt)				60

Setting the IC as the highest POD in the mixture would provide the highest total mixture ICEC, however, selection of the lowest POD as the reference chemical (IC) might offer the most biologically relevant approach. Clarification on criteria used for selecting the IC in RPF methodology is needed.

REVISED COMMENTS

No revisions to preliminary comments.

Below are five potentially relevant papers on low birthweight:

Figlio, D., Guryan, J., Karbownik, K., and Roth, J. (2014). The Effects of Poor Neonatal Health on Children's Cognitive Development, *American Economic Review*, 104(12): 3921–3955

Black, Sandra & Devereux, Paul & Devereux, Michael & Salvanes, Kjell. (2007). From the Cradle to the Labor Market? The Effect of Birth Weight on Adult Outcomes. *The Quarterly Journal of Economics*. 122. 409-439.

Almond, D., Doyle, J. J., Kowalski, A. E., & Williams, H. (2010). Estimating marginal returns to Medical Care: Evidence from at-risk newborns. *The Quarterly Journal of Economics*, 125(2), 591–634.

Almond, D.; Chay, K.Y.; and Lee, D.S. (2005). The Costs of Low Birth Weight. *The Quarterly Journal of Economics*, Volume 120 (3):1031-1083.

Barreca, A., Guldi, M., Lindo, J.M., and Waddell, G.R. (2011). Saving babies? Revisiting the effect of very low birth weight classification. *The Quarterly Journal of Economics*. 126 4, 2117-1223.

PRELIMINARY COMMENTS on EPA's draft Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water

11. Section 4.2 presents EPA's meta-analysis for the total cholesterol dose-response function.

B. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

- It would be helpful for EPA to list the studies that were excluded from the meta-analysis and a brief description of these studies. EPA mentions that studies performed on specific population subsets were not considered for inclusion in the meta-analysis. Yet, some studies that were included appear to include population subsets such as those living near chemical plant (Steenland et al., 2009), Inuit Population of Nunavik (Château-Degat et al., 2010), and Chinese Male Adults (Yang et al., 2018). I realize sensitivity analyses were done excluding certain studies, but it would be helpful to know what other studies, with particular subsets were also excluded.

C. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this

application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.

This comment may apply to both 1A and 1B:

- It would be helpful for EPA to comment on the quality of the studies included in the meta-analysis. In the Appendix, EPA mentions that all of the studies in the meta-analysis, except one (Lin et al. 2019) are cross-sectional with various methodological limitations. It would be helpful to know if the quality of the cross-sectional studies are viewed any differently than Lin et al. (2019) and what the implications of the analysis are when using studies of varying quality (if any).

2. Section 5.1 presents EPA’s life table approach methodology.

B. Please comment on the extent to which this analysis is scientifically supported and clearly described. To the extent improvements are suggested, please provide specific changes that are implementable in a U.S. national-level benefits analysis with readily available data.

Comments: Overall, the life table approach appears to be sound and follows similar life table approaches performed by US EPA in prior regulatory analyses, including the 2015 Steam Electric Rule (USEPA 2015) and the National Ambient Air Quality Standards for ground-level ozone (USEPA 2008). However, I do have some specific comments on particular aspects of the analysis for EPA to consider.

- I would like EPA to clarify how the life table approach differs in this application versus prior EPA applications. For instance, it appears that EPA has updated CDC prevalence statistics from the 2015 Steam Electric Rule (USEPA 2015). Other changes to key input data and assumptions of the model should be briefly discussed. A natural place to do this is when discussing prior applications (page 15).
- On page 14: “EPA adjusts the modeled population cohort to exclude individuals with pre-existing conditions.” How exactly is this done? Are the overall population numbers reduced (i.e., in Step 1 of Table 3)? By how much? What pre-existing conditions are included? Could a sensitivity analysis include these individuals as if they did not have pre-existing conditions? Can we assume they would be just as sensitive to changes in exposure to PFAS as the general public? By excluding them entirely, EPA reduces the overall mortality and morbidity impacts of the rule.
- On page 17, EPA states that “Each person included in the surviving current age-specific incident CVD subpopulation...is tracked for 5 additional years to estimate the number of CVD deaths occurring in that timeframe.” However, Table 5 seems to suggest that the five-year period significantly undercounts these impacts. There is no apparent major decline in mortality rates after year 4. Could EPA perform a sensitivity analysis that extrapolates the trend in post-acute mortality rates to more than five years?

- I found the description of the life table approach in Section 5.1 to be clear and easy to understand. However, I had a much harder time following the descriptions of the actual steps taken in Table 3. Is it possible to clarify Table 3? Perhaps add two additional columns that separate CVD and Non-CVD calculations under “Baseline Calculations” and “Treatment Scenario Calculations”? Or, splitting table 3 into calculations specific to baseline vs. treatment scenarios? Some of the steps come out of order, which is confusing. For instance, in the Treatment Scenario Calculations, Step 6 depends on Step 10, Step 1, and Step 12. Can these steps be explained in a linear fashion?
- It would be helpful to report the calibration factors used in Table 3. It would also be helpful to add a discussion as to how reasonable these magnitudes appear. Do these calibration factors suggest anything about how reasonable the projections from the ASCVD model are?
- EPA notes that the “ASCVD-based estimates may be inconsistent with the recent CVD prevalence statistics” (pg. 27). It would be helpful to know the level of inconsistency.
- It is my understanding that EPA will use the estimates from this report to inform subsequent economic analyses of the benefits of treating PFOA and PFOS. It would be helpful for EPA to discuss how the impacts captured in the life table will be linked with economic studies. It would also be helpful to discuss how the impacts captured by this table might differ from other endpoints captured by analyses of prior rules. For instance, EPA cites USEPA (2011) on page 15 as one example of a prior life table approach. Several cardiovascular impacts are monetized in USEPA (2011), including premature mortality, myocardial infarction, and cardiovascular hospital admissions. It would be helpful to understand how the estimated impacts in this study (i.e., fatal and non-fatal myocardial infarction, fatal and non-fatal ischemic stroke, and other CHD mortality) compare to those in prior EPA analyses.

References:

U.S. Environmental Protection Agency. (2008). *Final Ozone NAAQS Regulatory Impact Analysis*. (EPA 452/R-08-003). Research Triangle Park, North Carolina

U.S. Environmental Protection Agency. (2011). *The Benefits and Costs of the Clean Air Act from 1990 to 2020. Final Report - Rev. A*. Retrieved from https://www.epa.gov/sites/default/files/2015-07/documents/fullreport_rev_a.pdf

U.S. Environmental Protection Agency. (2015). *Benefit and Cost Analysis for the Effluent Limitations Guidelines and Standards for the Steam Electric Power Generating Point Source Category*. (EPA-821-R-15-005). US EPA, Office of Water, Washington DC 20460

4. Section 7 and Appendix A describe the limitations and uncertainties of the CVD risk reduction analysis.

B. Has EPA clearly described the individual contributions of the sources of uncertainty?

Comments: Overall, EPA reasonably describes the individual contributions of the sources of uncertainty. I have included a few suggestions for improvements below.

- Table 7 does not describe the impact of excluding the population of individuals with pre-existing conditions. Presumably this would underestimate the benefits of treatment.
- Given EPA's description of PFOA /PFOS exposure on other cardiovascular outcomes such as systolic blood pressure (page 31) and description of systolic blood pressures role in the ASCVD model (page 31), it is unclear why EPA did not include systolic blood pressure in this analysis. It would be helpful for EPA to provide further justification of this exclusion.
- There is no description or estimate of individuals that may engage in averting behaviors to avoid drinking contaminated water (switching to bottle water, filters). This exclusion suggests that the study overestimates the benefits of treatment.
- EPA does not describe the uncertainty in linking FIPS codes in the Woods & Poole database to PWS locations and the impact of this uncertainty on PWS population and race/ethnicity, sex, and age strata. I recommend adding a description of this uncertainty to Table 7.
- EPA does not describe the uncertainty in the baseline PFOA and PFOS drinking water concentrations. I recommend adding a description of this uncertainty to Table 7.
- It would be helpful for EPA to describe any cardiovascular impacts that are monetized in other EPA analyses that are not included here. For instance, EPA (2011), cited on page 15, monetizes cardiovascular hospitalizations. This would be a limitation of the study.

References

U.S. Environmental Protection Agency. (2011). *The Benefits and Costs of the Clean Air Act from 1990 to 2020. Final Report - Rev. A*. Retrieved from https://www.epa.gov/sites/default/files/2015-07/documents/fullreport_rev_a.pdf

REVISED COMMENTS

Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal (MCLG) for PFOA and PFOS in Drinking Water

MCLG CQ#1. Study Identification and Inclusion Chen, Post, **Pullen-Fedinick**

Health effects evidence synthesis and integration

The protocol for risk of bias assessment and, more importantly, how that approach was used in a synthesis of evidence for each particular health endpoint is not sufficiently clearly presented to be confidently judged for accuracy or transparency, or for consistency across health endpoints. This is especially important when a proposed systematic review protocol has not been previously registered or published. Beyond details provided about which studies are chosen for inclusion or exclusion, critical additional details are needed on how the particular seven domains were selected for internal validity/risk of bias/confidence rankings, on objective criteria for how deficiencies in certain/multiple domains contributed to an overall low confidence rating, and on the approach for the evidence synthesis process and the designations of strong vs. suggestive evidence, including whether a “tiered” approach (similar to a sensitivity analysis) was considered to evaluate whether interpretation or conclusions changed based on varied decisions about inclusion and rating of high, medium and low confidence studies across various study design domains.

Studies were downgraded for “study sensitivity”, for example if they had “limited exposure contrasts and/or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present” (page 46) Narrow exposure ranges should not automatically lead to downgrading of studies as these can still contribute informative data within that narrow range and do not necessarily bias to the null.

Finally, the reports mention exclusion of publications with specific population subsets. More transparency and information is needed on how this decision was reached and used as a basis for exclusion. If exclusions are being made based on certain population subsets, then did EPA consider providing data synthesis or estimates of effect in those particular population subsets?

MCLG CQ#2. Noncancer Hazard Identification DeWitt, **Post**, Savitz

Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

As also indicated in my response to Charge Question #1 above, the protocol for risk of bias assessment and, more importantly, how that approach was used in a synthesis of evidence for each particular health endpoint is not sufficiently clearly presented to be confidently judged for

accuracy or transparency, or for consistency across health endpoints. This is especially important when a proposed systematic review protocol has not been previously registered or published. Beyond details provided about which studies are chosen for inclusion or exclusion, critical additional details are needed on how the particular seven domains were selected for internal validity/risk of bias/confidence rankings, on objective criteria for how deficiencies in certain/multiple domains contributed to an overall low confidence rating, and on the approach for the evidence synthesis process and the designations of strong vs. suggestive evidence, including whether a “tiered” approach (similar to a sensitivity analysis) was considered to evaluate whether interpretation or conclusions changed based on varied decisions about inclusion and rating of high, medium and low confidence studies across various study design domains.

Cardiovascular endpoints/blood pressure

The rationale for including studies on blood pressure should be consistent with the rationale for including studies of lipids, and the clinical relevance of both should be treated comparably if using ASCVD risk calculator which includes both components (see CVD Charge questions below). Why rate as deficient studies that did not account for lipid lowering medications (this was the most common reason for a low confidence rating) but not apply the same rating for blood pressure medications?

4. Cancer

a. Cancer classification for PFOA/PFOS DeWitt, Post, Lipworth

PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a ‘likely carcinogen’ categorization which is a change from ‘suggestive’ in the 2016 HA. Does the panel agree with the ‘likely’ designation based on the new evidence? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

Based on the EPA’s *Guidelines for Carcinogen Risk Assessment* (2005), supporting data for the likely descriptor may include:

- Plausible associations between human exposure and cancer with some supporting experimental evidence (not necessarily carcinogenicity data from animal experiments);
- Positive tests in animal experiments in more than one species, strain, sex, site, or exposure routes, with or without evidence of carcinogenicity in humans;
- A positive tumor study that raises additional biological concerns such as early age at onset or a high degree of malignancy;
- A rare animal tumor response in a single experiment assumed to be relevant to humans;
- A positive tumor study strengthened by other links of evidence such as a plausible association between human exposure and cancer or evidence that agent or metabolite causes events generally known to be associated with tumor formation.

Based on these Guidelines and the available data, the three lower designations (not likely to be carcinogenic to humans, inadequate information to assess carcinogenic potential, and suggestive evidence of carcinogenic potential) are not appropriate for PFOA. Data from human and animal

studies, including new studies published since the 2016 review, are consistent with the examples provided in the EPA Guidelines supporting the designation of PFOA as “likely to be carcinogenic to humans.”

The evidence from epidemiologic studies is primarily based on the occurrence of kidney and testicular cancer. Since the publication of the 2016 document for PFOA, at least 8 additional epidemiological studies considering links between PFOA exposure and cancer have been published. At least one new general population nested case-control study conducted in the PLCO cohort (Shearer et al., 2021) supports previous positive associations with kidney cancer in individuals highly exposed to PFOA (elevated exposure from contaminated drinking water near PFAS producing facility) (Barry et al., 2013, Vieira et al, 2013). EPA Guidelines for Carcinogen Risk Assessment state that “When human data of high quality and adequate statistical power are available, they are generally preferable over animal data and should be given greater weight in hazard characterization and dose-response assessment, although both can be used.” The EPA determined that the new epidemiologic studies were all *medium confidence* studies, including the Shearer et al. study. While there is an absence of any *high confidence* epidemiologic studies, at least one new chronic cancer bioassay in rats (NTP, 2020) supports a plausible epidemiologic association between PFOA and cancer and supports previous evidence of tumorigenesis at multiple sites including testes, pancreas, and liver (Butenhoff et al, 2012). The 2020 NTP study concluded “clear evidence” of carcinogenic activity in rats exposed over a lifetime.

The available evidence does not support a higher designation of “carcinogenic to humans” which, according to EPA Guidelines, requires “convincing epidemiologic evidence of a causal association between human exposure and cancer.” However, the EPA document needs additional discussion of the “weight of the evidence” that supports the exclusion of this higher level of designation. It would enhance transparency and confidence to have an objective, well-described, systematic approach for evidence synthesis that incorporates the prior studies *and* the new studies. In fact, this “single integrative step” of weighing all the evidence after assessing the individual lines of evidence is emphasized in the EPA Cancer Guidelines. There is an absence of any *high confidence* epidemiologic study and the existing epidemiologic data suggest a plausible but not convincingly causal association. In the Shearer et al. study, the increased risk for kidney cancer in the highest PFOA exposure quartile was attenuated (from 2.63 to 2.19) and no longer statistically significant after adjusting for other PFAS. In addition, “an inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality.”

PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a ‘suggestive’ categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

Based on the EPA Guidelines, the available data support keeping the “suggestive” categorization for PFOS. According to those Guidelines, “suggestive” evidence of carcinogenic potential may include a positive cancer result from only a single animal or human study with additional studies

of mixed results. The epidemiologic evidence for the carcinogenicity of PFOS is mixed and/or studies suffer from methodological limitations that preclude firm conclusions. Of the 11 new studies identified since 2016, 8 were considered *medium confidence* and 3 were considered *low confidence*. There are no new animal toxicity studies identified. A single chronic cancer bioassay in rodents (Butenhoff et al. 2021) showed increases in tumors in the liver, thyroid gland, and mammary gland; these tumors did not appear in a dose-responsive pattern. Thus, the available data support a designation of “suggestive” which is unchanged since the 2016 categorization.

Further discussion of the Shearer et al. findings (classified as a medium confidence study) as they pertain to PFOS are needed, including in the weight of evidence section. In particular, the document should clearly indicate why the findings of that study were judged to be less definitive for PFOS than for PFOA. The magnitude of the association between PFOS and kidney cancer was lower than that for PFOA, and after adjustment for other PFAS the adjusted OR for the highest quartile was 1.14 and not statistically significant, but these data need to be presented clearly including a discussion of why the PFOS data from Shearer et al. were not considered sufficient for a higher designation of “likely carcinogenic.”

b. Cancer Slope Quantification

EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends. Fisher, Lipworth, Kamendulis

Lifetime RCC risk estimation relied on lifetime risk of kidney cancer in US males. RCC baseline risk is higher in Black individuals compared to White individuals. How does the CSF differ if different rates are applied?

7. Epidemiological Study RfD Derivation

a. EPA evaluated **potential confounding** as part of their study quality evaluation of the epidemiological studies and selected only ‘medium’ and ‘high’ quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies? Lipworth, Savitz, Slitt

The document emphasizes potential residual or uncontrolled confounding by SES in numerous places, and this contributes to confidence rankings for certain domains – and often contributes substantially to an overall low confidence rating for a study. “SES” is loosely used throughout the document and is a broad term with many contextual components; it would be helpful to tease this out further, to the extent possible, and to determine which components of SES are expected/suspected to confound associations of PFAS with particular health endpoints. If it is determined that SES is in fact not a strong confounder of particular associations between PFAS and health endpoints, as it may be for factors such as air pollution, then the blanket decision to exclude studies primarily on the basis of incomplete adjustment for SES is not justified.

Additional clarity is needed on how serious risk for residual confounding (low confidence) differs substantively from “lack of control for confounding” (critical deficiency, uninformative), and thus how these different decisions may have affected the results of moving studies forward for the RfD derivation.

Most studies have been conducted among predominantly White populations. Levels of individual serum PFAS levels in the general population have been shown to vary by race/ethnicity, as demonstrated in analyses of data from multiple NHANES cycles. In general, PFAS levels are lowest in Mexican Americans. Non-Hispanic White individuals have been consistently shown to have higher levels of PFOA compared to non-Hispanic Black individuals, while for PFOS levels are similar or somewhat lower among non-Hispanic Black compared to White individuals. Sensitivity analyses restricted to studies that included or separated diverse racial/ethnic groups would be worth considering.

https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume2_Mar2021-508.pdf

Calafat et al. <https://ehp.niehs.nih.gov/doi/10.1289/ehp.10598>

Calafat et al. <https://pubs.acs.org/doi/10.1021/es062686m>

Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water

1. Section 4.2 presents EPA’s meta-analysis for the total cholesterol dose-response function. Boyle, Chen, Lipworth, Savitz

a. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

b. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.

As a comment on the overall charge, the document needs to more clearly acknowledge that the epidemiologic literature does *not* provide support for a direct effect of PFAS on the risk of cardiovascular disease as a health endpoint (and, consistent with this, the MCLG documents concluded that there was not sufficient evidence for CVD to justify RfD calculations for that endpoint).

In general, in the steps prior to conducting the TC or HDLC meta-analysis, more details are needed on the protocol for risk of bias assessment/confidence rankings and whether that approach was used before considering the weight of the evidence and/or carrying studies forward for meta-analysis. This piece is critical for answering the question as to whether the meta-analysis approach or the use of a single dose-response study is appropriate. Beyond details provided about which studies are chosen for inclusion or exclusion, critical additional details are needed on how the particular domains were selected for internal validity/risk of bias/confidence rankings, on objective criteria for how deficiencies in certain/multiple domains contributed to an overall low confidence rating, and on the approach for the evidence synthesis process and the designations of strong vs. suggestive evidence, including whether a “tiered” approach (similar to a sensitivity analysis) was considered to evaluate whether interpretation or conclusions changed based on varied decisions about inclusion and rating of high, medium and low confidence studies across various study design domains.

Some additional concerns related to the basis for exclusion of studies from consideration. Studies were downgraded for “study sensitivity”, for example if they had “limited exposure contrasts and/or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present.” Narrow exposure ranges should not automatically lead to downgrading of studies as these can still contribute informative data within that narrow range and do not necessarily bias to the null. Additionally, the reports mention exclusion of publications with specific population subsets. More transparency and information is needed on how this decision was reached and used as a basis for exclusion. If exclusions are being made based on certain population subsets, then did EPA consider providing data synthesis or estimates of effect in those particular population subsets? It would be helpful to see the studies that were excluded due to being conducted in “specific population subsets” in order to judge the decision to exclude them. What is the objective basis for exclusion other than occupational populations? What is the rationale for determining that the Steenland et al. 2009 study (C8 cohort) is not a “special population” subset?

In the slope estimation, the associations for HDL-C and PFOA and PFOS were positive, albeit not statistically significant. Moreover, there was significant heterogeneity and small sample-size. I question the decision not to include HDL-C in the CVD risk reduction analysis simply on the basis of lack of statistical significance. EPA also concludes that the systematic review of HDLC associations found inconsistent and weak evidence to support PFOA or PFOS effects on HDLC; again, as indicated, description of an objective and transparent approach to this evidence synthesis is needed.

3. Section 5.2 presents EPA’s application of the atherosclerotic cardiovascular disease (ASCVD) risk model used to estimate the probability of hard CVD events corresponding to total cholesterol changes. Boyle, Hammitt, Lipworth, Olmstead

a. Please comment on the scientific validity of the ASCVD model application for estimating the probability of first time CVD events in various sub-populations and the extent to which it is clearly described.

b. Please comment on whether EPA's approach and assumption, of a uniform first CVD event hazard distribution over the 10-year period, is sufficiently robust given current data sources and literature. If additional distributional sources of information are suggested, please provide specific citations/sources for EPA's consideration.

c. Please comment on the scientific validity of using the ASCVD risk model for estimating reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA and PFOS in drinking water.

The ASCVD model represents a scientifically valid approach for estimating the probability of first time CVD events. However, there are many limitations of the model that need to be considered and the accuracy of the model predictions need to be discussed in more detail as they relate to EPA's application (some of these concerns also carry over to CVD CQ#4 on uncertainties/limitations).

Many studies have examined the discrimination and calibration of the pooled cohort equation (PCE) risk model, and both under-estimation and over-estimation of risk have been demonstrated depending on the characteristics of the study population, in particular in relation to age, race and socioeconomic factors.

For example, the PCE was derived from cohort data among individuals aged 40-79 years, and the model does not allow for changes in risk associations with increasing age (assigns fixed weights to each risk factor regardless of age). A recent study (Dalton et al; doi: [10.1111/jgs.16329](https://doi.org/10.1111/jgs.16329)) among older individuals aged 65+ demonstrated poor performance of the ASCVD model in predicting cardiovascular events in this older population, with the exception of white males aged 65-74. Of particular relevance, associations with systolic blood pressure, total cholesterol and diabetes weakened as a function of age.

Regarding socioeconomic factors, investigators in the REGARDS cohort of Black and White adults have shown that ASCVD risk is overestimated among those with less social deprivation, defined on the basis of income, education and living alone (Colantonio et al; <https://doi.org/10.1161/JAHA.117.005676>). Among those with more social deprivation, the PCE had good calibration or underestimated ASCVD risk. Thus, adding information on social deprivation resulted in modest improvement in risk classification.

A paper has recently been published in JAMA Cardiology, using the Framingham cohort study data, showing that inclusion of former smoking status, pack-years and years since quitting smoking improves ASCVD risk prediction among White individuals over the reference model with 2013 PCE variables (Duncan et al, doi:10.1001/jamacardio.2021.4990). The results require replication in other racial and ethnic groups, but this revised model with inclusion of detailed smoking variables could impact the results of EPA's exercise.

Whether or not all components in the ASVCD risk model reach the threshold for POD derivation (e.g. blood pressure) or statistical significance in a meta-analysis (e.g. HDL-C), I strongly question the decision not to consider PFOA and PFOS effects on other parameters in the ASCVD model when estimating avoided CVD risk as a result of reduction of PFOA and PFOS

in drinking water. In particular, for HDL cholesterol (even if an observed pooled effect estimate is not statistically significant) and blood pressure (including antihypertensive use), both of which are associated with cardiovascular outcomes. These factors have been shown to have similar discrimination for ASCVD risk in certain populations when included in the model.

PRELIMINARY COMMENTS

Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal (MCLG) for PFOA and PFOS in Drinking Water

1. Study Identification and Inclusion **Chen, Post, Pullen-Fedinick**

Health effects evidence synthesis and integration

The protocol for risk of bias assessment and, more importantly, how that approach was used in a synthesis of evidence is not sufficiently clearly presented to be confidently judged for accuracy or transparency, or for consistency across health endpoints. This is especially important when a proposed systematic review protocol has not been previously registered or published. Beyond details provided about which studies are chosen for inclusion or exclusion, critical additional details are needed on how the particular seven domains were selected for internal validity/risk of bias/confidence rankings, on objective criteria for how deficiencies in certain/multiple domains contributed to an overall low confidence rating, and on the approach for the evidence synthesis process, including whether a “tiered” approach (similar to a sensitivity analysis) was considered to evaluate whether interpretation or conclusions changed based on varied decisions about inclusion and rating of high, medium and low confidence studies across various study design domains.

Studies were downgraded for “study sensitivity”, for example if they had “limited exposure contrasts and/or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present” (page 46) Narrow exposure ranges should not automatically lead to downgrading of studies as these can still contribute informative data within that narrow range and do not necessarily bias to the null.

Finally, the reports mention exclusion of publications with specific population subsets. More transparency and information is needed on how this decision was reached and used as a basis for exclusion. If exclusions are being made based on certain population subsets, then did EPA consider providing data synthesis or estimates of effect in those particular population subsets?

2. Noncancer Hazard Identification DeWitt, **Post**, Savitz

Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

Cardiovascular endpoints/blood pressure

The rationale for including studies on blood pressure should be consistent with the rationale for including studies of lipids, and the clinical relevance of both should be treated comparably if using ASCVD risk calculator which includes both components (see CVD Charge questions below). Why rate as deficient studies that did not account for lipid lowering medications (this was the most common reason for a low confidence rating) but not apply the same rating for blood pressure medications?

4. Cancer

c. Cancer classification for PFOA/PFOS DeWitt, Post, Lipworth

PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a ‘likely carcinogen’ categorization which is a change from ‘suggestive’ in the 2016 HA. Does the panel agree with the ‘likely’ designation based on the new evidence? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

Although there is supporting animal and mechanistic evidence, from an epidemiologic perspective, this modified categorization is based on a single *medium confidence* study (Shearer et al. 2021) and in the absence of any *high confidence* epidemiologic studies. Again, for the cancer classification, it would help to have an objective, well-described approach for evidence synthesis that incorporated the prior studies and new study. US EPA Guidelines for Carcinogen Risk Assessment (EPA, 2005) state that “When human data of high quality and adequate statistical power are available, they are generally preferable over animal data and should be given greater weight in hazard characterization and dose-response assessment, although both can be used.” In addition, “an inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality.”

Consistency of the collective evidence is not convincingly supportive of the new designation. While the EPA document cites the paper by Bartell and Vieira for support, that review and meta-analysis included studies of occupational populations which were considered as “special population subsets” in EPA’s approach and excluded.

PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a ‘suggestive’ categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

d. Cancer Slope Quantification

EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends. Fisher, Lipworth, Kamendulis

Lifetime RCC risk estimation relied on lifetime risk of kidney cancer in US males. RCC baseline risk is higher in Black individuals compared to White individuals. How does the CSF differ if different rates are applied?

7. Epidemiological Study RfD Derivation

a. EPA evaluated **potential confounding** as part of their study quality evaluation of the epidemiological studies and selected only ‘medium’ and ‘high’ quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies? Lipworth, Savitz, Slitt

It is unclear how serious risk for residual confounding (low confidence) differs substantively from “lack of control for confounding” (critical deficiency, uninformative), and thus how these different decisions may affect the results of the RfD derivation.

The document emphasizes potential residual confounding by SES in numerous places and this contributes to confidence rankings for certain domains – and often contributes substantially to an overall low confidence rating for a study. “SES” is loosely used throughout the document and is a broad term with many contextual components – would be helpful to tease this out a bit, to the extent possible, and to determine a) which components of SES are expected/suspected to confound associations of PFAS with particular health endpoints; and b) if studies adjusted for potential confounding by *any* of these SES components vs. none.

Race/ethnicity – most studies have been conducted among White populations. Studies have reported higher concentrations of PFAS among Black individuals, who also have a higher incidence of several important outcomes including RCC. Sensitivity analyses restricted to studies that included/separated diverse racial/ethnic groups would be worth considering.

Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water

1. Section 4.2 presents EPA’s meta-analysis for the total cholesterol dose-response function. Boyle, Chen, Lipworth, Savitz

a. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

b. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level*

Goals for PFOA and PFOS in Drinking Water would add additional strengths for the CVD risk reduction application.

Would be helpful to see the studies that were excluded due to being conducted in “specific population subsets” in order to judge the decision to exclude them. What is the objective basis for exclusion other than occupational populations? What is the rationale for determining that the Steenland et al. 2009 study (C8 cohort) is not a “special population” subset?

For the four general population studies that were excluded due to inadequate data (page 4), did EPA consider contacting the authors to provide the needed information such as interquartile ranges, etc?

In the slope estimation, the associations for HDL-C and PFOA and PFOS were positive, albeit not statistically significant. Moreover, there was significant heterogeneity and small sample-size. I question the decision not to include HDL-C in the CVD risk reduction analysis simply on the basis of lack of statistical significance. EPA also concludes that the systematic review of HDLC associations found inconsistent and weak evidence to support PFOA or PFOS effects on HDLC; again, as indicated below, description of an objective and transparent approach to this evidence synthesis is needed.

As discussed in detail in the section above (Charge question 1), in the steps prior to conducting the TC or HDLC meta-analysis, more details are needed on the protocol for risk of bias assessment/confidence rankings and whether that approach was used before considering the weight of the evidence and/or carrying studies forward for meta-analysis. The evidence synthesis protocol should also include a “tiered” approach to evaluate whether results or conclusions change based on varied decisions about inclusion of high, medium and low confidence studies across various study design domains. This piece is critical for answering the question as to whether the meta-analysis approach or the use of a single dose-response study is appropriate.

3. Section 5.2 presents EPA’s application of the atherosclerotic cardiovascular disease (ASCVD) risk model used to estimate the probability of hard CVD events corresponding to total cholesterol changes. Boyle, Hammitt, Lipworth, Olmstead

a. Please comment on the scientific validity of the ASCVD model application for estimating the probability of first time CVD events in various sub-populations and the extent to which it is clearly described.

Many studies have examined the discrimination and calibration of the pooled cohort equation (PCE) risk model, and both under-estimation and over-estimation of risk have been demonstrated depending on the characteristics of the study population, in particular in relation to age, race and socioeconomic factors.

For example, the PCE was derived from cohort data among individuals aged 40-79 years, and the model does not allow for changes in risk associations with increasing age (assigns fixed weights to each risk factor regardless of age). A recent study (Dalton et al; doi: [10.1111/jgs.16329](https://doi.org/10.1111/jgs.16329))

among older individuals aged 65+ demonstrated poor performance of the ASCVD model in predicting cardiovascular events in this older population, with the exception of white males aged 65-74. Of particular relevance, associations with systolic blood pressure, total cholesterol and diabetes weakened as a function of age.

Regarding socioeconomic factors, investigators in the REGARDS cohort of Black and White adults have shown that ASCVD risk is overestimated among those with less social deprivation, defined on the basis of income, education and living alone (Colantonio et al; <https://doi.org/10.1161/JAHA.117.005676>). Among those with more social deprivation, the PCE had good calibration or underestimated ASCVD risk. Thus, adding information on social deprivation resulted in modest improvement in risk classification.

A paper has recently been published in JAMA Cardiology, using the Framingham cohort study data, showing that inclusion of former smoking status, pack-years and years since quitting smoking improves ASCVD risk prediction among White individuals over the reference model with 2013 pooled cohort equation variables. The results still require replication in other racial and ethnic groups. (Duncan et al, doi:10.1001/jamacardio.2021.4990), but this revised model with inclusion of detailed smoking variables could be considered for reclassification if the data allow.

b. Please comment on whether EPA's approach and assumption, of a uniform first CVD event hazard distribution over the 10-year period, is sufficiently robust given current data sources and literature. If additional distributional sources of information are suggested, please provide specific citations/sources for EPA's consideration.

c. Please comment on the scientific validity of using the ASCVD risk model for estimating reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA and PFOS in drinking water.

Whether or not all components in the ASVCD risk model reach the threshold for POD derivation (e.g. blood pressure) or statistical significance in a meta-analysis (e.g. HDL-C), I strongly question the decision not to consider PFOA and PFOS effects on other parameters in the ASCVD model when estimating avoided CVD risk as a result of reduction of PFOA and PFOS in drinking water. In particular, for HDL cholesterol (even if an observed pooled effect estimate is not statistically significant) and blood pressure (including antihypertensive use), both of which are associated with cardiovascular outcomes - see specific questions related to the analysis of blood pressure evidence in Charge Question 2 above. Lipids, blood pressure and diabetes have been shown to have similar discrimination for ASCVD risk in certain populations when included in the model.

See also comment above regarding recent evidence of a lack of relationship between total cholesterol and cardiovascular events among older patients.

Other comments

[Section 3.1. Can EPA explain why the 90th percentile \(.20 ug/L\) was used as "average" baseline concentration, as opposed to a different value such as median or mean?](#)

REVISED COMMENTS

Overall charge: EPA is seeking SAB comment on the extent to which the approach to estimating reductions in CVD risk associated with reductions in exposure to PFOA and PFOS in drinking water is scientifically supported and clearly described.

- Overall, the approach to estimate CVD risk reductions is clearly described. Because this approach requires many assumptions, listing all the assumptions would aid in the application of this approach and an assessment of its applicability.

12. Section 4.2 presents EPA’s meta-analysis for the total cholesterol dose-response function.

- i. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.
 - Please clarify the selection criteria of “general population” for study inclusion/exclusion. For example, the draft notes that studies on occupational populations were excluded because they represented greater exposure levels as compared to the general population (Appendix A, Section A.1.1). However, of the 14 studies included in the meta-analysis, the Steeneland et al. (2009) study was from a highly exposed population in the US, and so decision to include this study does not seem to be consistent with the stated selection criteria.
 - It would be helpful to describe the populations of the *excluded* studies that provided information on the relationship between PFAS and TC/HDLC. This corresponds to the 30 studies from the ATSDR-based literature review and the 27 studies from the EPA/OST-based review shown in Figure A-1. Description and justification (where needed) of the populations of excluded studies would help ensure that the study selection approach used is consistent.
 - For extraction of slope values for TC and HDLC, Section A.2.1 (pg. 6) states that when multiple models with different confounders were reported within a single study, *either* the most adjusted results or the main model results were selected. Is it common that these two criteria lead to different slopes being selected? If so, would this be reflected in the tests of publication bias?
- ii. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.
 - Given that the meta-analysis sensitivity results suggest a high degree of heterogeneity in the effect of PFOA/PFOS on TC, I do not believe using a single dose-response study is appropriate.
 - The impact of PFOA/PFOS on TC, derived from existing studies using observational data, is potentially subject to confounding, where the direction of the bias depends on the

nature of human behavior (e.g., avoidance behavior versus correlated exposures). For avoidance behavior, if individuals engage in defensive, health-protective measures (e.g., install filtration systems or buy bottled water), then the estimated relationship from human studies is net of such avoidance efforts. *Had* individuals *not* engaged in such avoidance behaviors, then the estimated impact of PFOA/PFOS may be larger (i.e., this would cause the effect to be underestimated). On the other hand, if individuals who are highly exposed to PFOA/PFOS in drinking water also have other habits that increase TC (e.g., frequently consume fast food), then this would cause the effect to be overstated. It would be useful to examine a set of studies that have, at minimum, some controls for confounders and/or some strategy to remove confounding factors (e.g., use of longitudinal data to remove individual-specific factors that both increase PFOA/PFOS exposure and TC) and show the sensitivity of the treatment impact with respect to such confounding effects.

13. Section 5.1 presents EPA’s life table approach methodology.

- C. Please comment on the extent to which this analysis is scientifically supported and clearly described. To the extent improvements are suggested, please provide specific changes that are implementable in a U.S. national-level benefits analysis with readily available data.
- The life table approach is clearly described in the text and figures 2 and 3 are clear. I had some difficulty following Table 3 on its own. The descriptions in the Appendix (with the equations) were very helpful. If possible, it would be useful to have the equation labels from Appendix B (B.3.2, B.3.3, B.3.4) in the Table 3 steps. Where possible, a mapping to Figure 3 would be useful as well.
 - This section lists previous regulatory analyses performed by the EPA that use life table calculations (Pg. 15). Since some of the pollutants have implications for CVD (e.g., PM2.5), it may be informative to highlight the key differences between the current application of the life table methodology and that in relevant regulatory analyses conducted previously.

4. Section 7 and Appendix A describe the limitations and uncertainties of the CVD risk reduction analysis.

- i. Has EPA clearly described the individual contributions of the sources of uncertainty
- Table 7 justifications have varying levels of detail. For example, some elements include a justification with citations, while others do not. If there is evidence supporting the assumption that PFOA and PFOS effects on TC are independent (Uncertainty #6), then source of information could be documented to be consistent with the details for other uncertainties. For Uncertainty #4 in Table 7, it would be helpful to clearly note what “high-quality data” means.
 - For the fraction of the population who smokes and has diabetes as an input into the ASCVD model (Uncertainty #10, from the top), it is not completely clear how the non-linearity leads to an underestimate in the description.
 - Similar to Uncertainty #12 (for the ASCVD model inputs), I believe there should be an element where unobserved human behavior (e.g., avoidance behavior, correlated exposures) could bias the impact of PFOA/PFAS on TC.
 - Related to the uncertainty with respect to the dynamic effects of serum PFOA/PFOS on TC (Uncertainty #3, #8, #14), appendix on pg. 20 notes: “Given the long half-lives of

PFOA and PFOS (with median half-lives of 2.7 and 3.5 years, respectively; Y. Li et al., 2018), current blood serum concentrations are expected to correlate well with past exposures.” Based on my reading of the draft to develop an MCLG, the half-lives of PFOA and PFOS in humans are not well understood, and so it is unclear to me whether there is enough evidence to justify the lack of concern for reverse causality.

- The number of PWS entry points is described to affect the proportion of the population that receives treatment (Pg. 9). Please clarify how this population share is extrapolated to systems with multiple entry points. Is this a source of uncertainty that should be considered? Related to the assumptions about PWSs, the PWS primary source water type is listed as a distinguishing characteristic of the hypothetical PWS (in this case, it was surface water). While the PWS primary source type might affect the treatment of PFOA/PFAS, am I correct that this does not have a material impact of the calculation of CVD risk proposed in this document? Please clarify.

PRELIMINARY COMMENTS

12/21/2021

CVD Risk Reduction Analysis Charge Questions

14. Section 4.2 presents EPA’s meta-analysis for the total cholesterol dose-response function.

- i. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

The primary selection criteria, “general population,” is somewhat unclear, and it would be helpful to clarify. Specifically,

- The document says that there were no meta-analyses of PFOA/PFOS and TC/HDL/C for the relevant age group of interest (pg. 11), so does that mean there were meta-analyses for this relationship populations outside of this age group that were excluded? If so, how do the resulting estimates compare with the one derived by the EPA?
- The meta-analysis developed uses a general population based on “non-workers” or “pregnant women”. The draft notes that studies on occupational populations were excluded because they represented greater exposure levels as compared to the general population (Appendix A, Section A.1.1). However, of the 14 studies included in the meta-analysis, the Steeneland et al. (2009) study was from a highly exposed population in the US; Chateau-Degat et al. (2010) was from a Canadian Inuit population.
- It would be helpful to describe the populations of the *excluded* studies that provided information on the relationship between PFAS and TC/HDL/C but were not considered to be general population. This corresponds to the 30 studies from the ATSDR-based literature review and the 27 studies from the EPA/OST-based review shown in Figure A-1.

For extraction of slope values for TC and HDL/C, Section A.2.1 (pg. 6) states that when multiple models with different confounders were reported within a single study, *either* the most adjusted results or the main model results were selected. Is it common that these two criteria lead to different slopes being selected? If so, would this be reflected in the tests of publication bias?

- ii. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.

It would be useful to examine a set of studies that have, at minimum, some controls for confounders and/or some strategy to remove confounding factors (e.g., use of longitudinal data to remove individual-specific factors that both increase PFOA/PFOS exposure and TC). This relates to my comments for Section 7 (below).

15. Section 5.1 presents EPA's life table approach methodology.

D. Please comment on the extent to which this analysis is scientifically supported and clearly described. To the extent improvements are suggested, please provide specific changes that are implementable in a U.S. national-level benefits analysis with readily available data.

The section lists previous regulatory analyses performed by the EPA that uses life table calculations (Pg. 15). Since some of the pollutants have implications for CVD (e.g., PM_{2.5}), it may be informative to highlight the key differences between the current application of the life table methodology and that in relevant regulatory analyses conducted previously.

The life table approach is clearly described in the text and figures 2 and 3 are clear. I had some difficulty following Table 3 on its own. The descriptions in the Appendix (with the equations) were very helpful. If possible, it would be useful to have the equation labels from Appendix B (B.3.2, B.3.3, B.3.4) in the Table 3 steps. Where possible, a mapping to Figure 3 would be useful as well.

Given that the meta-analysis sensitivity results suggest a high degree of heterogeneity in the effects of PFOA/PFOS on TC, it may be informative to present CVD risk reductions using the life table approach under a range of values for the PFOA/PFOS and TC relationship. For example, this could be shown in a figure similar to Figure 4 for the total reduction in cases under different scenarios.

4. Section 7 and Appendix A describe the limitations and uncertainties of the CVD risk reduction analysis.

i. Has EPA clearly described the individual contributions of the sources of uncertainty

I first list additional sources of uncertainty that I did not see from the draft that is potentially worth considering:

There are two estimates that involve significant uncertainty with respect to human behavioral interactions: (1) the impact of PFOA/PFOS on TC, and (2) the impact of TC on the first CVD event. For (2), the estimated impact of TC and the first CVD event is based on the ASCVD model. For (1), the evidence of PFOA/PFAS on TC is largely based on human studies. Similar to Uncertainty #12 (where the assumed independence between the ASCVD model inputs is likely to overestimate the effect of TC on CVD risk), confounders for (1) from human studies is likely to bias the effect of PFOA/PFOS on TC. The direction of the bias depends on type of human behavior considered:

- Avoidance behavior: If individuals know of the risks of PFAS in drinking water and have installed filtration systems privately (or buy bottled water), then the estimated

relationship from human studies is net of such avoidance efforts. *Had* individuals *not* engaged in such avoidance behavior, then the estimated impact of PFOA/PFOS may be larger (i.e., this would cause the effect to be underestimated).

- Correlated (and unobserved) behavior: If individuals who are highly exposed to PFOA/PFOS in drinking water also have other habits that increase TC (e.g., frequently consume fast food), then it is unclear whether the primary driver of TC is PFOA/PFOS. At minimum, this affects the magnitude of the estimate (i.e., this would cause the effect to be overstated).

Aside from issues of correlated behavior affecting the causal interpretation of the effect of PFOA/PFOS on TC noted above, there is significant heterogeneity in the magnitude of the estimates from the meta-analysis. This may have been the point of Uncertainty #4, but I was not completely clear.

The analysis assumes that non-drinking water exposure is independent of the drinking water PFOA/PFOS concentration (Pg. 10). I do not believe this was listed in the uncertainties table but should be considered since the treatment may affect the RSC.

PWS primary source water type is listed as a distinguishing characteristic of the hypothetical PWS (in this case, it was surface water). While the PWS primary source type might affect the treatment of PFOA/PFAS, am I correct that this does not have a material impact of the calculation of CVD risk? Please clarify.

The number of PWS entry points is described to affect the proportion of the population that receives treatment (Pg. 9). Please clarify how this population share is extrapolated to systems with multiple entry points. What is the magnitude of uncertainty based on this extrapolation?

Next, I list specific questions/comments for elements in Table 7 on uncertainties:

General comments: The table Details column has varying levels of detail (e.g., some include justification with citations, while others do not). It could be helpful to label the elements in the table.

For the fraction of population who smokes and has diabetes as an input into the ASCVD model (Uncertainty #10, from the top), it is not completely clear how the non-linearity leads to an underestimate in the description.

Related to the uncertainty with respect to the dynamic effects of serum PFOA/PFOS on TC (Uncertainty #3, #8, #14), appendix on pg. 20 notes: “Given the long half-lives of PFOA and PFOS (with median half-lives of 2.7 and 3.5 years, respectively; Y. Li et al., 2018), current blood serum concentrations are expected to correlate well with past exposures.” Based on my reading of the draft to develop an MCLG, the half-lives of PFOA and PFOS in humans are not well understood. Although this source of uncertainty was noted as not being analyzed in Table E-1 of the Appendix, this affects how reasonable the ASCVD model is when it starts its prediction for the population at age 40.

For Uncertainty #4, it would be helpful to clearly note what “high-quality data” means.

Dose-response function assumes PFOA and PFOS effects on TC are independent (Uncertainty #6). Is there evidence for or against this being the case? To be consistent with the details for other uncertainties (see general comment for this table), source of information could be documented.

REVISED COMMENTS

These comments are all relevant to the topic covered at the public meeting on Friday, January 7, 2022: *Peer Review of EPA's Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water*.

Responses to Charge Question #1A: Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

Response: I was surprised to see that all but one of the 11 studies in the total cholesterol (TC) meta-analysis were cross-sectional. Lin et al (2019) analyze longitudinal data, but only partially exploit the panel structure of their data in obtaining estimates of the PFOS/PFOA-TC relationship. They are able to include many controls for potential confounders (e.g., individual characteristics), but don't control comprehensively for unobservable characteristics – for example through the use of an individual fixed effect – that might mitigate concerns about endogeneity bias. My primary endogeneity concern is that individuals with a propensity toward high TC may also have a propensity for exposure to and/or accumulation of PFOS/PFOA, but I could also see reverse causality as a potential issue (individuals with high TC tend to accumulate more PFOS/PFOA). Another thing to note about the Lin et al. (2019) study is the high quality of the underlying RCT results that allow them to show that lifestyle modification mitigates the impact of PFOS/PFOA on TC, even in the high-risk group that their data describe (those likely to develop diabetes).

Because TC impacts are currently the only health effects for which the EPA has moved toward monetization (using the ASCVD model to estimate avoided premature mortality from reducing PFOA/PFOS, which can then be multiplied by VSL to estimate economic benefits), the tenuous connection at the first stage of this modeling effort (the literature on the PFOS/PFOA-TC connection) is likely to be a concern in the benefit estimation process for the MCLG, especially if any estimated CVD benefits represent a large share of estimated total benefits. It would be useful for the EPA to provide some additional information on which other health endpoints covered in our review, if any, are likely to be monetized, so that we can get a sense for how important the ASCVD results are likely to be in the big picture of benefit estimation.

Responses to Charge Question #3A: Please comment on the scientific validity of the ASCVD model application for estimating the probability of first time CVD events in various sub-populations and the extent to which it is clearly described.

Response: This approach is clearly described in Section 5.2. The ASCVD model is regularly used to extrapolate from large population averages to estimate risk for individual people. Thus, applying it to estimate risk at the level of “all people served by PWS” or even at the level of an individual PWS seems reasonable.

Responses to Charge Question #3B: Please comment on whether EPA's approach and assumption, of a uniform first CVD event hazard distribution over the 10-year period is sufficiently robust given current data sources and literature. If additional distributional sources of information are suggested, please provide specific citations/sources for EPA's consideration.

Response: I was not able to find a discussion of this approach either in the report, or in Appendix B (to which the report refers). (The word "uniform" shows up once in the appendices, on p. 67 in reference to another part of the analysis.) So I am not entirely sure that I understand the approach, but here is one thought: assuming a uniform distribution does not account for changes in risk over time. For example, Goff et al. (2014) recommend that 10-year CVD risk for an individual be re-estimated every 4-6 years in adults age 40-79, presumably because the risk of an event increases with age and behavioral factors. Unless one believes that population risk is shifting significantly over time, this may not be necessary for EPA's application of the ASCVD model for this purpose, and the assumption of uniform population risk over 10 years may be fine.

Responses to Charge Question #3C: Please comment on the scientific validity of using the ASCVD risk model for estimating reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA and PFOS in drinking water.

Response: The key question would seem to be whether one would expect CVD to respond similarly to an increase in total cholesterol from exposure to PFAS in drinking water as it does to increases in TC caused by other drivers (those likely to have been assumed by those who constructed the ASCVD risk model) - things like diet, genetics, etc. Given my expertise, I cannot comment on the viability of that assumption. I do see a paper in the literature that appears to summarize current thinking about the mechanisms that might explain the observed TC/PFAS association - it might be useful to engage the authors of this study on this question:

Andersen, Melvin E. et al. 2021. Why is elevation of serum cholesterol associated with exposure to perfluoroalkyl substances (PFAS) in humans? A workshop report on potential mechanisms. *Toxicology* 459: 152845.

Responses to Charge Question #4: Has EPA clearly described the individual contributions of the sources of uncertainty?

Response: The individual contributions of the sources of uncertainty are clearly described in Appendix E and Section 7 of the report (Table 7). I have two suggestions in this regard.

1. I suggest either adding a row to Table 7 on uncertainty related to HDLC effects, or adding a brief discussion of this to the top row on p. 31. That row in Table 7 focuses on systolic BP as an example of an excluded effect, which is why the "effect on estimate" column says "underestimate" (because if BP also increases, the CVD estimates would increase). But with respect to HDLC, where there are some weakly significant estimates in EPA's own analysis, inclusion of those effects would presumably reduce the CVD

estimates. So if you mention HDLC and BP in that row, the "effect on estimate" would switch to "uncertain." I think it would be clearest to just have two separate rows, for HDLC and all other excluded outcomes.

2. In the national modeling effort, EPA should identify from the long list in Table 7 the uncertainties that are likely the most significant, and then do some sensitivity analysis to demonstrate their potential importance. In some cases, Table 7 currently suggests that the "effect on estimate" is "uncertain" for aspects of the modeling that could be tested relatively easily. For example, in row 3 on p 31, wouldn't it be straightforward to simply use both the non-Hispanic white and non-Hispanic Black model for the other race/ethnicity groups (see my related comment above)? A good example of this kind of work appears in the row just below that one, which quantifies the likely impact of the assumptions regarding the fraction of the population that smokes and has diabetes -- more work like this is needed for the major uncertainties.

Additional peer-reviewed papers that might help with monetization of some of the health endpoints for EPA's benefit estimation process related to the PFOS/PFOA MCLG. Below are three papers from the economics literature on the links between low birthweight and education/income.

1. Chyna, E.; Gold, S.; and J. Hastings. 2021. The returns to early-life interventions for very low birthweight children. *Journal of Health Economics* 75:102400.
2. Fletcher, JM. 2011. The medium term schooling and health effects of low birth weight: Evidence from siblings. *Economics of Education Review* 30:517–527.
3. Bharadwaj, P.; Vellesen Loken, K.; and C Neilson. 2013. Early Life Health Interventions and Academic Achievement. *American Economic Review*, 103(5):1862–1891.

PRELIMINARY COMMENTS

Responses to Charge Question #3

- A. *Please comment on the scientific validity of the ASCVD model application for estimating the probability of first time CVD events in various sub-populations and the extent to which it is clearly described.*

This approach is clearly described in Section 5.2. The ASCVD model is regularly used to extrapolate from large population averages to estimate risk for individual people. Thus, applying it to estimate risk at the level of “all people served by PWS” or even at the level of an individual PWS seems reasonable.

It would be helpful if EPA could explain why they chose to use the non-Hispanic Black ASCVD model coefficients for Hispanic, Asian American, and American Indian/Native American people, rather than non-Hispanic white coefficients, given that the text of the report indicates that Goff et al. (2014) recommend using the non-Hispanic white

coefficients for this purpose. The coefficients vary quite a bit across these groups in Appendix B Table B.3. How much of a difference would it make in the Table 6 results to take the approach recommended by Goff et al. (2014)? (Table 7 suggests that the direction of the effect of this assumption on the estimate is “uncertain,” but it seems like this would be straightforward to test.)

- B. ***Please comment on whether EPA’s approach and assumption, of a uniform first CVD event hazard distribution over the 10-year period is sufficiently robust given current data sources and literature. If additional distributional sources of information are suggested, please provide specific citations/sources for EPA’s consideration.***

I was not able to find a discussion of this approach either in the report, or in Appendix B (to which the report refers). (The word "uniform" shows up once in the appendices, on p. 67 in reference to another part of the analysis.) So I am not entirely sure that I understand the approach, but here is one thought: assuming a uniform distribution does not account for changes in risk over time. For example, Goff et al. (2014) recommend that 10-year CVD risk for an *individual* be re-estimated every 4-6 years in adults age 40-79, presumably because the risk of an event increases with age and behavioral factors. Unless one believes that population risk is shifting significantly over time, this may not be necessary for EPA’s application of the ASCVD model for this purpose, and the assumption of uniform population risk over 10 years may be fine.

- C. ***Please comment on the scientific validity of using the ASCVD risk model for estimating reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA and PFOS in drinking water.***

The key question would seem to be whether one would expect CVD to respond similarly to an increase in total cholesterol from exposure to PFAS in drinking water as it does to increases in TC caused by other drivers (those likely to have been assumed by those who constructed the ASCVD risk model) - things like diet, genetics, etc. Given my expertise, I cannot comment on the viability of that assumption. I do see a paper in the literature that appears to summarize current thinking about the mechanisms that might explain the observed TC/PFAS association - it might be useful to engage the authors of this study on this question:

Andersen, Melvin E. et al. 2021. Why is elevation of serum cholesterol associated with exposure to perfluoroalkyl substances (PFAS) in humans? A workshop report on potential mechanisms. *Toxicology* 459: 152845.

Responses to Charge Question #4

- A. ***Has EPA clearly described the individual contributions of the sources of uncertainty?***

The individual contributions of the sources of uncertainty are clearly described in Section 7 of the report (Table 7). I have a two suggestions in this regard.

1. I suggest either adding a row to Table 7 on uncertainty related to HDLC effects, or adding a brief discussion of this to the top row on p. 31. That row in Table 7

focuses on systolic BP as an example of an excluded effect, which is why the "effect on estimate" column says "underestimate" (because if BP also increases, the CVD estimates would increase). But with respect to HDLC, where there are some weakly significant estimates in EPA's own analysis, inclusion of those effects would presumably reduce the CVD estimates. So if you mention HDLC and BP in that row, the "effect on estimate" would switch to "uncertain." I think it would be clearest to just have two separate rows, for HDLC and all other excluded outcomes.

2. In the national modeling effort, EPA should identify from the long list in Table 7 the uncertainties that are likely the most significant, and then do some sensitivity analysis to demonstrate their potential importance. In some cases, Table 7 currently suggests that the "effect on estimate" is "uncertain" for aspects of the modeling that could be tested relatively easily. For example, in row 3 on p 31, wouldn't it be straightforward to simply use both the non-Hispanic white and non-Hispanic Black model for the other race/ethnicity groups (see my related comment above)? A good example of this kind of work appears in the row just below that one, which quantifies the likely impact of the assumptions regarding the fraction of the population that smokes and has diabetes -- more work like this is needed for the major uncertainties.

REVISED COMMENTS

Responses to Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Study Identification and Inclusion

Charge Question 1: *EPA used systematic review methods consistent with the current ORD systematic review practice to ensure transparency and completeness of literature identification, sorting, and study quality evaluation. Is the process clearly described? Please identify additional peer-reviewed studies that the panel is aware of that could inform toxicity value derivation.*

Response: These assessments will provide the basis for the PFOA and PFOS MCLGs. They therefore have large practical impacts and will likely receive extensive attention and scrutiny. For this reason, it is stressed that a strong and transparent rationale for all decisions and conclusions and a thorough description of all steps in the evaluation of the scientific literature be provided.

It is recognized that EPA plans to propose drinking water standards that will be supported by these MCLG documents within a short timeframe, and that substantial resources would be needed to make the revisions suggested below for all endpoints included in the drafts. The draft EPA evaluations, as well as evaluations by other scientists, indicate that the human evidence for noncancer effects is strongest for effects on the liver, immune system, serum lipids, and fetal growth. The effects with the strongest evidence from animal studies could also be identified; these would likely include hepatic, immune system, and developmental effects, and possibly others. A possible approach to making the suggested revisions with the available resources would be to focus on revisions for those endpoints that have been concluded to have the strongest evidence, rather than for all endpoints.

General comments

Section 1.0 (Background) states: “Fit-for-purpose systematic review methods, also consistent with current EPA methods, were used to develop the toxicity values within the timeline to rule proposal and in order to follow a transparent and scientifically robust process to identify, evaluate, and synthesize the best available science.” However, the specifics of the “fit-for-purpose” systematic review methods that were used to allow for completion of the assessment “within the timeline to rule proposal” do not appear to be provided. For example, information should be provided on any modifications to the Office of Research and Development's systematic review approaches presented in the draft EPA ORD staff handbook for developing IRIS assessments (USEPA, 2020), which is cited later in the document.

Within Section 2.0 (Methods for PFOA/PFOS Health Effects Systematic Review), Section 2.1 on the criteria for study inclusion states that: “Systematic review methods used were largely

consistent with the recent draft ORD staff handbook for developing IRIS assessments (EPA, 2020...).” As written, it is not clear that the systematic review approaches from EPA (2020) were used for just the study identification and inclusion components of the toxicity assessment but not for the other components of the assessment such as evidence synthesis and integration. It As discussed later in my response to this charge question and my response to the Noncancer Hazard Identification charge question, the lack of a consistent approach for evidence synthesis and integration for the different health outcomes is problematic. This issue was also mentioned in several public comments.

Reliance on results of literature search used for 2016 HESDs

As discussed in the draft PFOA and PFOS documents and at the December 6 SAB PFAS panel meeting, current systematic review approaches were not used for the literature searches and study inclusion steps in the 2016 HESDs. At the December 6 meeting, there was a discussion of whether the literature review and study inclusion approaches for the 2016 HESDs had missed key studies, and I stated that I did not have concerns that key studies were missed.

I am now revising my earlier statement because I later noticed that a key study of immunotoxicity is not cited in the 2016 HESD for PFOS. Specifically, Dong et al. (2011 <https://link.springer.com/article/10.1007%2Fs00204-011-0661-x>), which reported that PFOS caused a dose-related decrease in serum levels of sheep red blood cells (SRBC)-specific IgM in mice, was not included in the 2016 HESD. Dong et al. (2011) is a key study because it was used as the critical study for the PFOS Reference Doses and drinking water guidelines established by three states (MN, NH, WA; reviewed in Post, 2021 <https://setac.onlinelibrary.wiley.com/doi/full/10.1002/etc.4863>). After noticing that this key study was not included in the 2016 HESD, I am no longer completely confident that other important studies were not also omitted.

Consideration of human studies included in 2016 HESDs

The rationale for not considering human studies that were included in the 2016 HESDs is not clear or supportable. There is no reason to believe that the earlier studies are less relevant or of lesser quality than the newer studies. Consideration of all human studies is especially important because conclusions about the level of evidence for human health effects, which are generally observational rather than experimental, are based on the overall weight of evidence from all relevant data. My comments on this issue are also included in my response to the first charge question in the Noncancer Hazard Identification section.

The 2016 HESD for PFOA concluded that there was substantial evidence for human effects of PFOA, as follows: "Human epidemiology data report associations between PFOA exposure and high cholesterol, increased liver enzymes, decreased vaccination response, thyroid disorders, pregnancy-induced hypertension and preeclampsia, and cancer (testicular and kidney)" and that "...human data identified significant relationships between serum levels and specific indicators of adverse health effects..." The reason that human studies were not considered as the basis for PODs and RfDs in the 2016 HESDs was not related to the quality of the studies themselves or to a lack of overall evidence for human effects, but was rather due to the lack of a toxicokinetic

model to relate internal dose (serum levels) to external exposure (administered dose); the 2016 PFOA HESD states that human data were not used as the basis for PODs and RfDs because of "lack [of] the exposure information for dose-response modeling." This is no longer an issue because EPA has now accepted a model (Verner et al., 2016) that can relate serum PFOA and PFOS levels to external dose during developmental lifestages and throughout life, and it has concluded that the human data for PFOA and PFOS can be used in dose-response modeling.

The evaluation of the association of PFOA and high cholesterol in occupationally exposed workers in the draft PFOA document illustrates the need to consider all epidemiology studies, including those considered in the 2016 HESD, in making strength of evidence conclusions. As stated in Section 3.3.5.1.2.1, the 2016 HESD concluded that there was "relatively consistent and robust" evidence of an association of PFOA and increased serum cholesterol in the occupational worker studies. However, the draft PFOA document reviewed only the three newer occupational worker studies not included in the 2016 HESD, all of which were rated as "low confidence." The draft PFOA document states that these newer studies "suggest no association between PFOA and TC in workers" and that "differences in findings from occupational studies between the 2016 [HESD] and this review may be attributable to limitations of occupational studies in this review." There does not appear to be a supportable rationale for making a conclusion based on only three low confidence studies when other potentially stronger studies are also available. As such, it seems clear that the overall strength of evidence conclusion should consider both the older and newer worker studies.

Another important aspect of this issue is that specific human epidemiology studies included in the 2016 HESDs may be preferable to the more recent studies for POD development for some health endpoints. As an illustration of this point, the PFOA and PFOS RfD are based on a dataset from Grandjean et al. (2012), a study that was included in the 2016 HESDs. Specifically, the RfDs are based on serum PFOA or PFOS levels at age 5 and tetanus or diphtheria vaccine antibody concentrations at age 7 in Cohort 3 (born 1997-2000) from Grandjean et al. (2012). Subsequent studies from the same research group (Mogensen et al., 2015; Grandjean et al., 2017) re-published this dataset and/or used this dataset in some of their analyses, but the BMDL (from Budtz-Jorgensen and Grandjean, 2018) that was used as the POD for the final RfD comes only from the dataset originally presented in Grandjean et al. (2012). If the subsequent papers that included the dataset from Grandjean et al. (2012) in additional analyses had not been published, the Grandjean et al. (2012) study and dataset used as the basis for the RfDs would not even have been considered for POD development. This example clearly demonstrates why the older human studies included in the 2016 HESD should be considered.

Additional details relevant to this point are: Grandjean et al. (2012) is not included in the table summarizing epidemiology studies (Table C-7) in the PFOA and PFOS documents, and there is no systematic review evaluation of Grandjean et al. (2012) in HAWC. If the dataset from Grandjean et al. (2012) remains the basis for the PFOA and PFOS RfDs, a HAWC systematic review of this study should be performed and included in the final document.

PECO criteria, literature search strategy, screening process, and study evaluation

In the draft PFOA and PFOS documents, the list of "relevant forms" of PFOA and PFOS is incomplete in the PECO criteria for human and animal studies of health effects of PFOA and PFOS (Table 2). Table 2 does not include salts of PFOA such as ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, and potassium perfluorooctanoate, or salts of PFOS such as lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate (K+PFOS), ammonium perfluorooctanesulfonate, or sodium perfluorooctanesulfonate, although these salts are listed in the PECO criteria for ADME and mechanistic studies (Tables 4 and 5).

The omission of salts of PFOA and PFOS from the table of PECO criteria for human and animal studies is problematic because a salt of PFOA (e.g., APFO) or PFOS (e.g., K+PFOS) was used as the test compound in all of the toxicity studies of PFOA and PFOS in laboratory animals reviewed in the draft documents. These salts completely dissociate to the anionic form (PFOA, PFOS) in the body, water, and other environmental media. While it is likely that the publications reporting the animal toxicity studies include the term "PFOA" or "PFOS" as well the salt of PFOA or PFOS that was used, the salts should be included from the "relevant forms" in the PECO criteria.

I noticed that the salts were not listed in Table 2 while reviewing the summary of Convertino et al. (2018) in the Hazard Identification section of PFOA. Convertino et al. (2018) is a human study in which advanced cancer patients were orally dosed with APFO. (While not relevant to this comment on PECO criteria, problematic issues with this study are discussed in my response to the first Noncancer Hazard Identification charge question.) The draft PFOA document states that subjects were dosed with "ammonium perfluorooctanoate (APFO), a PFOA precursor" and that this study "differed from the other studies in several ways" including that "participants ingested APFO rather than being exposed to PFOA." However, APFO is not a "PFOA precursor" in the conventional sense, and, as above, salts were the test substances in animal studies. Also, while it is true that human exposure from drinking water and other environmental media is to the anionic form, PFOA (since salts disassociate in water), occupationally exposed workers were exposed to APFO. For example, Lundin et al. (2009 https://journals.lww.com/epidem/Fulltext/2009/11000/Ammonium_Perfluorooctanoate_Producti_on_and.25.aspx) state that they "conducted a mortality study in a cohort of 3993 employees of an ammonium perfluorooctanoate (APFO) manufacturing facility. APFO rapidly dissociates to PFOA in blood."

Additionally, there are some inconsistencies among different sections of the draft document as to the durations of the studies that were included/not included, and clarification is needed. For example, Table 2 of the PFOA document states: "Studies with less than 28 days of dosing, with the exception of reproductive or developmental studies, should be tagged as supplemental," but Section 3.1.3, which is about literature search results for animal toxicology studies, states, "of the 32 animal studies that met the inclusion criteria, most studies had either short-term (n = 15) or developmental (n = 12) study designs." The inclusion of short-term studies other than developmental studies in Section 3.1.3 does not seem to be consistent with the information in Table 2. Possibly relevant to this issue, non-developmental studies with a shorter than 28 day

duration were considered in the 2016 HESD. The 2016 HESD states on p. 4-4: "A number of studies identified adverse effects following low dose exposures over durations of 7 to 38 days. The studies fall into two clusters, those evaluating developmental or reproductive effects and those with a focus on immunological effects." For example, DeWitt et al. (2008), which is a key study from the 2016 HESD, is a 15-day study.

For study evaluations, the domains that were evaluated for the human and animal studies are can be found in the HAWC database. However, the domains that were evaluated are not shown within the PFOA or PFOS documents in either the text or a table. For clarity, it is suggested that these domains also be included in the documents so that readers can easily find this information.

Regarding studies selected for dose-response evaluation, it is stated in the PFOA and PFOS documents that "Studies were evaluated for use in POD derivation on the basis of study design, study quality evaluation, and data availability." As written, it is unclear that the overall weight of evidence that PFOA and PFOS causes the effects was also considered in selecting the endpoints used for POD derivation, and this should be clarified.

It is also stated in the draft PFOA and PFOS documents that: "For human evidence, all study designs were considered; for animal evidence, only animal studies with at least two exposure groups and with high or medium for study quality were considered." It is unclear if this means that human studies with low confidence were considered. If all human studies were considered, the rationale for considering human studies, but not animal studies, that have low confidence should be provided.

Data extraction

The Dose-response Studies subsection of the Data Extraction section in the PFOA and PFOS documents states: "Data extraction was conducted for most studies that were included in the literature inventory, except those excluded as described below. ... Extractions were limited to outcomes of interest and/or the most sensitive LOAEL." It is not clear whether this means the most sensitive LOAEL based on internal dose (serum PFOA or PFOS level) or administered dose. For most human studies, the doses are based on serum levels (internal dose). For animal studies, the lowest LOAEL based on administered dose may not correspond to the lowest LOAEL based on internal dose (serum level) because of toxicokinetic (half-life) differences between species or sexes (e.g., much quicker excretion rate in female vs. male rats). While evaluating this issue for all studies for which it is relevant may not be possible due to resource limitations, it is suggested that this potential uncertainty in the approach be acknowledged.

Additionally, it is stated in this same section that that "...low confidence studies when medium and high confidence studies (e.g., on an outcome) were available" did not undergo data extraction." This appear to contradict the statement in Section 2.4 that low confidence animal studies were not considered and that "all study designs" (not specifying study confidence level) were considered for human studies. This information should be clarified and should be consistent in the two sections.

Information on where the ADME and mechanistic data that were extracted can be found does not appear to be included in documents. If the data extracted from these studies are not publicly available, this should be stated.

More importantly, mechanistic data are not summarized in this document, except for summary tables of the number of studies with each type of mechanistic information that were identified. Discussion of the mode(s) and/or mechanism(s) of action for toxicity is normally an important component of toxicity assessments such as these PFOA and PFOS assessments. As discussed in the draft ORD staff handbook for developing IRIS assessments (EPA, 2020), such an evaluation can provide information about the human relevance of effects observed in animal species and the plausibility of effects observed in humans, among other areas of potential uncertainty. EPA did provide a rationale for concluding that mechanistic data does not need to be considered in cancer and non-cancer hazard identification and that this information can be added later. While recognizing that it may not be possible to include an evaluation of mechanistic data for all health effects, a mechanistic or mode of action evaluations for the noncancer endpoint(s) selected as critical endpoints for RfD(s) and for the weight of evidence carcinogenicity would help to provide support for EPA's conclusions.

Evidence synthesis

The evidence synthesis/integration approach presented in the Systematic Review section is generally clear and appropriate. However, the Health Effects Evidence Synthesis and Integration sections on each health outcome in the Hazard Identification Section 3.3 do not appear to consistently follow the process presented in the Systematic Review Section 2.6. For example, it is stated in the Systematic Review section that "a summary discussion that addresses considerations regarding causation as adapted from Hill (1965)" is provided for each health outcome. However, this was not consistently done in the Evidence Synthesis and Integration sections in Hazard Identification (Section 3.3). Also, as mentioned above, there is frequently little or no discussion of mechanistic information in the Hazard Identification section, with only a reference to the section in the 2016 HESD on this topic and a statement that an updated evaluation will be completed after the SAB review.

Furthermore, the content, format, and terminology are inconsistent among the evidence synthesis sections for different health endpoints. EPA stated at the December 16, 2021 SAB meeting that a structured approach was not used for evidence synthesis and acknowledged the lack of consistency among evidence synthesis sections. They stated that different sections were written by different scientists who used professional judgement as to the terms used and the way conclusions are presented. These inconsistencies make it difficult to compare the conclusions of the different health effects sections.

Specifically, the conclusions in different Evidence Integration sections do not use consistent terminology. For example, terms which appear to have similar meanings are used ("*suggestive evidence*," "*moderate evidence*," and "*consistent evidence*"; "*inconsistent evidence*" and "*mixed evidence*"), but no definitions of these terms are provided.

Furthermore, the overall nature of the conclusions for different health effects is not presented consistently (e.g., "*suggestive evidence for an association of PFOS with [the health outcome]*", "*suggestive evidence that PFOA impacts [the health outcome]*", or "*suggestive evidence for an effect of PFOA on [the health outcome]*"). It is unclear from the wording of these conclusions whether they are intended to apply to the evidence for association of the effect with PFOA or to the overall evidence that PFOA causes the effect; this is obviously an important distinction.

Additionally, information is not presented in a consistent manner in the sections on human and animal evidence for different health outcomes. As just one example (from the PFOA document), human studies considered in the 2016 HESD are discussed in detail for some endpoints (e.g., male reproductive, Section 3.3.2.1.1) but are not mentioned for other endpoints (e.g., birth weight), and no rationale is provided for why these studies are or are not discussed for each health outcome. As a second example (also from the PFOA document), the study selection criteria are presented for some endpoints (e.g., Developmental, Section 3.3.1.2) but not for other endpoints (e.g., Reproductive).

Also, it is stated that: "The syntheses of human and animal health effects evidence focused on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of studies." The meaning of "exposure context" here is unclear. It should be clarified whether this refers to the exposure levels that are relevant to environmental exposures, or to something else.

Finally, it is also stated that: "Low confidence studies were used [for evidence synthesis] if few or no studies with higher confidence were available to help evaluate consistency, or if the study designs of the low confidence studies addressed notable uncertainties in the set of high or medium confidence studies on a given health effect." However, Section 2.4.1 says that low confidence animal studies were not considered. This inconsistency should be clarified.

To address these issues, it is suggested that a structured, consistent process and consistent terminology be used for analysis and synthesis of animal evidence, human evidence, and overall evidence be used. One example of such an approach is presented in Chapters 9 and 11 of the draft ORD staff handbook for developing IRIS assessments (EPA, 2020), and an example of the application of this approach in toxicity assessment of another PFAS is found in Sections 3.2 and 4.1 of the draft EPA IRIS assessment of perfluorobutanoic acid (PFBA) at https://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=543579. As stated above, this may not be possible for all health outcomes included in the draft document due to resource limitations. If this is the case, a structured approach should be used to evaluate the evidence for those endpoints that have been concluded to have the strongest evidence.

Additional studies

It is recommended that EPA consider the following additional studies, as well as additional studies suggested by other panel members:

Additional epidemiology studies on associations of PFAS and breastfeeding issues: Nielsen et al. (2021).

<https://www.sciencedirect.com/science/article/pii/S0013935121015073>

Timmerman et al. (2021). <https://academic.oup.com/jcem/advance-article-abstract/doi/10.1210/clinem/dgab638/6369501?redirectedFrom=fulltext>

Additional epidemiology studies on associations of PFAS and vaccine antibody response:

Shih et al. (2021).
<https://www.tandfonline.com/doi/epub/10.1080/1547691X.2021.1922957?needAccess=true>

Timmermann et al. (2022):
<https://www.sciencedirect.com/science/article/pii/S0013935121010069?via%3Dihub>

Additional epidemiology study on associations of PFAS with infectious disease:

Timmermann et al. (2020). <https://ehp.niehs.nih.gov/doi/10.1289/EHP6517>

Dalsager et al. (2021)
<https://www.sciencedirect.com/science/article/pii/S0160412021000192?via%3Dihub/>

Bulka et al. (2021).
<https://www.sciencedirect.com/science/article/pii/S0269749121001974?via%3Dihub1999e2016>

Additional epidemiology study on associations of PFAS with bone health:

Buckley et al. (2021). <https://ehp.niehs.nih.gov/doi/pdf/10.1289/EHP9424>

Banjabi et al. (2020). <https://pubmed.ncbi.nlm.nih.gov/32485360/>

Noncancer Hazard Identification

Charge question 1: Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

List of health effects/outcomes

The list of health effect/outcome categories included in the draft PFOA and PFOS documents appears to be complete and appropriate.

General comments

As a general comment, these assessments will provide the basis for the PFOA and PFOS MCLGs. They therefore have large practical impacts and will likely receive extensive attention and scrutiny. For this reason, it is stressed that a strong and transparent rationale for the conclusions about strength of evidence for health outcomes be provided.

The intent of this charge question was not clear regarding "strong vs. suggestive evidence designations for the various health outcome categories" because a conclusion of "strong evidence" was not made for any health outcome for PFOA and was only made for once (for altered serum lipid levels) for PFOS. At the December 16, 2021 meeting, EPA clarified the intent of the charge question. EPA stated that the question was not intended to ask about "strong vs. moderate evidence designations." It was intended to ask whether the evidence is strong enough to perform dose-response (develop PODs) for the endpoints selected for POD development and whether the evidence for any additional health endpoints is strong enough for POD development. As such, my response below discusses general issues about the strength of evidence designations and selection of endpoints and studies for POD development. Some points in this response are related to my responses to the Systematic Review charge question.

It is recognized that EPA plans to propose drinking water standards that will be supported by these MCLG documents within a short timeframe, and that substantial resources would be needed to make the revisions suggested below for all endpoints included in the drafts. The draft EPA evaluations, as well as evaluations by other scientists, indicate that the human evidence for noncancer effects is strongest for effects on the liver, immune system, serum lipids, and fetal growth. The effects with the strongest evidence from animal studies could also be identified; these would likely include hepatic, immune system, and developmental effects, and possibly others. A possible approach to making the suggested revisions with the available resources would be to focus on revisions to the human, animal, and mechanistic evidence sections and evidence integration sections for those endpoints which have been concluded to have the strongest evidence, rather than for all endpoints.

Effects on health endpoints related to the liver, immune system, serum lipids, and fetal growth were generally consistently reported for PFOA and PFOS. Many of these studies evaluated biomarkers for these effects (e.g., increased serum levels of lipids or liver enzymes, decreased antibody response to vaccines, decreased birthweight). While most studies did not evaluate the number of subjects with a clinically abnormal value, one or more studies of each of these four effects reported an association of PFOA and/or PFOS with increased risk of a clinically abnormal value (e.g., tetanus or diphtheria antibodies levels below a clinically protective level; clinically defined low birth weight or small for gestational age; clinically defined high cholesterol; clinically defined elevated ALT). In studies in which this was not specifically

evaluated, an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. An increase in the number of individuals within the population with clinically defined abnormal values is of public health concern, and exposure to a drinking water contaminant should not cause an increased risk of having a clinically abnormal value. Furthermore, the available evidence indicates an association between PFOA and PFOS and increased risk of infectious disease. This is discussed in more detail later in my response to this charge question and in my response to the later charge question on the selection of critical effect and critical study.

Comments on evidence designations and selection of studies/endpoints for PODs

The sections on Human Evidence and Animal Evidence for the specific health effects/outcomes are often difficult to follow due to the highly summarized presentation of the information and the inconsistent presentation of information and conclusions for different health outcomes (discussed in detail below). Specifically, consistent terminology and a consistent approach was not used for the synthesis of human, animal, and mechanistic evidence and integration of these three types of evidence to make overall hazard conclusions, and it is recommended that a consistent approach be used.

It is suggested that a format or template be developed so that the information can be presented consistently for each endpoint. Summary tables that present basic information for each study, such as study population (e.g., general population, children, pregnant women, occupational, etc.) for epidemiology studies and lab animal species for toxicology studies, exposure range or dose levels, and overall results for the endpoints evaluated (e.g., significant increase, significant decrease, or no effect) would be helpful to understanding the overall human and animal evidence.

Terms used for weight of evidence conclusions

The level of evidence designations and the strength of evidence conclusions in the Evidence Integration sections are difficult to evaluate because a consistent approach and terminology were not used. At the December 16 meeting, EPA stated that consistent language and a structured approach were not used to describe the level of evidence for different health effects because the health endpoints sections were written by different individuals and the conclusions are based on professional judgment. EPA also acknowledged that terms such as "suggestive evidence," "moderate evidence," and other seemingly interchangeable terms are used in sections on different health outcomes, that these terms are not defined in the draft PFOA and PFOS documents, and that there is no intended difference among these seemingly similar terms. Additionally, no strength of evidence term is used in some cases, such as "...the evidence indicates an association between increased serum levels of PFOS and decreased antibody production following routine vaccinations in children," and "EPA concluded the impaired IgM response reported in [animal studies] supported the human results and this endpoint was considered for POD derivation [for PFOA]."

To address these issues, it is suggested that consistent descriptors be defined and used for human, animal, and overall strength of evidence conclusions for each endpoint. As an example, Table

11-5 in the draft ORD staff handbook for developing IRIS assessments (EPA, 2020) "Evidence integration judgments for characterizing potential human health hazards in the evidence integration narrative" provides the following descriptors: "evidence demonstrates", "evidence indicates [likely]", "evidence suggests but is not sufficient to infer", "evidence inadequate", and "strong evidence supports no effect." Criteria and examples for applying these descriptors are also provided. Such an approach would be consistent with the use of consistent descriptors, and consistent criteria for selection of descriptors, for the domains and overall confidence conclusions in the individual study evaluations earlier in the overall process. It would also be consistent with the use of descriptors and criteria for selection of the descriptors for weight of evidence for carcinogenic potential in the EPA (2005) Guidelines for Carcinogen Risk Assessment.

As above, it is recognized that time limitations may prevent incorporation of these suggestions for all health outcomes. A possible approach to address this issue would be to focus on those endpoints which have been concluded to have the strongest evidence, rather than for all endpoints.

Type of conclusion to which strength of evidence evaluation applies

There is also inconsistency among the health outcomes sections in the draft PFOA and PFOS documents in the language regarding the specific type(s) of conclusion to which the strength of evidence terms apply. For example, it may be stated that there is a certain level of evidence (e.g., suggestive) for "associations of PFOA [or PFOS] with [the effect]", while in other cases, it is stated that there is a certain level of evidence (e.g., suggestive) that PFOA or PFOS "impacts [the effect]" or a certain level of evidence "for [the effect]" (or similar language). It is not clear whether these different terms are intended to distinguish between the level of evidence for an association versus the level of evidence supporting a plausible relationship between PFOA or PFOS and the effect. It is recommended the intended meaning of the strength of evidence conclusions for each health outcome be clarified and that consistent terminology be used for these strength of evidence conclusions.

Section 2.6 in the Systematic Review section states that "a summary discussion that addresses considerations regarding causation as adapted from Hill (1965)" is provided for each health outcome. However, such a discussion does not appear to be included for many or most of the health outcomes in the Noncancer Hazard Identification section. Including such a summary discussion of considerations for causation adapted from Hill (1965) would address the issue mentioned above.

As above, it is recognized that time limitations may prevent incorporation of these suggestions for all health outcomes. A possible approach to address this issue would be to focus on those endpoints which have been concluded to have the strongest evidence, rather than for all endpoints.

Consideration of mechanistic information

Evaluation of the mode(s) and/or mechanism(s) of action for toxicity is normally an important part of toxicity assessments such as these PFOA and PFOS assessments. However, there is frequently little or no discussion of mechanistic information in the draft documents, with only summary tables of the number of studies with each type of mechanistic information that were identified, a reference to the section in the 2016 HESD on this topic, and a statement that an updated evaluation will be completed after the SAB review. Mechanistic/mode of action data can help to inform conclusions about the human relevance of effects observed in animal species and the plausibility of effects observed in humans, among other areas of potential uncertainty. It is recommended that an evaluation of mechanistic/mode of action data be included for those effects considered as the potential basis for the RfDs, or, at a minimum, for the effect(s) selected as the basis for the final RfD.

Consideration of human studies included in 2016 HESDs

The rationale for not considering human studies that were included in the 2016 HESDs is not clear or supportable. There is no reason to believe that the earlier studies are less relevant or of lesser quality than the newer studies. Consideration of all human studies is especially important because conclusions about the level of evidence for human health effects, which are generally observational rather than experimental, are based on the overall weight of evidence from all relevant data. My comments on this issue are also included in my response to the Systematic Review charge question.

The 2016 HESD for PFOA concluded that there was substantial evidence for human effects of PFOA, as follows: "Human epidemiology data report associations between PFOA exposure and high cholesterol, increased liver enzymes, decreased vaccination response, thyroid disorders, pregnancy-induced hypertension and preeclampsia, and cancer (testicular and kidney)" and that "...human data identified significant relationships between serum levels and specific indicators of adverse health effects..." The reason that human studies were not considered as the basis for PODs and RfDs in the 2016 HESDs was not related to the quality of the studies themselves or to a lack of overall evidence for human effects, but was rather due to the lack of a toxicokinetic model to relate internal dose (serum levels) to external exposure (administered dose); the 2016 PFOA HESD states that human data were not used as the basis for PODs and RfDs because of "lack [of] the exposure information for dose-response modeling." This is no longer an issue because EPA has now accepted a model (Verner et al., 2016) that can relate serum PFOA and PFOS levels to external dose during developmental lifestages and throughout life, and it has concluded that the human data for PFOA and PFOS can be used in dose-response modeling.

The evaluation of the association of PFOA and high cholesterol in occupationally exposed workers in the draft PFOA document illustrates the need to consider all epidemiology studies, including those considered in the 2016 HESD, in making strength of evidence conclusions. As stated in Section 3.3.5.1.2.1, the 2016 HESD concluded that there was "relatively consistent and

robust" evidence of an association of PFOA and increased serum cholesterol in the occupational worker studies. However, the draft PFOA document reviewed only the three newer occupational worker studies not included in the 2016 HESD, all of which were rated as "low confidence." The draft PFOA document states that these newer studies "suggest no association between PFOA and TC in workers" and that "differences in findings from occupational studies between the 2016 [HESD] and this review may be attributable to limitations of occupational studies in this review." There does not appear to be a supportable rationale for making a conclusion based on only three low confidence studies when other potentially stronger studies are also available. As such, it seems clear that the overall strength of evidence conclusion should consider both the older and newer worker studies.

Another important aspect of this issue is that specific human epidemiology studies included in the 2016 HESDs may be preferable to the more recent studies for POD development for some health endpoints. As an illustration of this point, the PFOA and PFOS RfD are based on a dataset from Grandjean et al. (2012), a study that was included in the 2016 HESDs. Specifically, the RfDs are based on serum PFOA or PFOS levels at age 5 and tetanus or diphtheria vaccine antibody concentrations at age 7 in Cohort 3 (born 1997-2000) from Grandjean et al. (2012). Subsequent studies from the same research group (Mogensen et al., 2015; Grandjean et al., 2017) re-published this dataset and/or used this dataset in some of their analyses, but the BMDL (from Budtz-Jorgensen and Grandjean, 2018) that was used as the POD for the final RfD comes only from the dataset originally presented in Grandjean et al. (2012). If the subsequent papers that included the dataset from Grandjean et al. (2012) in additional analyses had not been published, the Grandjean et al. (2012) study and dataset used as the basis for the RfDs would not even have been considered for POD development. This example clearly demonstrates why the older human studies included in the 2016 HESD should be considered.

Additional details relevant to this point are: Grandjean et al. (2012) is not included in the table summarizing epidemiology studies (Table C-7) in the PFOA and PFOS documents, and there is no systematic review evaluation of Grandjean et al. (2012) in HAWC. If the dataset from Grandjean et al. (2012) remains the basis for the PFOA and PFOS RfDs, a HAWC systematic review of this study should be performed and included in the final document.

Selection of health effects for POD development

As discussed in Dr. Weihsueh Chiu's preliminary responses, the hazard identification (strength of evidence for a health outcome) and selection of studies for POD development are sequential steps that should be discussed separately. There may be strong evidence for a hazard even if no studies are suitable for POD derivation, and, conversely, there may be studies appropriate for POD derivation in the absence of strong evidence for a hazard. However, these two steps are often not clearly separated in the draft documents, and this issue should be addressed in the final document.

Additionally, the rationale and criteria for selection of endpoints for POD development are not always clearly presented. PODs can potentially be used as the basis for the Reference Dose.

Therefore, it is important to clearly demonstrate that the endpoints selected for POD development are well established, sensitive, adverse or precursor to adverse, and that endpoints from animal studies are relevant to humans.

Furthermore, the information about development of PODs for effects described as having "suggestive" evidence in different sections of the draft documents is internally inconsistent. The documents state (PFOA - p. 317; PFOS – p. 290) that human studies showing an "association" for an effect were used for PODs, but it then goes on to indicate that human health effects with "suggestive" evidence were not used for PODs, as follows: “Well-conducted ... human studies were prioritized for POD derivation and compared to PODs derived from animal data when possible when the human data provided an association between PFOA and an adverse effect. Such human studies were available for immunotoxicity, developmental, serum lipid, and hepatic effects. For other health effects where the epidemiological data were suggestive of adverse effects, dose response data from the animal studies were prioritized.” Although the text quoted above indicates that human effects with suggestive evidence were not used for PODs, the human effects that were used for PODs (immune, developmental, serum lipids [for PFOA], hepatic) were described as having "suggestive" or "moderate" evidence; a conclusion of "strong" evidence was provided only for PFOS and increased serum lipids. While recognizing that EPA did not use approaches from the draft ORD IRIS staff handbook (EPA, 2020) for strength of evidence determinations in the draft PFOA and PFOS documents, it should be noted that EPA (2020) does not recommend that PODs for health endpoints with “suggestive” evidence be used as the basis of toxicity values (i.e., Reference Doses). Because of the uncertainty associated with such PODs, EPA (2020) recommends that they be used only for range finding and prioritization.

To address this issue, EPA should clarify the internal inconsistencies mentioned above. Additionally, it is suggested that EPA consider reevaluating its strength of evidence conclusions for some human endpoints, including (but not necessarily limited to) decreased immune response, increased liver enzymes, increased serum lipids (for PFOA), and decreased fetal growth to determine if they are better described as having “likely” or "strong" evidence rather than “suggestive” or "moderate" evidence. A conclusion of "likely" or "strong" evidence would provide additional support for development of a POD to be used as the basis for Reference Dose.

Based on the EPA (2020) criteria for “likely” evidence,³ and the assumption that “strong evidence” mentioned in the charge question is equivalent to “evidence demonstrates” or “likely

³ For “evidence indicates (likely)”, there should be “an evidence base that indicates that [PFOA] exposure likely causes [the health effect] in humans, although there may be outstanding questions or limitations that remain...,” and “this conclusion level is used if there is robust animal evidence supporting an effect and slight-to-indeterminate human evidence, or with moderate human evidence when strong mechanistic evidence is lacking. This conclusion level could also be used with moderate human evidence supporting an effect and slight or indeterminate animal evidence, or with moderate animal evidence supporting an effect and slight or indeterminate human evidence. In these scenarios, any uncertainties in the moderate evidence are not sufficient to substantially reduce confidence in the reliability of the evidence, or mechanistic evidence in the slight or indeterminate evidence base (e.g., precursors) exists to increase confidence in the reliability of the moderate evidence.”

evidence” in EPA (2020), there appears to be sufficient evidence to classify additional endpoints as "strong" or “likely,” particularly if the studies included in the 2016 HESD and more recent studies published after the ending date of the literature search for the current draft are considered. As mentioned in my response to the systematic review charge question, consideration of at least the earlier studies from the 2016 HESD is necessary for determining the overall weight of evidence for each health outcome.

Using immunosuppression as just one example, the Evidence Integration section (3.3.4.4) for immune effects of PFOA states: “The evidence of an association between PFOA exposure and immunosuppressive effects in human studies is moderate based on largely consistent decreases in antibody response following vaccination ... in two medium confidence, overlapping birth cohorts.” Consideration of studies that evaluated vaccine response in populations in other locations, including older studies from the 2016 HESD and recent studies not included in the current draft (some have been suggested by SAB panel members), could potentially support the conclusion that the evidence for decreased antibody response to vaccines is stronger than "moderate."

Also relevant to strength of evidence for immunosuppression, the Human Evidence subsections of the Immune sections review studies of PFOA and PFOS and infectious disease, many of which found associations with increased risk. Additionally, Pachkowski et al. (2019 <https://www.sciencedirect.com/science/article/abs/pii/S0013935118304286?via%3Dihub>) concluded that the studies available through 2018 "provide evidence for an association between general population levels of PFOS exposure and infectious disease, a clinical meaningful measure of health risk," although evidence for PFOA was not reviewed. Several recent studies not cited in the draft documents⁴ provide further support for this conclusion for both PFOA and PFOS. These include not cited in draft documents include: Timmermann et al. (2020 <https://ehp.niehs.nih.gov/doi/10.1289/EHP6517>); Dalsager et al. (2021 <https://www.sciencedirect.com/science/article/pii/S0160412021000192?via%3Dihub/>); Bulka et al. (2021 <https://www.sciencedirect.com/science/article/pii/S0269749121001974?via%3Dihub1999e2016>) However, the Evidence Integration subsections of the Immune sections do not provide overall conclusions on infectious disease and do not even mention infectious disease. The reason for this omission is unclear, especially since immunosuppression is the critical effect for the RfD. Weight of evidence conclusions should be developed for infectious disease, and these conclusions may provide further support for the choice of decreased antibody response to vaccination as the critical effect for the RfD.

Need for clarifications in Hazard Identification information on serum cholesterol

It is important that issues related to the strength of evidence for PFOA and PFOS and increased serum cholesterol be discussed clearly and thoroughly, especially since this effect is a major part of the basis for the separate evaluation of cardiovascular disease risk. The draft document states that PFOA and PFOS caused decreased serum lipids in some animal studies while lipids

are increased in human studies, and that this interspecies difference may be due to the "difference in serum lipid composition between humans and commonly used rodent models" and that "food consumption may confound these results, as diet is a major source of lipids, yet studies do not consistently report a fasting period before serum collection."

Other potentially important explanations for this human versus animal difference in the effect of PFOA and PFOS on serum lipids should be discussed in the documents. These include lower human exposure levels compared to the doses used in animal studies (see discussion below), differences in the fat content of human diets versus rodent lab diet, and differences in the activity of PPAR-alpha in humans and laboratory animals. These issues were investigated in studies reviewed in DWQI (2017 <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>) including Tan et al. (2012, <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0061409>) and Rebholz et al. (2016 <https://www.sciencedirect.com/science/article/pii/S2214750015300822?via%3Dihub>), as well as newer studies such as Schlezinger et al. (2020, <https://www.sciencedirect.com/science/article/abs/pii/S0041008X20303306?via%3Dihub>).

In general, human and rodent data suggest that the effects of PFOA and other PFAS on lipid formation and storage results from the balance of different effects which may act in opposite directions (Das et al., 2017 <https://www.sciencedirect.com/science/article/abs/pii/S0300483X16303390?via%3Dihub>). The decrease in serum lipids at the higher doses used in animal studies is believed to be due to activation of PPAR-alpha (DWQI, 2017). PPAR-alpha is also active in humans, as demonstrated by the use of PPAR-alpha activating drugs to decrease high cholesterol in humans. However, PFOA, PFOA, and other PFAS do not activate PPAR-alpha in humans at lower environmentally relevant doses, and the increased serum lipids associated with PFOA, PFOS, and other PFAS may result from activation of other receptors/biological pathways involved with lipid metabolism that act in the opposite direction.

Convertino et al. (2018) is a study of advanced cancer patients who were given extraordinarily high doses of PFOA. The draft PFOA document acknowledges concerns and limitations for this study. It states that "participants dosed with extremely high levels of ammonium perfluorooctanoate (APFO), a PFOA precursor, in an open-label, nonrandomized, phase 1 trial, were found to have reduced levels of total cholesterol with increasing plasma PFOA concentrations." It further states that Convertino et al. (2018) "differed from the other studies in several ways. First, all participants were solid-tumor cancer patients who failed standard therapy. Second, participants ingested APFO rather than being exposed to PFOA. Third, participants' plasma PFOA concentrations were several orders of magnitude higher than those reported in the general population," and that "it is unclear if these factors explained the inverse association between PFOA and total cholesterol." This study was rated as "low confidence" by EPA.

Convertino et al. (2018) may arguably fulfill the PECO criteria for health effects studies of PFOA in humans shown in Table 2. However, in my opinion, Convertino et al. (2018) does not appear to be appropriate for consideration in hazard identification of PFOA. I agree with the NJDEP (2020) conclusion that Convertino et al. (2018) "is not useful in the evaluation of

potential health effects of chronic drinking water exposure to PFOA in the general population," and that "limitations of this study include small sample size, very short length, limited power of study, and potential altered metabolic state of study group consisting of late-stage cancer patients. Observations in these patients cannot be considered relevant to healthy individuals because their nutritional and physiological status was likely affected by their severe illness."

The draft PFOA document acknowledges some of these problematic issues with Convertino et al. (2018). If consideration of this study remains in the document, it is suggested that the additional information provided below be included:

An earlier abstract about this study (Macpherson et al., 2010

<https://www.sciencedirect.com/science/article/abs/pii/S135963491072098X?via%3Dihub>)

stated that one of the patients dosed experienced drug related toxicity (DLT) consisting of "grade 5 renal failure and transaminitis" (indicative of liver damage), and that these effects were noted as "possibly drug related." This indicates the potential for PFOA to cause renal and hepatic toxicity in humans, and that it is unclear why the observation of "possibly drug related" kidney and liver toxicity reported by Macpherson et al. (2010) was not mentioned by Convertino et al. (2018).

Also, as mentioned above, the plasma PFOA levels in the subjects in this study were extraordinarily high. The plasma PFOA levels in the 10 exposure categories shown in Figure 4 of Convertino et al. (2018) ranged from ~4000 ng/ml to ~630,000 ng/ml. Cholesterol was decreased only in the three highest exposure categories (approximately 262,000 ng/ml or higher plasma PFOA), but not in the seven lower exposure categories that also had extremely high plasma PFOA levels of up to approximately 200,000 ng/ml. The plasma PFOA levels at which cholesterol was decreased are many orders of magnitude above those found in the general population or in communities with contaminated drinking water. They are higher than the highest serum or plasma PFOA levels in occupationally exposed workers in data summarized in Table 5-27 of ATSDR (2021 <https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf>), and they are similar to the serum PFOA levels at which cholesterol is decreased in animal studies, presumably through activation of PPAR-alpha. The observation of decreased cholesterol at these extremely high plasma concentrations is consistent with the effects of PPAR-alpha activating drugs that reduce serum cholesterol in humans. In contrast, the increased cholesterol associated with PFOA in the general population and in individuals exposed through contaminated drinking water likely occurs through a different mechanism that is operational at much lower PFOA concentrations.

Selection of specific studies for POD development

A clearer explanation should be provided throughout as to why specific studies were selected for POD development when there are a number of possible choices. As one example, the BMDL presented in Dong et al. (2019) was used by EPA as the POD for increased serum cholesterol. However, no information is provided in the PFOA document as to why Dong et al. (2019) was selected for dose-response for serum lipids from the many studies that are available.

Furthermore, the BMD modeling section (Appendix B) states that few details are provided by the

authors about several aspects of the BMD modeling presented in the Dong et al. (2019) publication; this lack of information does not appear to support use of this BMDL as a POD.

It is possible, although not stated in the draft documents, that Dong et al. (2019) was selected because studies included in the 2016 HESD were not considered. Again, it is strongly recommended that older studies that were included in the 2016 HESD be considered for POD development, and it is noted that some of these older studies were considered in the draft "Analysis of CVD Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water." As discussed in my response to the Systematic Review charge question, there is no reason to assume that the more recent studies are preferable to the older studies, as illustrated by the choice of a dataset from Grandjean et al. (2012) as the basis for the final RfDs for both PFOA and PFOS in the draft documents.

Furthermore, the list of studies and endpoints considered for POD development in Table 15 is not totally consistent with the information in the text and the dose-response results in Appendix B. For example, some of the studies/endpoints (corpora lutea, body weight) stated in the text of the PFOA document to have been selected for dose-response/POD development were not modeled, while other endpoints (kidney weight) stated not to be selected for dose-response/POD development were modeled. Also, it is stated in the text that prenatal loss from Lau et al. (2006) was modeled, but the data from Wolf et al. (2007), not Lau et al. (2006), were actually modeled.

There is also inconsistency among the evidence integration sections for different health outcomes regarding information about whether any human and/or animal studies and, if so, which ones, were selected for POD development. For example, the human evidence integration discussions for developmental effects do not state whether any of the human studies were selected for POD development, although several birthweight studies were later selected (Table 15) for both PFOA and PFOS. As another example, the evidence integration sections for immune effects in both documents do not state that a human study of vaccine response (which eventually was used as the basis for the final RfD) was selected for POD development. In contrast, the evidence integration sections for some other endpoints specifically mention whether or not any studies were selected for POD development, and, if so, which studies were selected.

Finally, while it is stated (in the text quoted above - PFOA - p. 317, first paragraph; PFOS – p. 290, final paragraph) that PODs were developed from human data for hepatic effects, human data for hepatic effects was not used for POD development (as stated for PFOA on 148, for PFOS on p. 133, and in the charge question below).

Additional endpoints for POD derivation

I am not aware of any additional endpoints that should be considered for POD derivation.

Charge Questions 2 and 3: *Elevation of liver serum biomarkers in humans is frequently used an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater*

than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.

Charge Questions 2 and 3, Part A. *Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.*

EPA's rationale for not considering the ALT endpoint from human studies for derivation of a POD, particularly for PFOA, is not completely clear and does not appear to be supportable. As stated in the charge question, increased ALT is indicative of liver damage. EPA (2002) guidelines for RfD development (<https://www.epa.gov/sites/default/files/2014-12/documents/rfd-final.pdf>) state that a RfD should be based on an adverse effect or a precursor to an adverse effect, and this guidance supports considering ALT for RfD development.

The draft EPA PFOA document discusses Darrow et al. (2016), which reported an association between modeled PFOA exposure and increased risk of clinically elevated ALT in a population with elevated PFOA exposure from contaminated drinking water. Gallo et al. (2012 <https://pubmed.ncbi.nlm.nih.gov/22289616/>), which was not included in the draft PFOA document but was reviewed in the 2016 HESD, also found an association between measured serum PFOA levels and increased risk of clinically elevated ALT in the same study group. As discussed in my more general response to the first Noncancer Hazard Identification charge question, although the increase in clinically elevated ALT was specifically evaluated in only those two studies, it is expected that this does occur whenever there is an increase in ALT overall.

Dr. Deborah Cory-Slechta's preliminary response to this charge question provide important additional information to support the conclusion that increased ALT should be considered for POD derivation. She provided information indicating that increased ALT is associated with increased risk of liver-related morbidity and mortality.

Additionally, a recent review of epidemiological evidence for health effects of PFOA by Steenland et al. (2020, <https://www.sciencedirect.com/science/article/pii/S0160412020320808>) noted that: "There is also evidence that effects on ALT are more pronounced among obese subjects, who are at higher risk of nonalcoholic fatty liver disease (Lin et al. 2010 https://journals.lww.com/ajg/Abstract/2010/06000/Investigation_of_the_Associations_Between_Low_Dose.24.aspx; Jain and Ducatman 2019, https://journals.lww.com/joem/Abstract/2019/04000/Selective_Associations_of_Recent_Low.5.a.spx)."

Futhermore, the public comments submitted by an occupational physician who is a PFAS researcher provide an important clinical perspective on the importance of the increases in ALT associated with PFOA, and it is recommended the EPA consider these comments. These comments indicate that the charge question's statement that increases in ALT of less than 2-fold are not clinically relevant is not accurate, and that the percent increases in ALT (such as 6% in Darrow et al., 2016) associated with PFOA are clinically relevant. The comments also indicate that the current clinical cutoffs for elevated ALT are lower than those used in Darrow et al. (2016), which reported an increase in number of subjected with clinically elevated ALT even using the higher older clinical cutoff values. Additionally, the comments state that increases in serum ALT and serum lipids are both related to hepatic toxicity, and that these effects should be considered together rather than in isolation.

It is also noted that California EPA (2021; <https://oehha.ca.gov/media/downloads/crn/pfoapfosphgdraft061021.pdf>) selected increased risk of clinically elevated serum ALT as the basis for its draft Reference Dose for PFOA. In their evaluation, California EPA (2021) considered some of the issues related to use of elevated ALT in humans as a critical effect that are discussed in the charge question above. It is suggested that EPA review the California EPA (2021) rationale for its decision to use elevated ALT as the critical effect for Reference Dose development.

Charge Questions 2 and 3, Part B. *Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.*

As mentioned in my response to the Systematic Review charge question above, I recommend that the epidemiological studies included in the 2016 HESD be considered when evaluating the weight of evidence for epidemiological effects of PFOA and PFOS, including the studies reporting associations with increased ALT.

Additionally, as noted by Steenland et al. (2020), a recent study of adults from a community with elevated exposure to PFOA from contaminated drinking water showed that PFOA "was associated with cytokeratin 18 M30, a marker of hepatocyte apoptosis (Bassler et al., 2019), and a mechanism of disease progression in nonalcoholic fatty liver disease." Bassler et al. (2019, <https://www.sciencedirect.com/science/article/abs/pii/S0269749118341599>) provides further evidence that PFOA causes liver cell injury, and it is suggested that EPA consider this study.

The draft PFOA document (p. 133, last paragraph) states that Jin et al. (2020) found increased odds of nonalcoholic steatohepatitis in a medium confidence study. It is unclear why it is then stated that Darrow (2016) is the only medium confidence study of liver disease, since nonalcoholic steatohepatitis, reported by Jin et al. (2020), is a liver disease.

Additionally, hepatic effects of PFOA in laboratory animals may be relevant to the potential for PFOA to cause fatty liver in humans. It is suggested that a discussion of steatosis caused by PFOA and other PFAS in mice (Das et al., 2017 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5994610/>) be added to Section 3.3.3.2.3. It is also suggested that Quist et al. (2015, <https://journals.sagepub.com/doi/full/10.1177/0192623314551841>), which reported

histopathological changes in mouse liver that are observable only with electron microscopy at very PFOA low doses, be discussed here.

c) Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

Cancer

Charge Question 1. *Cancer classification for PFOA/PFOS*

Charge Question 1, Part A. *PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a 'likely carcinogen' categorization which is a change from 'suggestive' in the 2016 HA. Does the panel agree with the 'likely' designation based on the new evidence? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.*

Designation of PFOA as 'likely carcinogen'

Based on the results of human and animal studies of PFOA presented in in the draft document, I agree that it is appropriate to designate PFOA as "Likely to Be Carcinogenic to Humans."

As discussed in the draft EPA PFOA document, PFOA was associated with increased risk of testicular and kidney cancer in studies of a large population with elevated exposure from contaminated drinking water (Barry et al., 2013; Vieira et al., 2013) and with increased risk of kidney cancer in a large general population study (Shearer et al., 2020).

PFOA also caused testicular Leydig cell, pancreatic acinar cell, and/or hepatocellular tumors in male Sprague-Dawley rats in three chronic studies (Butenhoff et al., 2012 [also reported by Sibinski, 1987]; Biegel et al., 2001; NTP, 2020). In NTP (2020), the incidence of both malignant and benign tumors was increased, and the incidence of pancreatic tumors was very high in all dosed groups of males (2020). However, the occurrence of malignant tumors and the very high incidence of pancreatic tumors is not mentioned in the draft PFOA document, and it should be added because it adds to the weight of evidence for PFOA's carcinogenic potential. In There was also a marginal increase in hepatocellular carcinomas and uterine adenocarcinomas, and non-significant increases in benign and malignant pancreatic acinar cell tumors, in females in NTP (2020). The lower response in females was stated by NTP (2020) to be consistent with the lower plasma PFOA levels due to the rapid excretion of PFOA in female rats.

Mode of action analyses (2016 PFOA HESD; DWQI, 2017

<https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>) have concluded that the rat liver tumors caused by PFOA may not be relevant to humans, although this is not a settled issue. However, the mode of action for other types of tumors caused by PFOA in rats has not been established, and, as specified in the EPA (2005) Guidelines for Carcinogen Risk Assessment, they are considered relevant to humans.

An additional study that is relevant to the mode of action for carcinogenicity of PFOA is the initiation-promotion study in rainbow trout (Benninghoff et al., 2012 <https://academic.oup.com/toxsci/article/125/1/69/1668619>). This study was discussed in regard to carcinogenicity of PFOS in preliminary comments from other SAB Panel members and at the

January 6 SAB Panel meeting. In this study, PFOA significantly increased the number of tumors and the diameter of liver tumor compared to controls treated with the same initiator. The increase in number of liver tumors was greater for PFOA than for PFOS, and PFOS did not cause a significant increase in tumor diameter. As reviewed in DWQI (2017, <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>), the overall significance of this study is that PFOA increased hepatic tumors in "rainbow trout, a species used as a model for human liver carcinogenesis because it is insensitive to peroxisome proliferation [e.g., PPAR-alpha activation], suggest[ing] that PFOA promotes liver tumor development through an estrogenic mechanism." This study is therefore important to understanding the mode of action of hepatic tumors caused by PFOA because, although it is not a settled issue, it has been suggested that rodent liver tumors caused by PFOA and other PFAS occur through PPAR-alpha activation that is not relevant to humans.

Rationale for 'likely carcinogen' designation

The designation of PFOA as "Likely to Be Carcinogenic to Humans" would have large practical impacts because the MCLG for "likely carcinogens" is zero. For this reason, it is stressed that it is especially important for EPA to provide a strong and transparent rationale for this conclusion. As discussed above, human, animal, and mode of action studies support designation of PFOA as "Likely to Be Carcinogenic to Humans." However, the rationale for this designation is not adequately provided in the draft document. Specifically, a discussion of how the relevant data fulfill the criteria for designation as a "likely" carcinogen from the EPA (2005) Guidelines for Carcinogen Risk Assessment is not presented.

Specifically, the EPA (2005) Guidelines for Carcinogen Risk Assessment provide several examples of data that support the descriptor "Likely to Be Carcinogenic to Humans." The PFOA document should demonstrate that the available data for PFOA are consistent with one or more of the three examples below:

- "an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments."
- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans."
- "a positive tumor study that is strengthened by other lines of evidence, for example, ... plausible (but not definitively causal) association between human exposure and cancer."

The PFOA document should also demonstrate that data relevant to carcinogenic potential of PFOA are stronger than in the four examples below from the EPA (2005) guidelines for the descriptor "Suggestive Evidence of Carcinogenic Potential."

- "a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor 'Likely to Be Carcinogenic to Humans.' The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system."

- "a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed."
- "evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence (such as structure-activity relationships)."
- "a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend."

Additionally, the draft PFOA document (p. 343-344) discusses a genotoxic versus non-genotoxic mode of action for carcinogenicity of PFOA, followed by the conclusion that PFOA is considered "Likely to Be Carcinogenic to Humans." As such, it should be made clear that the designation of "likely to be carcinogenic to humans" is independent of whether the mode of action is genotoxic or non-genotoxic.

Finally, the weight of evidence evaluation (i.e., determination of the appropriate descriptor for carcinogenic potential, such as "suggestive" or "likely") is part of the Hazard Identification component of the risk assessment, not the Dose-Response component. As such, the Weight of Evidence section (section 4.2) should be moved from the Dose-Response section to the Hazard Identification section (e.g., Evidence Integration) on cancer.

Charge Question 1, Part B. PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a 'suggestive' categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

The information on new cancer studies for PFOS identified since the 2016 HESD and the significance of this new information for weight of evidence for carcinogenicity of PFOS should be expanded and clarified.

In the draft PFOS document, the Hazard Identification section on cancer (Section 3.3.1.7) discusses that Shearer et al. (2021) showed an association of PFOS with kidney cancer. Comparison of the discussion of this topic in Section 3.3.1.7 of the PFOS document with the discussion of the same topic in the PFOA document indicates that some, but not all, of the specific analyses that were statistically significant for PFOA were not significant for PFOS.

Specifically, the draft EPA PFOS document states (p. 286-287):

"PFOS was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma) in a medium confidence study {Shearer, 2021, 7161466}. The study reported a statistically significant increase in risk in the highest exposure quartile and per doubling of PFOS concentration. After adjusting for other PFAS the association remained elevated in the highest quartile (i.e., adjusted OR=1.14), but it was no longer statistically

significant and was lower than the second quartile; additionally, there was no association when evaluated on a per doubling of PFOS."

This can be compared to the information from the draft EPA PFOA document (p. 309):

"PFOA was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma (RCC)) {Shearer, 2021, 7161466}. This large medium confidence case-control study nested within the NCI's Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO) reported a statistically significant increase in risk of kidney cancer in highest exposure quartile and per doubling of PFOA concentration. Even after adjusting for other PFAS the association remained significant in analyses on a per doubling increase in PFOA. The increase in the highest exposure quartile remained and the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS), but it was no longer statistically significant."

However, the draft PFOS document does not mention that the Shearer et al. (2021) results were used for a CSF for PFOA, and it does not discuss why the PFOS results are not considered to be as definitive as the PFOA results. Additionally, the cancer weight of evidence section (Section 4.2.1) of the draft PFOS document does not mention Shearer et al. (2021) at all in its discussion of new PFOS cancer studies identified since the 2016 HESD. These issues should be addressed in the final document.

Li et al. (2022, <https://www.sciencedirect.com/science/article/pii/S0013935121015188>) is a recent study that may be relevant to weight for carcinogenicity of PFOS, and it is suggested that it be discussed. This study reported an increased incidence of kidney cancer in a Swedish population exposed to drinking water contaminated with a mixture of PFAS, with PFOS predominating.

An additional study that is relevant to the mode of action for carcinogenicity of PFOS is the initiation-promotion study in rainbow trout (Benninghoff et al., 2012 <https://academic.oup.com/toxsci/article/125/1/69/1668619>). This study was discussed in regard to carcinogenicity of PFOS in preliminary comments from other SAB Panel members and at the January 6 SAB Panel meeting. In this study, PFOS significantly increased the number of tumors compared to controls treated with the same initiator; this increase was greater for PFOA than for PFOS. PFOS did not cause a significant increase in tumor diameter compared to controls treated with the same initiator, while this effect was significant for PFOA. As reviewed in DWQI (2017, <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>), the overall significance of this study is that PFAS increased hepatic tumors in "rainbow trout, a species used as a model for human liver carcinogenesis because it is insensitive to peroxisome proliferation [e.g., PPAR-alpha activation], suggest[ing] that PFOA promotes liver tumor development through an estrogenic mechanism." As discussed above, this study is important to understanding the mode of action of hepatic tumors caused by PFOA because it has been suggested that rodent liver tumors caused by PFOA occur through PPAR-alpha activation that is not relevant to humans. However, this is not an issue for PFOS because the rodent liver tumors caused by PFOS do not appear to be PPAR-alpha dependent. Specifically, DWQI (2018 <https://www.state.nj.us/dep/watersupply/pdf/pfos-recommendation-appendix-a.pdf>) concluded that "available data do not support the conclusion that PFOS causes liver effects through a PPAR α -dependent mode of action at the doses that resulted in tumors in [the chronic rat study conducted by] Butenhoff et al., 2012."

Finally, as mentioned for PFOA above, the weight of evidence evaluation (i.e., determination of the appropriate descriptor for carcinogenic potential, such as "suggestive" or "likely") is part of the Hazard Identification component of the risk assessment, not the Dose-Response component. As such, the Weight of Evidence section should be moved from the Dose-Response section to the Hazard Identification section (e.g., Evidence Integration) on cancer.

Charge Question 2. Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends.

Does SAB support the selection of this CSF in the derivation of a risk specific dose for PFOA (i.e., the concentration of PFOA in drinking water that would have a one-in-1-million chance of an increased cancer risk)? If not, please provide input on the strengths and weaknesses of the other candidate CSFs that EPA derived.

It was unclear what was meant by "peak exposure" in this charge question since this term is not mentioned or discussed in the draft EPA PFOA document. At the December 16, 2021 SAB meeting, EPA clarified that the term "peak exposure" should be disregarded, and that input is requested on the development of the CSF in general.

I support the use of a CSF based on human epidemiological data derive a risk specific dose for PFOA. The other CSFs derived by EPA are based on animal data, and it is preferable to base a CSF on human data when appropriate human data are available.

However, as reviewed in detail in California EPA (2021 <https://oehha.ca.gov/media/downloads/crn/pfoapfosphgdraft061021.pdf>), several human studies show an association of PFOA with cancer including Shearer et al. (2013), Vieira et al. (2013), Barry et al. (2013), and Steenland and Woskie (2012). EPA did not provide the rationale for selecting a CSF from Shearer et al. (2021) instead of one of the other studies. Specifically, California EPA (2021) derived CSFs for PFOA and kidney cancer in terms of serum PFOA level (ng/ml)⁻¹ from a study of the general population (Shearer et al., 2021) and a study of communities exposed to PFOA in drinking water (Vieira et al., 2013), and the CSF recommended in California EPA (2021) (which is a draft) is the average of these two CSFs. The draft EPA PFOA document does not provide its rationale for the selection of Shearer et al. (2021) rather than Vieira et al. (2013) or one of the other studies as the basis of its CSF, and such a rationale should be provided.

Additionally, the draft EPA PFOA document states that the CSF for Shearer et al (2021) is based on the modeling approach used by California EPA (2021), and CSF shown in the the corrected version of Table 25 in the draft PFOA document is the California EPA (2021) central tendency estimate serum level CSF from Shearer et al. (2021). At the December 16, 2021 meeting, it was stated that EPA ORD independently replicated the modeling performed by California EPA. However, the details of the modeling and its results are not shown in the draft PFOA document, and this should be included in the final EPA document.

Table 25 of the draft PFOA document shows the CSF from Shearer et al. (2021) in terms of administered dose (ng/kg/day)⁻¹ as well as in terms of serum PFOA (ng/ml)⁻¹. However,

California EPA (2021) does not provide the CSF in terms of administered dose. EPA's determination of the CSF in terms of administered dose $(\text{ng/kg/day})^{-1}$ from the CSF in terms of PFOS serum level $(\text{ng/ml})^{-1}$ is not mentioned either in the main part of the document or in Appendix Section B.1.5.1, which briefly discusses the calculation of the cancer slope factor from Shearer et al. (2021).

From my own calculations, it appears that the serum level CSF was converted to the administered dose CSF with a clearance factor of 0.12 ml/kg/day. This clearance factor was not mentioned or provided in the document, and I calculated it from a half-life of 2.7 years and a volume of distribution of 170 ml/kg, which are the values stated to have been selected by EPA in Sections 3.2.3 and 3.2.4 earlier in the document. Applying this clearance factor to the serum level CSFs shown in Table 25 results in the administered dose CSFs that are shown. However, as above, neither the clearance factor or its use in determination of the administered dose CSFs are mentioned in the draft document. Additionally, although the numerical value of the central tendency slope factor (0.00178) is shown correctly in Appendix B.1.5.1, the units shown, $(\text{ng/kg/day})^{-1}$ instead of $(\text{ng/ml})^{-1}$, are incorrect. The development of the clearance factor and its use in determining that administered dose CSFs from the serum level CSFs should be clearly and completely described in the final document.

Also, Table 25 of the draft PFOA document also shows the 95% upper confidence level of the CSF from Shearer et al. (2021). However, the 95% upper confidence level CSF is not provided in California EPA (2021), and as mentioned above, no information on the modeling results that provided this value are shown in the draft EPA document. Also, it is not clear from the information in the draft PFOA document whether the central tendency CSF or the upper 95th percentile CSF would be used to develop a risk specific dose, and this should be clarified.

Finally, I agree with EPA's decision not to apply age-dependent adjustment factors (ADAFs) to the PFOA CSFs. Although not mentioned in the draft EPA PFOA document, the EPA (2005) Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (https://www.epa.gov/sites/default/files/2013-09/documents/childrens_supplement_final.pdf) recommend that application of ADAFs for carcinogens known to act through a mutagenic mode of action, and this is not the case for PFOA.

Charge Question 2 (continued).

Toxicokinetic Models

Charge Question 1. Human model

Charge Question 1, Part A. *For endpoints observed in adults, EPA used a steady-state approach to calculate the HED, which assumes a relatively constant exposure and clearance during adulthood. Please comment on this method of HED calculation. Are there alternative approaches that EPA should consider? If so, please describe the rationale for recommending this approach(es).*

Clarification needed about consideration of prenatal and breastfeeding exposures in modeling of serum levels at age 5

A complete discussion of how prenatal and breastfeeding exposures were considered in development of the POD_{HED} for decreased vaccine response from exposure at age 5 should be provided, including clarification of what is meant by "the dose to mothers & children that results in the same serum concentration at 5 years of age." Information on this topic is especially important because these PODs are the basis for the final PFOA and PFOS Reference Doses in the draft documents.

In the draft PFOA document, it is not clear from the information provided in Section 4.1.3.2 (Toxicokinetic Model for Human Dosimetry) that prenatal and breastfeeding exposures were considered in development of the POD_{HED} for decreased vaccine response from exposure at age 5. This is confusing, particularly because the excerpt from Section 4.1.3.2 of the draft PFOA document copied below (bolding added) indicates that the steady-state assumption was used to develop the POD_{HED} for all of the human endpoints (including the POD_{HED} based on exposure at age 5), and that exposures during early lifestages were only considered in animal developmental studies.

"This updated [Verner et al., 2016] model was used to simulate the HED from the animal PODs that were obtained from BMD modeling of the animal studies (Section B.2). It was also used to simulate selected human studies to obtain a chronic dose that would result in the internal POD obtained from dose-response modeling. **For PODs resulting from chronic exposure, such as a long-term animal study or a human study, the steady state approximation was used** to calculate a HED that would result in the same dose metric after chronic exposure. **For PODs from exposure to developmental animals, the life-stage developmental model was used** to calculate a HED that results in the same dose metric during the developmental window selected by the Dose-Response team."

In the draft PFOS document, it is indicated that prenatal and breastfeeding exposure was considered in development of the POD_{HED} for decreased vaccine response from exposure at age 5, and EPA confirmed this at the December 16, 2021 SAB meeting. However, this topic is not discussed in the text; it is only mentioned in a footnote to the POD_{HED} for decreased antibody response to tetanus and diphtheria vaccines in Table 21 as follows: "Calculated as the dose to mothers & children that results in the same serum concentration at 5 years of age. Note that the model predicted slightly different serum concentrations for male and female children, so the lower HED was selected to be more health protective."

Need to consider maternal and child drinking water ingestion rates

Although the draft documents develop Reference Doses, not MCLGs, EPA should consider how a Reference Dose based on serum PFOA levels at age 5 can be used to develop a drinking water concentration (MCLG) that is protective for all lifestages. It is unclear how a "dose to mothers & children that results in the same serum concentration at 5 years of age" can account for the different drinking water rates in adults (mothers) and children.

The draft documents discuss differences between the Verner et al. (2016) and Goeden et al. (2019) models for predicting serum PFAS levels in early life. However, the draft documents do not recognize that the two models have different purposes and provide different information. The Verner et al. (2016) model predicts infant and child serum PFOA or PFOS levels resulting from a constant daily PFOA or PFOS dose (ng/kg/day) to the mother and to the child after weaning.

As above, it is not clear how a Reference Dose from the Verner et al. (2016) model, which predicts serum PFOA or PFOS levels at age 5 years from a constant daily dose to the mother and the child, can be used to develop an MCLG that considers both exposure through breastfeeding and post-weaning and the changing drinking water consumption rates up to age 5.

In contrast, the Goeden et al. (2019) model considers both age-specific toxicokinetic factors and the changing drinking water intakes at different age periods. Although the Goeden et al. (2016) publication presents the application of the model to PFOA, the model can also be applied to other PFAS by using chemical-specific values for half-life, volume of distribution, and other chemical-specific factors. As reviewed in Post (2021 <https://setac.onlinelibrary.wiley.com/doi/full/10.1002/etc.4863>), at least four states (MN, NH, MI, WA) have used this model to develop drinking water guidelines for PFOA, PFOS, PFNA, and/or PFHxS.

The Goeden et al. (2019) model predicts the serum PFOA or PFOS levels at any age (including infancy, childhood, and adulthood) that result from maternal and child consumption of drinking water with a certain concentration (ng/L) of PFOA or PFOS. Specifically, it considers the maternal drinking water intake rate which impacts PFOA or PFOS levels in breastmilk, and the varying drinking water intake rates in children of different ages after weaning. The daily water intake (L/kg/day) in young children is much higher than in older individuals, and it varies during different childhood age periods up to age 5.

The draft PFOA and PFOS documents state that the Verner et al. (2016) model was selected because "the Goeden et al. (2019...) model did not account for the decrease in concentration that occurs due to growth dilution which plays a substantial role in the PK of growing infants and children." Public comments submitted by the scientists who developed the Goeden et al. (2019) model indicate that this statement and some other information about this model in the draft PFOA and PFOS documents are not accurate, and it is recommended that EPA consider these public comments. Additionally, the higher dose received by young children due to their higher drinking water consumption would tend to counteract the growth dilution effect. For example, Goeden et al. (2019; Figure 6) predicts that, from a certain concentration of PFOA or PFOS in drinking water, the serum PFOA or PFOS level in a 5 year old child who was breastfed for 6 months is about 2.8-fold higher than at steady-state in adulthood. Serum levels at age 5 years would be even higher from breastfeeding for one year, as was assumed by EPA (p. 333, last paragraph of PFOA document).

Based on the comments above, it is recommended that EPA reconsider its choice of the Verner et al. (2016) model and consider whether the Goeden et al. (2019) model is more appropriate for use in development of the PFOA and PFOS Reference Doses and MCLGs.

Charge Question 1, Part B. Two key parameters are the half-life and volume of distribution, which were used to calculate clearance. Half-life and volume of distribution were assumed to be constant across sex and age groups because of a lack of strong quantitative data to parametrize changes across sex and age. Please comment on the strengths and weakness of the use of this assumption and the choice of these parameters by the EPA. Please describe the rationale for alternative recommended approaches. 2 For endpoints observed in human neonates or children, EPA used a one-compartment TK model to simulate dosimetry during pregnancy and a two-compartment TK model (one-compartment models for the mother and the child) to simulate dosimetry during lactation, to calculate the HED for each POD. Please comment on the

strengths and weaknesses of this choice of model structure for the task of predicting dosimetry in the human fetus and child compared to dosimetry in mice and rats in the similar lifestages. Please provide the rationale for any alternative recommended approaches.

I have no comments on this specific question. However, Section 4.1.3.2 of the draft PFOA and PFOS documents discuss the parameters from the Verner et al. (2016) model that were modified by EPA, and these do not include the volume of distribution or half-life. For both PFOA and PFOS, the volume of distribution used in Verner et al. (2016) is identical to the value stated to have been selected by EPA in Section 3.2.4 of the draft documents (0.17 L/kg for PFOA; 0.23 L/kg for PFOS). However, the half-lives of 3.8 years for PFOA and 5.4 years for PFOS used by Verner et al. (2016) differ from the half-lives of 2.7 years for PFOA and 3.8 years for PFOS stated to have been selected by EPA in Sections 3.2.3 of the draft documents. It is unclear whether EPA used the half-life values that it selected or those selected by Verner et al. (2016) when applying the Verner et al. (2016) model. Also, if EPA did not use the half-lives it selected when applying the Verner et al. (2016) model, it is unclear where they were actually used in the EPA evaluations. This information should be clarified.

Additionally, please see my response to Part A regarding questions about how varying drinking water ingestion rates at different lifestages should be incorporated into the model that to predict dosimetry from exposure to PFOA and PFOS in drinking water in the human fetus and child.

Charge Question 1, Part C. *The key chemical-specific parameters that describe the transfer of the chemical from the mother to the child during gestation and lactation are the maternal to fetal serum ratio and the ratio of maternal serum to milk PFOA/S concentration. These ratios were assumed to be constant during gestation and lactation, respectively. Another important parameter is the rate of milk ingestion, which is chemical-independent and varies throughout lactation. Please comment on the strengths and weaknesses of the choice of parameters for fetal to maternal partitioning and partitioning into breastmilk, as well as the choice for lactation rate. Please also comment on the choice to assume that fetal to maternal partitioning and partitioning to breastmilk did not vary in time. Please describe whether there are other methods you would recommend to account for these changes over time and across development.*

I do not have a response to this charge question.

Charge Question 2. Animal model

Charge Question 2, Part A. *After a review of the available toxicokinetic models for PFOA/S predictions in laboratory animals, EPA selected the Wambaugh et al. (2013) model because it was parametrized using all species of interest, demonstrated good agreement with training and test datasets, and used a single, biologically motivated, model structure across all species. Does the panel agree with selecting this model? If not, please describe the rationale for alternative recommended approaches for the calculation of the internal dose metrics in adult animals.*

The animal model (Wambaugh et al., 2013) was not used to develop the Reference Doses presented in the draft PFOA and PFOS documents because they are based on human epidemiology studies. However, if a decision is made to use Reference Doses based on animal studies in the final document, the animal model will become an important part of the basis for the MCLGs.

A general common about development of PODs from animal studies is that serum/plasma data from the study itself (e.g., from at the end of the dosing period), rather than the serum levels predicted by modeling, could be used when appropriate serum or plasma PFOA/PFOS data are available. It is suggested that EPA consider the pros and cons of this approach, since use of the data from the study itself could reduce uncertainty. It should be noted that four of the eight states whose PFAS drinking water guidelines were reviewed by Post (2021, <https://setac.onlinelibrary.wiley.com/doi/full/10.1002/etc.4863>) used an approach based on serum PFAS levels measured at the end of dosing in development of Reference Doses for PFOA and PFOS.

Additionally, the PFOA document (p. 330) states: “The purpose of the animal PBPK model is to make predictions of internal dose in lab animals used in toxicity studies or in humans. Therefore, to evaluate its predictive utility for risk assessment, a number of dose-metrics across life stages were selected for simulation in a mouse, rat, monkey, or human.” However, toxicokinetic parameters appear to be presented only for animal species but not humans in Section 4.3.1, and application of the model to humans is not discussed. As such, it should be clarified whether or how this model was used to simulate human exposures.

Charge Question 2, Part B. *The animal model parameters were obtained through a Bayesian inference parameterization which produced wide credible intervals for some parameter values, but relatively tight credible intervals for the predicted serum concentration. Does the panel agree with using the median values of the estimated animal parameter distributions for prediction of serum concentration and internal dose metrics?*

I do not have a response to this question.

Charge Question 2, Part C. *Based on visual inspection of model predictions to the calibration datasets, EPA utilized sex-independent parameters for PFOS. The male-specific parameters were used for all rat-specific PFOS predictions including predictions in pregnant and nursing dams and the female-specific parameters were used for all mouse-specific PFOS predictions because the parameter values obtained from fitting the female-specific rat data and male-specific mouse data were not consistent with the overall TK parameters for PFOS and produced poor fits to the training and test datasets. Does the panel agree with this approach and justification for this assumption for PFOS? If not, please describe other approaches that could be considered?*

I do not have a response to this question.

Charge Question 2, Part D. *EPA assumed a one compartment model for the developing infant based on the lack of infant-specific toxicokinetic data from rats and mice. This model utilizes averages of half-life and volume of distribution from the literature coupled with physiologically relevant lactational parameters for pup nursing. Does the panel agree with the decision to use this model structure for infant animals? If not, please provide data on infant-specific changes during the animal lactational-period that could be used to account for toxicokinetic differences between the adult and infant rats and mice.*

I do not have a response to this question.

Charge Question 2, Part E. *Several parameters dictate the transfer of chemical from the mother to her pup. Does the panel agree with the selection of these parameters for the animal model? If*

not, please provide your justification and alternative parameters. I do not have a response to this question.

Charge Question 2, Part F. *For neonatal animals, EPA assumed no sex differences in clearance in neonatal animals based on the lack of identification of sex-dependent differences in PFOA/S toxicokinetics from the available data. Does the panel agree with this assumption? If not, please provide your justification and available data on sex differences in neonatal rats. I do not have a response to this question.*

Epidemiological Study RfD Derivation

Charge Question 1. *EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only 'medium' and 'high' quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?*

Although exposures to PFOA, PFOS, and other PFAS are often correlated, the observed associations of health endpoints with PFOA and PFOS are highly unlikely to actually be due to co-exposure to PFOA (in the case of PFOS) and vice versa for PFOS, or to other PFAS. However, the quantitative dose-response relationship for the association of a health effect with PFOA or PFOS may be impacted by co-exposure to other PFAS that also cause the same effect, and this is a relevant consideration in development of PODs for PFOA and PFOS from human study. Therefore, information, if any, on potential impact of co-exposure to PFOA (for PFOS), PFOS (for PFOA), and other PFAS on the dose-response relationship should be discussed for each of the epidemiological studies selected for POD derivation. This information should include whether the impact of other PFAS was evaluated, and, if so, the results of the evaluation.

The BMDLs for decreased antibody response to vaccines that was published by Budtz-Jorgensen and Grandjean (2018) were selected as PODs for the final PFOA and PFOS RfDs in the draft documents. Budtz-Jorgensen and Grandjean (2018) considered the issue of the impact of co-exposure to PFOA, PFOS, and other PFAS on the dose-response for each PFAS individually, and they accounted for this effect in development of BMDs and BMDLs for PFOA and PFOS.

EPA also selected five human studies for development of PODs for decreased birthweight caused by PFOA, and four of these five studies were also used for development of PODs for this effect for PFOS. Information on potential impact of PFOA and other PFAS on associations of PFOS with decreased birthweight, and vice versa for PFOA, in each of these studies should be discussed in the PFOA and PFOS documents. For example, Chu et al. (2020) evaluated the impact of adjustment for PFOA and PFOS on the effect of another PFAS (CIPFESA), but it appears that the impact of PFOA on PFOS and vice versa were not evaluated. Additionally, it appears that Sagiv et al. (2018) did not evaluate potential impact of co-exposure to other PFAS; the HAWC evaluation states that "there is some minor concern over potential bias due to confounding by other PFAS." The sensitivity analysis conducted by Starling et al. (2017) does not appear to support an association with PFOS for birthweight after co-exposure to other PFAS is considered. This sensitivity analysis is not mentioned in the HAWC evaluation of this study,

and it should be reviewed by EPA to determine if this study is appropriate for dose-response for the effects of PFOS on birthweight. Finally, as noted in the HAWC file, Wikstrom et al. (2020) did not consider the impact of co-exposure to other PFAS; the authors discuss this as a limitation of their study.

Additionally, BMDLs from the same human study (Dong et al., 2019) were used as PODs for increased serum cholesterol for both PFOA and PFOS. As stated in the HAWC evaluation for Dong et al. (2019), there was "no discussion of potential confounding across PFAS."

Charge Question 2. *Studies of developmental immune health outcomes (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]) after PFOA/S exposure identified associations with very low doses of either PFOA or PFOS with developmental immune effects. The RfD for this outcome was selected as the critical effect because it was the lowest among the candidate RfDs for PFOA or PFOS and can result in severe illness. Does the panel agree with the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS?*

Charge Question 2, Part A. *If so, please explain your justification.*

I agree that the critical effect and the critical study selected by EPA are appropriate as the basis of an RfD. Effects used as the basis for an RfD must be well established, adverse or a precursor to an adverse effect, and relevant to humans (for effects from animal studies). In a review of the six studies of PFOS and antibody response to vaccines published through 2018, Pachkowski et al. (2019)

<https://www.sciencedirect.com/science/article/abs/pii/S0013935118304286?via%3Dihub>)

concluded that this effect is well established, as follows: "The six studies differ in the ages at which PFOS exposure and vaccine antibodies were measured, the time between inoculation and the measurement of antibody levels, the vaccine antibodies that were measured, the study populations, and the study designs. Nevertheless, the observation of an association of decreased vaccine antibodies with some measure of PFOS exposure for at least one vaccine antibody in all but one study supports an association between increased PFOS serum levels and decreased antibody response across different populations and different study designs." This effect is also adverse, as discussed in Dr. Jamie Dewitt's detailed preliminary response to this charge question. Therefore, decreased antibody response to vaccines is clearly an appropriate choice as the basis for an RfD,

Epidemiological evidence indicating that PFOA and PFOS increase the risk of infectious disease is also relevant to use of an endpoint related to immunosuppression as the critical effect. As stated by Pachkowski et al. (2019), "A decrease in vaccine antibodies and an increase in childhood infections are mutually consistent, since both are indicative of immunosuppression." Pachkowski et al. (2019) concluded that the studies available through 2018 "provide evidence for an association between general population levels of PFOS exposure and infectious disease, a clinical meaningful measure of health risk." (PFOA was not reviewed in this publication.) The Human Evidence subsections of the Immune sections in Hazard Identification also review studies of PFOA and PFOS and infectious disease, many of

which found associations with increased risk, and several recent studies not cited in the draft documents⁵, provide further support for this conclusion for both PFOA and PFOS. However, for an unknown reason, increased risk of infectious disease is not mentioned in the Evidence Integration sections and no strength of evidence conclusions for this effect are provided. This needs to be addressed in the final document.

Additionally, animal studies showing that PFOA and PFOS cause immunotoxicity, including decreased response to foreign antibodies (e.g., sheep red blood cells), are reviewed in the draft PFOA and PFOS documents, Pachkowski et al. (2019), and elsewhere. The results of these animal studies provide further support for decreased antibody response to vaccines in humans as the critical effect.

I also support selection of Grandjean et al. (2012) as the critical study, provided that the additional analyses discussed in my response to Part C of this charge question are completed. As discussed in DWQI (2018) and Pachkowski et al. (2019), an earlier limitation in the use of Grandjean et al. (2012) and other human studies of PFAS and decreased antibody response to vaccines for Reference Dose derivation was lack of information on the dose-response for PFOA and PFOS individually. This issue was subsequently addressed by Budtz-Jorgensen et al. (2018). As mentioned in my response to the charge question on confounding above, these authors considered the impact of co-exposure to PFOA, PFOS, and other PFAS on the dose-response for each individual PFAS, and they accounted for this effect in development of BMDs and BMDLs for PFOA and PFOS.

Charge Question 2, Part B. *If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of chronic RfDs.*

I do not have a specific suggestion for an alternative critical study or effect. As above, decreased antibody response to vaccines is an appropriate critical effect and Grandjean et al. (2012) is an appropriate critical study. However, as discussed at the December 6 SAB PFAS panel meeting, other health effects from human studies may also potentially be appropriate as the basis for additional candidate RfDs.

Charge Question 2, Part C. *Are any additional analyses or rationales needed to increase the confidence in the chronic RfDs for PFOA and PFOS?*

My response to this question addresses several issues that are important in supporting the RfDs that are based on a serum PFOA/PFOS levels in children at age 5 and decreased vaccine response: 1) the need for an expanded explanation for use of human data as the basis for RfDs; 2) the need to clarify the duration of exposure to which the RfDs apply; 3) the need for more detail on Faroe Islands studies of PFAS and antibody response to vaccines and 3) the need for EPA to provide additional support for the PODs from Grandjean et al. (2012) that are the basis of the PFOA and PFOS RfDs.

⁵ Recent studies not cited in draft documents include: Timmermann et al. (2020 <https://ehp.niehs.nih.gov/doi/10.1289/EHP6517>); Dalsager et al. (2021 <https://www.sciencedirect.com/science/article/pii/S0160412021000192?via%3Dihub/>); Bulka et al. (2021 <https://www.sciencedirect.com/science/article/pii/S0269749121001974?via%3Dihub1999e2016>)

Need for expanded explanation for use of human data as basis for RfDs

I agree with the overall EPA conclusion that there is strong evidence that very low exposure to PFOA and PFOS (i.e., within the general population exposure range) increases the risk of several health effects that are of public health concern, and I support the use of human epidemiology data as the basis for the Reference Doses. That being said, there is a need for an expanded explanation of the rationale for the preferential use of human studies for POD derivation, resulting in much lower PODs than if animal data were used. It is important that the rationale for this decision be thoroughly explained and supported because it is a major change from the approach used in the 2016 HESD, which concluded that human data were precluded from use for PODs and RfDs.

Need for clarification of duration(s) of exposure to which Reference Doses (RfDs) apply

It is critical that EPA clarify the duration(s) to which the PFOA and PFOS Reference Doses apply. At the December 16, 2021 SAB meeting, EPA stated that the RfDs apply to shorter-than-chronic as well as chronic exposure. However, the draft documents state that PFOA and PFOS RfDs are intended for chronic exposure, and their application to shorter exposure durations is not mentioned. These RfDs are based on an effect in childhood (decreased antibody levels in response to vaccines at age 7) that results from exposure in childhood (serum PFOA or PFOS levels at age 5). Although the critical effect results from shorter-than-chronic exposure, these RfDs are also considered to be protective for chronic exposure because they are more sensitive (i.e., have lower PODs) than chronic effects. Relevant to this point, the PFOA document (p. 338) states that no adjustment for subchronic to chronic exposure is needed (i.e., UF of 1) because "...the developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure," and "... the developing immune system is recognized as a susceptible lifestage; therefore, exposure during this time window can be considered more relevant than lifetime exposure," and the PFOS document (p. 309) includes very similar statements.

As such, these RfDs are applicable to shorter (subchronic and/or short-term) exposures as well as chronic exposure, in contrast to many other contaminants (e.g., GenX) for which subchronic RfDs are higher than chronic RfDs (see EPA [2021] toxicity evaluation for GenX at https://www.epa.gov/system/files/documents/2021-10/genx-chemicals-toxicity-assessment_tech-edited_oct-21-508.pdf).

Clarity from EPA regarding the exposure duration(s) to which the RfDs apply is extremely important in situations of exposure to PFOA and PFOS. In addressing situations of drinking water contamination, the duration of exposure to which MCLGs and MCLs based on these RfDs apply has practical implications for the timeframe (e.g., acute, short term, longer term) in which exposure to contaminated drinking water should be stopped when the MCL is exceeded.

It should be noted that the EPA (2009) Provisional Drinking Water Health Advisories for PFOA and PFOS were stated to apply to short-term exposure. See

<https://www.epa.gov/sites/default/files/2015-09/documents/pfoa-pfos-provisional.pdf>.

Additionally, the EPA (2016) Lifetime Health Advisories for PFOA and PFOS, which are based on developmental effects, are stated to apply to both short-term (weeks to months) and lifetime (chronic) exposure, as follows: "... short-term exposure to PFASs can result in a body burden that persists for years and can increase with additional exposures. Thus, EPA recommends that the lifetime HA for PFOA [or PFOS] of 0.07 µg/L apply to both short-term (i.e., weeks to months) scenarios during pregnancy and lactation, as well as to lifetime-exposure scenarios."

Need for more detail on Faroe Islands studies of PFAS and antibody response to vaccines

Section 3.4.4.1.1 on human evidence for immunosuppression in the draft PFOA document (p. 151) discusses four studies of PFAS and antibody response to vaccines from the Faroe Islands (Grandjean et al., 2012; 2017a, 2017b; Mogensen et al., 2015). Among these studies, two different cohorts were evaluated, and associations of maternal and/or child (at different age points) serum PFAS levels and antibody response to vaccination at different ages are reported. The text here is difficult to follow, and does not clearly and accurately indicate that, among these studies, maternal serum PFAS were measured late in pregnancy or two weeks after delivery (depending on the cohort), offspring serum PFAS levels were measured at 18 months, 5 years, 7 years, and/or 13 years (depending on the cohort), and vaccine antibodies were measured at 5 years pre-booster, 5 years post-booster, 7 years, and 13 years (depending on the cohort). In order to review the information from these studies, it was necessary for me to make a table of the cohort(s), age points for serum PFAS, and age points for vaccine response analyzed in each study. Adding such a table here would be helpful, especially since this endpoint and one of these studies were selected as the critical endpoint and study for the RfD.

Need for inclusion of supporting documentation for BMD modeling from Budtz-Jorgensen and Grandjean (2018) and EPA's review of the modeling

EPA did not independently replicate the BMD modeling conducted by Budtz-Jorgensen and Grandjean (2018), and EPA's review of this BMD modeling by EPA is not discussed in the draft PFOA and PFOS modeling. Details of the modeling and the modeling output are not included in the Budtz-Jorgensen and Grandjean (2018) publication. However, at the December 16, 2016 meeting, EPA stated that the authors provided a supplemental document with details of the BMD modeling. They also stated that the basis for the BMD modeling was reviewed by the EPA Office of Research and Development.

It is important that all information on the BMD modeling of the data from Grandjean et al. (2012) be publicly available, especially since the results of this modeling are the basis of the final EPA RfDs for both PFOA and PFOS. This point was also emphasized in several public comments. It is therefore strongly recommended that the supplemental document provided by the authors of Budtz-Jorgensen and Grandjean (2018), as well as the details and conclusions of EPA's review of this BMD modeling, be included in the final PFOA and PFOS documents. While it seems unlikely that the supplemental document contains confidential information such as personal identifiable information for the study subjects, a version with the confidential information redacted could be provided if such information is included in the document.

Additionally, both California EPA (2021, <https://oehha.ca.gov/media/downloads/crn/pfoapfosphgdraft061021.pdf>) and EFSA (2020, <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6223>) considered using decreased antibody response to vaccination from Grandjean (2012) as the basis for their toxicity factors (California EPA RfDs; EFSA Tolerable Weekly Intakes) for PFOA and PFOS. Both agencies provide their rationales for not selecting the data from Grandjean et al. (2012) as the basis for their toxicity factors. It is recommended that EPA review the evaluations of Grandjean et al. (2012) provided by California EPA (2021) and EFSA (2020) to determine if any of the conclusions made by these agencies should be considered.

Need for rationale for selection of BMDL from Grandjean et al. (2012) for age 5 serum levels and age 7 vaccine response and piecewise model

The PFOA and PFOS RfDs are based on data from Grandjean et al. (2012), a study that was included in the 2016 HESDs. Specifically, the RfDs are based data from Grandjean et al. (2012) on serum PFOA or PFOS levels at age 5 and tetanus or diphtheria vaccine antibody concentrations at age 7 in a cohort born in 1997-2000. Subsequent studies (e.g., Mogensen et al., 2015; Grandjean et al., 2017) that evaluated exposure and effects at other age points and/or included an additional cohort also used the data from Grandjean et al. (2012) in some of their analyses, but the BMDLs (from Budtz-Jorgensen and Grandjean, 2018) that are used as the PODs for the final RfDs come only from data originally presented in Grandjean et al. (2012).

Presumably because it was included in the 2016 HESDs, Grandjean et al. (2012) is not included in the tables summarizing epidemiology studies (Table C-7) in the PFOA and PFOS documents, and there is no systematic review evaluation of Grandjean et al. (2012) in HAWC. As such, it is not clear whether or not the full systematic review evaluation of the various domains included in the HAWC evaluations was performed for the Grandjean et al. (2012) study. Since data from Grandjean et al. (2012) is used as the basis for the final RfDs, it is especially important that a systematic review of this study be performed. It should be clarified that this study was systematically reviewed, if this is the case. If it was not systematically reviewed, it should be.

Budtz-Jorgensen and Grandjean (2018) developed BMDs and BMDLs for maternal serum PFOA and PFOS and age 5 (pre-booster) vaccine antibodies, and for age 5 serum PFOA and PFOS and age 7 vaccine antibodies. EPA selected the BMDLs for age 5 serum PFOA or PFOS and age 7 tetanus (PFOA) or diphtheria (PFOS) vaccine antibody as PODs, and ultimately as the basis for the RfDs. However, the fact that the PODs are based on age 5 serum data and age 7 antibody response does not appear to be mentioned in the text or tables in any of the subsections of Section 4.0 on dose-response for non-cancer endpoints. The only place this choice appears to be mentioned is in Appendix B.1.1 where the BMD modeling is discussed. Even in Appendix B.1.1, the fact that Budtz-Jorgensen and Grandjean (2018) also developed BMDs and BMDLs for maternal serum PFOA and PFOS and age 5 antibody response is not mentioned.

An explanation should be provided about why the BMDLs for age 5 serum PFAS and age 7 antibody response were selected rather than the BMDLs for maternal serum PFAS and age 5 antibody response. The reason for this decision is unclear because the BMDLs for maternal serum PFOA and antibody response to vaccines for the piecewise model (the model that was selected by EPA, see below) are lower, and the ratios between the BMDs and the BMDLs are

smaller, than for the BMDLs for the piecewise model for age 5 serum PFOA at age 5 years and tetanus antibody response at age 7 years that was selected by EPA.

Also, the rationale in the first paragraph of section B.1.1 of the draft PFOA and PFOS documents for selection of the BMDLs for the piecewise model instead of the linear model is not clear and should be clarified. Finally, in the last sentence of this paragraph in the draft PFOA document, "PFOS exposure" should be "PFOA exposure."

Charge Question 3. *The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.*

I do not have a response to this question.

Charge Question 4. *EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for PFOA and PFOS.*

i. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

The application of an uncertainty factor of 10 for inter-individual human variability and 1 for the other four uncertainty factors (interspecies, duration of exposure, NOAEL-to-LOAEL, and database) is appropriate and sufficiently protective. As discussed in the draft EPA PFOA and PFOS documents, there is no basis to select a factor other than the default value of 10 for intra-human variability. An interspecies uncertainty factor of 1 (i.e., no adjustment is made) is appropriate because the RfDs are based on human data. A value of 1 is also appropriate for the uncertainty factor for exposure duration because the critical effects (decreased antibody response to tetanus or diphtheria vaccine from exposure at age 5) result from shorter-than-chronic exposure, and they are more sensitive than chronic effects of PFOA and PFOS. Therefore, the RfDs based on these effects are expected to also be protective for chronic effects. A NOAEL-to-LOAEL uncertainty factor of 1 is appropriate because the RfDs are based on BMDLs, and a

database uncertainty factor of 1 is appropriate because it is not expected that effects that have not been adequately studied would be more sensitive than the critical effects.

ii. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

The rationale for the selection of uncertainty factors is clearly and thoroughly presented in both the text and Table 22 in Section 4.1.5 of the draft PFOA and PFOS documents.

Relative Source Contribution

Charge Question 1. *EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources. Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at: <https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS.*

Charge Question 1, Part A. *Are you aware of additional relevant exposure data that EPA should consider in developing the RSCs for PFOA and PFOS? If so, please provide citations.*

Please see information on daily dietary exposures to PFOA and PFOS and NHANES serum levels for PFOA and PFOS in my response to Part ii below.

Charge Question 1, Part B. *Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS is adequately supported and clearly described.*

For reasons discussed later in this response, I agree that the recommended RSC of 20% is appropriate and scientifically supportable for MCLGs that are based on the PFOA and PFOS RfDs developed in the draft documents. However, the rationale presented in the draft documents for selecting the 20% RSC is not consistent with the approach provided in the EPA (2000) guidance for RSC development that is cited. It is important that the rationale for selecting an RSC of 20% be revised to be consistent with the approach provided in the EPA (2000) guidance.

As stated at the beginning of the RSC sections of the draft PFOA and PFOS documents (Section 5)⁶ and in the charge question, an RSC is applied to ensure that total exposure from all sources

⁶ The draft EPA PFOA and PFOS documents state that: "EPA applies an RSC when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant (i.e., PFOA/PFOS) does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water...; the remainder of the exposure equal to the RfD is allocated to other potential sources."

does not exceed the Reference Dose. The RSC is the portion of the Reference Dose that is allocated to drinking water, based on the portion of the Reference Dose that is known or assumed to come from non-drinking water sources. For example, if it is known that exposures from non-drinking water sources (food, consumer products, air, dust, etc.) are 40% of the Reference Dose, then 60% of the Reference Dose is allocated to drinking water and the RSC is 60%. The highest value recommended for the RSC is 20%, and if it is known that exposure to more than 80% of the Reference Dose comes from non-drinking water sources, the RSC is set at 20%. Additionally, the default RSC is 20% when there is insufficient information to determine a chemical-specific value. (See EPA, 2000, including Figure 4-1, Decision Tree used to determine RSC.)

Because the RSC is based on the portion of the RfD that comes from non-drinking water sources, the choice of the RSC depends on the numerical value of the RfD. The RSC will decrease as the RfD decreases since the non-drinking water exposures represent a higher proportion of a lower RfD. Because the RfDs used in the 2016 EPA Health Advisories and in state drinking water guidelines are several orders of magnitude higher than the RfDs presented in the draft documents, the RSCs used in the 2016 HESD and by states (discussed on p. 347-348 of the PFOA document) are not relevant to selection of the RSC in the current draft EPA PFOA and PFOS assessments.

As above, the relevant parameter for selection of the RSC is the percentage of the RfD that comes from non-drinking water sources. Therefore, actual PFOA or PFOS exposures from drinking water are not relevant to RSC selection. Specifically, the percentage of total PFOA/PFOS exposure that comes from drinking water and the concentrations of PFOA/PFOS in drinking water at locations where the MCL will be applied are not relevant to RSC selection.

The important concept that actual exposures from drinking water are irrelevant to RSC selection does not appear to be correctly presented in the draft documents. Specifically, the following text from the PFOA and PFOS documents does not accurately reflect the EPA (2000) guidance and should be removed: “For disproportionately affected subpopulations, such as the occupationally exposed or site-impacted (e.g., by a particular source or industry) where there may be higher average PFAS concentrations in drinking water, it may be appropriate to apply an RSC greater than 20 percent if there is sufficient information to quantitatively characterize sources other than drinking water. This is a less conservative approach from a public health perspective and would result in a higher MCLG for those disproportionately affected subpopulations.”

Additionally, Section 5.1.4 of the draft PFOA document and Section 5.4 of the draft PFOS document on Recommended RSC discuss several studies (for both PFOA and PFOS - Hu et al., 2019; East et al., 2021; Gebbink et al., 2015; also for PFOS - Jogsten et al., 2012), that estimate that the percentage of total exposure to PFOA or PFOS that comes from drinking water is less than 20%. The draft documents (PFOA - p. 360; PFOS – p. 327) state that these “*estimates support a 20% RSC for drinking water.*” However, these data on percentage of total exposure that comes from drinking water are not relevant to selection of the RSC since, as discussed above, the actual percentage of exposure from drinking water does not affect the choice of the RSC.

Although the rationale for a 20% RSC in the draft document needs clarification, available data on non-drinking water exposures to PFOA and PFOS clearly support the choice of an RSC of 20% for MCLGs based on the RfDs presented in the draft document. Estimates of daily dietary exposure to PFOA in studies from North America and Europe reviewed by the DWQI (2017, <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>) range from 0.16 ng/kg/day to 6.2 ng/kg/day, and the lower bound estimate of median daily dietary exposure for adults from EFSA (2020, <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6223>) is 0.18 ng/kg/day, with higher values for younger age groups. The RfD of 1.5×10^{-9} mg/kg/day (0.0015 ng/kg/day) in the draft PFOA document is two orders of magnitude below the lowest of these dietary estimates. Similarly, PFOS RfD of 7.9×10^{-9} mg/kg/day (0.0079 ng/kg/day) is almost two orders of magnitude lower than the EFSA (2020) lower bound estimate of median daily dietary exposure to PFOS in adults of 0.58 ng/kg/day. Additionally, there are non-drinking water exposures from other sources such as consumer products and house dust. Therefore, it is clearly evident that exposures from non-drinking water sources far exceed the RfD, indicating the choice of the default RSC of 20%.

An RSC of 20% is also supported by data on serum PFOA and PFOS levels from the U.S. general population. The serum PFAS levels associated with the RfDs can be determined by applying an Uncertainty Factor of 10 to the $POD_{Internal\ Dose}$ (e.g., human serum levels at the $PODs$) of 1.7×10^{-4} mg/L (0.17 ng/ml) from Table 21 of the draft PFOA document and 5.4×10^{-4} mg/L (0.54 ng/ml) from Table 21 of the draft PFOS document. The serum levels associated with the RfDs, 0.017 ng/ml for PFOA and 0.054 ng/ml for PFOS, are far below even the lower percentiles for serum PFOA and PFOS in the U.S. general population in the most recent (2017-18) NHANES data (obtained from <https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Laboratory&CycleBeginYear=2017>). In the 2017-18 NHANES, the 5th percentile serum PFOA level, presumably representing those with little or no drinking water exposure, is 0.48 ng/ml, which is 28-fold higher than the serum level associated with the RfD of 0.017 ng/ml. Similarly, the 5th percentile serum PFOS level, also presumably representing those with little or no drinking water exposure, is 1.08 ng/ml, which is 20-fold higher than the serum level associated with the RfD, 0.054 ng/ml. Therefore, serum PFOA and PFOS levels in the lowest 5th percentile of the general population (presumably without exposure to contaminated drinking water) far exceed 100% of the RfD, supporting the default RSC of 20%.

Responses to Charge Questions on EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Charge questions

16. *The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, an assumption of dose addition for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA's supplementary guidance (EPA, 2000) states: "The common mode-of-action (MOA)*

assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration).” This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which “similarity” can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.

C. Please comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.

Assumption of dose additivity

All of the component-based approaches for assessing the risks of PFAS mixtures (HI, TOSHI, RPF, Mixture BMD) included in the draft mixtures framework document are based on the assumption of dose additivity. Sections 2 and 3 of the draft document present information supporting the assumption of dose additivity for chemical mixtures in general, including mixtures of PFAS. The information in these sections supports the conclusion that toxicological interactions of chemical mixtures are usually additive or close to additive. It also supports the conclusion that dose additivity is a public health protective assumption that usually does not underestimate the toxicity of a mixture. Based on the information presented by EPA, I agree with the assumption of dose additivity for evaluation of the toxicity of PFAS mixtures, in the absence of chemical-specific information indicating that another type of toxicological interaction should be assumed.

Section 3.4 of the draft document discusses data that indicates a common mode of action and dose additivity for PFAS. For example, the draft document discusses that Wolf et al. (2014) reported additivity for PPAR-alpha activation in binary mixtures of PFOA and four other PFAS in cultured cells transfected with the mouse or human PPAR-alpha receptor. While I support the dose additivity assumption for reasons discussed above, the discussion of studies of toxicological interactions in PFAS mixtures in the EPA mixtures framework document should be expanded to also include studies that do not indicate dose additivity and/or a common mode of action for PFAS. Some of these studies are summarized below. Acknowledging and including this additional information will increase transparency and help to characterize the uncertainties associated with the assumption of dose additivity.

A recent study, Nielsen et al. (2021, <https://pubmed.ncbi.nlm.nih.gov/34743024/>), that was not included in the draft EPA document did not find dose additivity for activation of PPAR-alpha by PFAS mixtures in cultured cells transfected with a full length human PPAR-alpha construct. It is suggested that discussion of Nielsen et al (2021) be included in the final EPA mixtures framework document. As was previously reported by other studies, Nielsen et al. (2021) found that the potency (EC50) for PPAR-alpha activation varied among the seven PFAS tested. Nielsen et al. (2021) also reported that the efficacy (maximal PPAR-alpha activation compared to positive control) was lower for PFASs than for PFCAs, and that a general concentration

addition (GCA) model that considers differences in both potency and efficacy among PFAS predicts the PFAS interactions better than an RPF approach that considers only differences in potency. They further conclude that an effect summation model can also likely predict the interactions at low concentrations. Additional studies that report non-additive interactions of PFAS include Kjeldsen and Bonefeld-Jorgensen (2013, <https://pubmed.ncbi.nlm.nih.gov/23764977/>) who studied PFAS activation of the estrogen and androgen receptor in a cultured cell line transfected with these receptors; Ojo et al. (2020, <https://pubmed.ncbi.nlm.nih.gov/32247900/>) who studied effects of binary and ternary PFAS mixtures on cell viability of a human liver cell line, HepG2; Ding et al. (2013) who studied interactions of PFOA and PFOS for lethality in zebrafish; and Menger et al. (2020, <https://pubmed.ncbi.nlm.nih.gov/31877453/>) who studied behavioral effects in zebrafish of nine PFAS individually and a mixtures of equal concentrations of all nine PFAS;

Surprisingly, no published mammalian studies of defined mixtures of PFAS were available to EPA for inclusion in the draft mixtures framework document. As discussed in the draft EPA document, a recent EPA study of rat developmental toxicity of mixtures of PFOA and PFOS (Conley et al. - Appendix A of draft EPA document) indicates additive toxicity for developmental effects of these two PFAS.

However, a very recent paper not cited in the draft EPA document, Marques et al. (2021, <https://www.sciencedirect.com/science/article/abs/pii/S0300483X21002444>) indicates that toxicological interactions of a mixture of PFOA, PFOS, and PFHxS in mice can be additive, synergistic, or antagonistic for specific hepatic and metabolic effects after perinatal exposure. As stated by Marques et al. (2021): "The PFAS mixture had very distinct effects when compared to single compound treatment. With regard to liver weights and liver to body weight ratios increases, the PFAS mixture data were analogous to the effects seen with PFOA treatment. However, unlike PFOA, the serum ALT level, did not increase in the PFAS mixture. In the case of liver lipids, only the PFAS mixture in combination with HFD [high fat diet] feeding decreased total cholesterol in the pups and increased total lipid in the pups. However, liver triglycerides were increased with all three single PFAS treatments with the SD [standard diet], and in treatment with the PFAS mixture with SD, there was no change compared to control... These results suggest that there are multiple pathways in which PFAS could add, synergize, or antagonize specific effects, and warrants further investigation of dose response data with model predictions of additivity." These results also suggest that co-exposure to other PFAS may impact the toxicokinetics of individual PFAS, as follows: "PFOS levels in pup and dam serum were lower in the PFAS mixture compared to PFOS treatment alone." Especially since it is the only peer reviewed study of mixtures of PFAS in mammalian species that has been identified, it is recommended that discussion of this study be added to the EPA mixtures document.

Assumption of similarity of toxicity endpoint rather than MOA

The charge question states: "For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather

than MOA," and "As an emerging chemical class, MOA data is limited or not available for many PFAS." The draft EPA mixtures framework document emphasizes that the EPA (2000) mixtures risk assessment guidance states (as also stated in the charge question): "The common mode of action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." However, it is not completely clear how "duration" is incorporated into the approaches based on similar toxic endpoint that are proposed in the draft EPA mixtures framework document, and this should be clarified.

Furthermore, while it is undoubtedly true that there are little or no MOA data for many PFAS, information from *in vivo* studies indicates that the mode(s) of action for several key toxicological effects differ among several well-studied PFAS. It is suggested that EPA include this information in its discussion of approaches based on a common apical endpoint, rather than a common mode of action. For example, PFOA, PFNA and PFOS cause the same general types of hepatic toxicity. However, as reviewed by Post et al. (2017, <https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.2002855>) and the New Jersey Drinking Water Quality Institute (DWQI, 2017 <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf> for PFOA; DWQI, 2015 <https://www.state.nj.us/dep/watersupply/pdf/pfna-health-effects.pdf> for PFNA, hepatic effects of PFOS in rodents are primarily PPAR- α independent, while hepatic effects of PFOA and PFNA involve substantial contributions from both PPAR- α dependent and independent processes. Likewise, while the developmental effects of PFOA, PFOS, and PFNA are generally similar, most (but not all) developmental effects of PFOA (reviewed in DWQI, 2017) and PFNA (reviewed DWQI, 2015) in mice are PPAR- α dependent, while the developmental effects of PFOS (reviewed in DWQI, 2019 <https://www.state.nj.us/dep/watersupply/pdf/pfos-recommendation-appendix-a.pdf>) appear to be independent of PPAR- α .

Consideration of human data

The examples of mixtures assessments provided in the draft mixtures framework document are based on the four PFAS that currently have final EPA Reference Doses (PFOA, PFOS, PFBS, GenX); all of these Reference Doses are based on animal data. However, the Reference Doses for PFOA and PFOS and the cancer slope factor for PFOA in the draft PFOA and PFOS MCLG approaches documents are based on human data, and additional toxicity factors based on human data may be developed in the future for other PFAS. It is suggested that EPA consider how toxicity factors based on human data could be used in evaluations of PFAS mixtures, including mixtures in which toxicity factors for some PFAS are based on animal data and toxicity factors for other PFAS are based on human data.

Development of toxicity factors for PFAS for which final EPA toxicity factors are not available

The draft EPA mixtures framework document (p. 33, last paragraph) discusses that toxicity values are needed to address PFAS (and other contaminants) for which final EPA toxicity factors have not been developed. The draft EPA document also discusses that several states have developed toxicity factors for several PFAS for which there are no EPA toxicity factors (see Post, 2021, <https://setac.onlinelibrary.wiley.com/doi/epdf/10.1002/etc.4863>). As noted in the

draft EPA document, EPA has developed guidance for development of subchronic and chronic oral RfDs, and most or all states follow this EPA guidance.

I fully agree with EPA recommendations that toxicity values for PFAS should be developed by scientists with appropriate expertise and that their basis should be transparent. However, the recommendation that such toxicity values "undergo independent peer review" does not appear to be appropriate for inclusion in the EPA mixtures framework document. This recommendation is not specific to toxicity values used in mixtures assessments and would apply equally to toxicity values used in chemical-by-chemical approaches for addressing PFAS in drinking water or other media. It is important to recognize that each state has its own processes (established in legislation, regulation, or by policy) for development of such toxicity values, and that these processes may or may not include formal peer review. In fact, the Minnesota Department of Health oral toxicity values mentioned in the draft mixtures framework document for potential use in HI calculations (p. 33, first paragraph) do not undergo external peer review.

In some states, advisory bodies consisting of scientific experts develop toxicity values and recommend them to state environmental agencies. These toxicity value recommendations may be posted for public comment as drafts and revised as appropriate in response to the public comments before finalization. While such a process may not be considered to be a formal "independent peer review," it is a rigorous process that considers extensive scientific input from outside of the agency that will use the toxicity factor. A recommendation in the EPA PFAS mixture framework for "external peer review" of toxicity values developed by states could potentially be used as the basis for challenges to the validity of such state processes that may not include formal "external peer review." If such a recommendation is to be included in the EPA mixtures framework document, it is strongly suggested that it be broadened to recommend the opportunity for scientific input and review in general, rather than specifically "external peer review."

Use of NAMs data in component-based mixtures approaches for PFAS

The potential use of new approach methodologies (NAMs; e.g., high throughput assays, read-across) data for hazard identification and dose-response evaluation in assessment of PFAS mixtures is mentioned in several places in the draft mixtures framework document (p. 12, 27, 34, 37, 52). I agree with the draft document's statement (p. 41) that the use of NAMs data to develop screening level toxicity Reference Values (RfVs) will allow for consideration of toxicity of "data-poor PFAS" detected in environmental media that would not otherwise be considered.

However, it should be recognized that the potential use of NAMs data to address environmental contaminants that lack sufficient human or animal data for traditional toxicity factor (e.g., Reference Dose) development is not specific to PFAS mixtures assessment. This is a key issue for both chemical-by-chemical and mixtures assessment of PFAS and other contaminants. This issue has become especially important because chemical-specific toxicity factors cannot be developed for several PFAS (e.g., perfluoropentanoic acid; perfluoroheptanoic acid) that commonly occur in drinking water, because there are no or virtually no data on their toxicity in animals or humans. Compounding this problem, EPA has adopted a policy of minimizing

animal studies in its toxicology research, despite the obvious high impact of even the small number of recent animal studies from ORD which have provided key information on developmental effects of several PFAS of current concern. Examples of these high impact *in vivo* studies of PFAS of current concern are: GenX - Conley et al. (2019, <https://pubmed.ncbi.nlm.nih.gov/30920876/>) and Conley et al. (2021, <https://pubmed.ncbi.nlm.nih.gov/33126064/>); Nafion Byproduct 2 - Conley et al., (2021; <https://www.sciencedirect.com/science/article/pii/S0160412021006814>); and mixtures of PFOA and PFOS - the recent studies highlighted in the draft mixtures framework document.

Current EPA risk assessment guidance does not provide for the use of NAMs data as the basis toxicity factors such as Reference Doses, and state environmental agencies generally follow EPA risk assessment guidance in developing health-based standards and guidance values for environmental contaminants. Therefore, states would face difficulties in justifying and implementing either a chemical-specific or a mixture-based standard or guidance value based on NAMs data for contaminants (PFAS or others) in drinking water or other environmental media.

Regarding this issue, EPA stated at the December 16, 2021 SAB meeting that it does not plan to develop guidance for use of NAMs data to develop toxicity factors in the near future. EPA also stated that the use of NAMs in mixtures assessment is currently "quite abstract," and that it is not expected that NAMs data will be used as the basis for standards or guidance values in the near future. EPA clarified that that an approach based on NAMs data might be used to get a sense of whether PFAS detected in drinking water pose a risk in the absence of traditional toxicity data and that EPA hopes to develop case studies using NAMs data to evaluate the potential risk of PFAS mixtures. This clarification of how EPA envisions the use of NAMs data in PFAS mixtures assessments is not included in the draft EPA mixtures framework document, and it should be added to the final document.

B. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).

I do not recommend an alternative methodology. As above, I agree that an approach based on dose additivity and a common toxicity endpoint/health effect can be used as a default approach for PFAS mixtures and that this approach is public health protective. As above, the uncertainties associated with this approach should be more thoroughly and clearly presented along with the information that supports this approach.

17. Section 4.3 (Hazard Index; HI) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and hypothetical exposure information. The example calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as "GenX chemicals").

A. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.

In general, the screening level Hazard Index (HI) approach, in which Reference Values (RfV) for the mixture components are used regardless of the effect on which the RfVs are based, is appropriate for initial screening of whether exposure to a mixture of PFAS poses a potential risk that should be further evaluated.

The Target Organ Specific Hazard Index (TOSHI) approach, in which RfVs based on a common effect are used, provides a more refined estimate of whether exposure to a mixture of PFAS poses a potential risk. However, as stated in the draft EPA mixtures framework document (p. 42), some PFAS present in a PFAS mixture may not have been tested for some of the health effects of concern.

B. Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment.

The proposed HI methodology (to be used for both the screening HI and the TOSHI approaches) uses Health-based Water Concentrations (HBWCs). As such, chemical-specific HBWCs, not just chemical toxicity factors (e.g., Reference Doses, Minimal Risk Levels), are needed for each PFAS included in the HI or TOSHI evaluation. Development of such HBWCs would require additional effort beyond what is needed for toxicity factor development. Additionally, as shown in Table 4-3 (p. 39) of the draft EPA mixtures framework document, development of HBWCs requires chemical-specific toxicity factors (e.g., Reference Doses) and chemical-specific exposure assumptions (ingestion rates, Relative Source Contribution factors). Additionally, HBWCs may apply to different exposure durations (short-term, subchronic, chronic). The EPA mixtures framework document should consider whether it is appropriate to use HBWCs based on different exposure assumptions and/or different exposure durations in HI evaluation of PFAS mixtures.

For example, the HBWCs used in the examples of the HI approach (Section 4 of the draft EPA mixtures framework document) are the EPA (2016) Health Advisories (HAs) for PFOA and PFOS. As shown in Table 4-3, the PFOA and PFOS HAs are based on the drinking water ingestion rate for lactating women which is higher than the default adult ingestion rate. The ingestion rate for lactating women was selected because PFOA and PFOS are transferred to breastmilk, and exposure to PFOA and PFOS in breastfed infants (via maternal consumption of PFOA/PFOS-contaminated drinking water) is higher than in infants who consume formula prepared with the contaminated water or older individuals. However, ingestion rates for subgroups other than lactating women (e.g., infants, children, default adults) may be appropriate for HBWCs for other PFAS. For example, the ingestion rate for lactating women is not likely to be appropriate for HBWCs for PFBS or GenX, since there is no information to indicate that GenX or PFBS are present in breastmilk. Additionally, the EPA (2016) Health Advisories are stated to apply to both short-term (weeks to months) and chronic exposures, while HBWCs for

other PFAS might apply to different exposure duration(s). As above, EPA should consider these issues in developing the HI methodologies for PFAS mixtures that uses HBWCs.

An additional comment on the presentation of the HI approach in the draft document relates to the examples provided in Tables 4-4 and 4-5. In the example in Table 4-4, the individual concentrations of 20 ng/L for PFOA and PFOS are below the HBWCs of 70 ng/L for these chemicals and the combined concentration of PFOA and PFOS is also below 70 ng/L. It is therefore not unexpected that the HI is below 1 for the combined concentration. In the example in Table 4-5, individual concentrations of PFOA and PFOS of 400 ng/L exceed the HA of 70 ng/L, so it is not unexpected that the HI for the combined concentration (and for each individual PFAS) exceeds 1. It would be useful to provide an additional example in which the concentration of each individual PFAS is below its HBWC (e.g., the HA), yet the HI that considers both PFAS exceeds 1. For example, 40 ng/L for PFOA and 50 ng/L for PFOS.

18. Section 4.4 (Relative Potency Factor; RPF) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and HFPO dimer acid and GenX chemicals.

A. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.

It appears that the RPF approach is a reasonable methodology for estimating risks associated with PFAS mixtures. However, it would be helpful if additional explanation is provided to clarify the conceptual differences between the TOSHI approach and the RPF approach, since both are based on health effect-specific values (RfVs or RPFs) for the individual PFAS in the PFAS mixture. Although the relevant information is provided in the draft EPA document, it should be more clearly explained and indicated that the RPF approach is based on a specific effect (e.g., decreased offspring body weight) while the TOSHI approach is based on a general category of effects (e.g., developmental effects). For example, Table 4-6 shows a POD_{HED} (LOAEL) for “Developmental Effect: Decreased Pup Body Weight” for PFOA of 0.0109 mg/kg/day. However, Tables 4-7 and 4-8 refer only to “Developmental Effect” RPFs, without mentioning that the values are specific to decreased offspring body weight. It is important that this point is clarified because, for example, the 2016 HESD for PFOA provides a lower POD_{HED} (LOAEL) of 0.0053 mg/kg/day for a different developmental effect, reduced ossification and accelerated male puberty in mouse offspring.

B. Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.

The RPF approach is based on the assumptions of dose additivity and use of a common health effect/toxicity endpoint as a surrogate for a common MOA, as discussed in Charge Question 1

above. As such, the comments on the scientific basis of these assumptions from Charge Question 1 apply here as well.

The scientific basis for the RPF approach presented in the draft EPA mixtures framework document is strengthened by the use of PODs from animal studies that are based on human equivalent doses (HEDs) rather than administered doses. In contrast, the PFAS RPFs based on BMDs for a 5% increase in relative liver weight from subchronic exposure to male rats developed by Bil et al. (2020, <https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.4835>), which are being used to address PFAS mixtures by some European environmental authorities, are based on administered dose and do not consider differences among PFAS regarding animal-to-human toxicokinetic extrapolation.

19. Section 4.5 (Mixture BMD) of the framework document demonstrates the application of a component-based mixture approach using established EPA dose-response modeling (i.e., benchmark dose; BMD) of hypothetical PFAS dose-response data, and hypothetical exposure information.

A. Please provide specific feedback on whether the Mixture BMD approach is a reasonable methodology for estimating what is in essence a mixture-based point-of-departure. If not, please provide an alternative.

This methodology appears to be reasonable. It is based on the same assumptions (dose additivity and use of a common health endpoint, in the absence of knowledge of a common MOA, as the TOSHI and RPF approaches. However, the practical utility of this approach is unclear and difficult to envision. The output of this approach is a BMD in units of mg/kg/day for the total concentration of a mixture of specific PFAS in specific proportions. At the December 16, 2021 SAB meeting, EPA stated that a Mixture BMD could be used to characterize a unique site or exposure and that it is applicable when it is fairly certain that the composition of the mixture is relatively stable. However, it is unclear what benchmark the Mixture BMD could be compared to in order to determine whether or not there is a potential risk from a mixture of PFAS in drinking water or other environmental media.

B. Please provide specific feedback on whether the proposed Mixture BMD methodology in the framework is scientifically supported for PFAS mixture risk assessment.

It is stated that an advantage of this approach is that only PODs (NOAELs, LOAELs, BMDs) rather than RfVs (RfDs, HBWCs) are needed. However, the RPF approach is also based on PODs, rather than HBWCs or RfDs.

Additionally, it is not clearly stated that PODs based on HEDs will be used in the Mixtures BMD approach. At the December 4 SAB PFAS panel meeting, EPA clarified that PODs based on HEDs are to be used in all approaches included in the mixtures framework document, including the Mixtures BMD approach. This should be clarified in the final document.

PRELIMINARY RESPONSES to Charge Questions for SAB Review of the Proposed Approach to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Study Identification and Inclusion

Charge Question 1: *EPA used systematic review methods consistent with the current ORD systematic review practice to ensure transparency and completeness of literature identification, sorting, and study quality evaluation. Is the process clearly described?*

Response: As a general comment, these assessments will provide the basis for the PFOA and PFOS MCLGs. They therefore have large practical impacts and will likely receive extensive attention and scrutiny. For this reason, it is especially important that a strong and transparent rationale for all decisions and conclusions and a thorough description of all steps in the evaluation of the scientific literature be provided. Specific comments on the systematic review approach used by EPA follow:

Section 1.0 (Background) states: “Fit-for-purpose systematic review methods, also consistent with current EPA methods, were used to develop the toxicity values within the timeline to rule proposal and in order to follow a transparent and scientifically robust process to identify, evaluate, and synthesize the best available science.” However, the specifics of the “fit-for-purpose” systematic review methods that were used to allow for completion of the assessment “within the timeline to rule proposal” do not appear to be provided. For example, information should be provided on any modifications of the Office of Research and Development’s systematic review approaches presented in the draft EPA ORD staff handbook for developing IRIS assessments (USEPA, 2020), which is cited later in the document,

Within Section 2.0 (Methods for PFOA/PFOS Health Effects Systematic Review), Section 2.1 on the criteria for study inclusion states that: “Systematic review methods used were largely consistent with the recent draft ORD staff handbook for developing IRIS assessments (EPA, 2020...).” As such, it appears that the systematic review approaches from EPA (2020) were used for just these components of the toxicity assessment, but not for the other components of the assessment described in other parts of Section 2 (which discusses systematic review for all steps in the process). At the December 16, 2021 SAB meeting, EPA confirmed that the systematic review approach from EPA (2020) or another structured approach was not used for other components of the toxicity assessment such as evidence synthesis and integration. The lack of a structured approach for the overall health effects evaluation process was stated to be problematic by one of the public commenters (Dr. Katie Pelch) at the December 16, 2021 SAB meeting.

Comments are provided on each subsection of Section 2 below:

2.1.1 Incorporation of Data from the 2016 Health Effects Support Documents

p. 11. The rationale for not considering human studies that were included in the 2016 HESDs is not clear and does not appear to be supportable. The 2016 HESD for PFOA concluded that there was strong evidence for human effects of PFOA, as follows: "Human epidemiology data report associations between PFOA exposure and high cholesterol, increased liver enzymes, decreased vaccination response, thyroid disorders, pregnancy-induced hypertension and preeclampsia, and cancer (testicular and kidney)" and that "...human data identified significant relationships between serum levels and specific indicators of adverse health effects..."

The reason for exclusion of the human studies as the basis PODs and RfDs in the 2016 HESDs was not related to the quality of the studies themselves or to a lack of overall evidence for human effects, but was rather due to the lack of a toxicokinetic model to relate internal dose (serum levels) to external exposure (administered dose); the 2016 PFOA HESD states that human data were not used as the basis for PODs and RfDs because of "lack [of] the exposure information for dose-response modeling."

EPA has now reviewed and accepted a model (Verner et al., 2016) that can relate serum PFOA and PFOS levels to external dose during developmental lifestages and throughout life, and it has concluded that the human data for PFOA and PFOS can be used in dose-response modeling. As such, the rationale for excluding the earlier human studies from current consideration is not clear and does not appear to be supportable. Consideration of the earlier human studies is particularly important because conclusions about the level of evidence for human health effects should be based on the overall weight of evidence from all relevant data, including both earlier and more recent studies.

Additionally, there is inconsistency among the different health outcomes sections as to how the epidemiology studies included in the 2016 HESDs are discussed and considered. The earlier studies should be considered in a standardized way for all health outcomes, or a rationale for deciding whether or not to discuss the earlier studies for a specific health outcome should be provided. As just one example from the PFOA document, the numerous epidemiology studies of birth weight and related fetal growth effects included in the 2016 HESD are not mentioned at all in the Section 3.3.1 on developmental effects. In contrast, the studies of male and female reproductive effects included in the 2016 HESD and the overall conclusions regarding reproductive effects in the 2016 HESD are discussed in some detail in Section 3.3.2 on reproductive effects. Similar discussions of epidemiology studies and conclusions from the 2016 HESDs are also found in sections on several other health outcomes.

Relevant to the need to consider the earlier human studies, it is important to recognize that a specific human epidemiology study included in the 2016 HESDs may be preferable to the more recent studies for POD development for some health endpoints. As an illustration of this point, the PFOA and PFOS RfDs are based on data from Grandjean et al. (2012), a study that was included in the 2016 HESDs. Specifically, the RfDs are based on serum PFOA or PFOS levels at age 5 and tetanus or diphtheria vaccine antibody concentrations at age 7 in Cohort 3 (born 1997-2000) from Grandjean et al. (2012).

Subsequent studies from the same research group (Grandjean et al., 2017; Mogensen et al., 2015) evaluated associations of measured or modeled serum PFOA at age point(s) other than 5 years and antibody response at age points other than 7 years, and/or included Cohort 5 (born in 2007-09). Some of these studies (e.g., Mogensen et al., 2015; Grandjean et al., 2017) also used the data from Grandjean et al. (2012) in some of their analyses, but the BMDL (from Budtz-Jorgensen and Grandjean, 2018) used as the POD for the final RfD comes only from data originally presented in Grandjean et al. (2012). If the subsequent papers that included additional analyses of some of the data from Grandjean et al. (2012) had not been published, the data from Grandjean et al. (2012) that was ultimately selected as the critical dataset for the RfD would not even have been considered for POD development.

Relevant to this point, Grandjean et al. (2012) is not included in the tables summarizing epidemiology studies (Table C-7) in the PFOA and PFOS documents, and there is no systematic review evaluation of Grandjean et al. (2012) in HAWC. As such, it is not clear whether or not the full systematic review evaluation of the various domains included in HAWC was performed for the Cohort 3 analysis from Grandjean et al. (2012) as part of the HAWC evaluation of Grandjean et al. (2017), a later study that used the same data in some of its analyses. Since the data used as the basis for the final RfDs come from Grandjean et al. (2012), it should be clarified that this study was systematically reviewed, if this is the case, and, if it was not systematically reviewed, it should be.

2.1.2 PECO Criteria for the Updated PFOA Health Effects Systematic

Review In general, this is topic clearly described.

However, there are some inconsistencies about this information among different sections of the draft document as to the durations of the studies that were included/not included, and clarification is needed. For example, Table 2 of the PFOA document states: "Studies with less than 28 days of dosing, with the exception of reproductive or developmental studies, should be tagged as supplemental," but Section 3.1.3, which is about literature search results for animal toxicology studies, states, "of the 32 animal studies that met the inclusion criteria, most studies had either short-term (n = 15) or developmental (n = 12) study designs." The inclusion of short-term studies other than developmental studies in Section 3.1.3 does not seem to be consistent with the information in Table 2. Possibly relevant to this issue, non-developmental studies with a shorter than 28 day duration were reconsidered in the 2016 HESD. The 2016 HESD states on p. 4-4: "A number of studies identified adverse effects following low dose exposures over durations of 7 to 38 days.

The studies fall into two clusters, those evaluating developmental or reproductive effects and those with a focus on immunological effects." For example, DeWitt et al. (2008), which is a key study from the 2016 HESD, is a 15 day study.

2.2 Updated Literature Search

Strategy This strategy is clearly described.

2.3 Screening Process

These criteria are clearly described.

2.4 Study evaluation.

The domains that were evaluated for the human and animal studies are found in the evaluation of each study in the HAWC database. However, the domains that were evaluated (e.g., selection and performance, exposure methods, outcome methods/results presentation, confounding, etc.) are not included in the text or a table in the PFOA or PFOS documents. It is suggested that these domains be included in the documents to inform the readers about the factors considered in study evaluation.

2.4.1 Dose-response studies

It is stated in the PFOA and PFOS documents that "Studies were evaluated for use in POD derivation on the basis of study design, study quality evaluation, and data availability." As written, it is unclear that the overall weight of evidence that PFOA and PFOS causes the effects from all relevant studies was also considered in selecting the endpoints that are included for POD derivation, and this should be clarified.

It is also stated in the draft PFOA and PFOS documents that: "For human evidence, all study designs were considered; for animal evidence, only animal studies with at least two exposure groups and with high or medium for study quality were considered." It is unclear if this means that human studies with low confidence were considered. If all human studies were considered, the rationale for considering human studies, but not animal studies, that have low confidence should be provided.

2.5 Data extraction

2.5.1 Dose-response studies

Some of the statements in this section of the PFOA and PFOS documents are not completely clear and need to be clarified:

"Data extraction was conducted for most studies that were included in the literature inventory, except those excluded as described below. ... Extractions were limited to outcomes of interest and/or the most sensitive LOAEL." Does this mean the most sensitive LOAEL based on internal dose (serum PFOA level) or administered dose? For most human studies, the doses are based on serum levels (internal dose). For animal studies, the lowest LOAEL based on administered dose may not correspond to the lowest LOAEL based on internal dose (serum level) because of differences in toxicokinetics (half-life) between species or sexes (e.g., much quicker excretion rate in female vs. male rats). While evaluating this issue for all studies for which it is relevant may not be possible due to resource limitations, it is suggested that this potential uncertainty in the approach be acknowledged.

It is stated that "...low confidence studies when medium and high confidence studies (e.g., on an outcome) were available" did not undergo data extraction." This appears to contradict the statement in Section 2.4 that low confidence animal studies were not considered and that "all study designs" (not specifying study confidence

level) were considered for human studies. This information should be clarified and should be consistent in the two sections.

2.5.2 ADME studies

Information on where the ADME data that were extracted can be found does not appear to be included in documents. If the data extraction from these studies are not publicly available, this should be stated.

2.5.3 Mechanistic studies

Again, there is no information about where the mechanistic data that were extracted can be found in the documents. If the data are not publicly available, this should be stated.

More importantly, mechanistic data are not summarized in this document, except for summary tables of the number of studies with each type of mechanistic information that were identified. EPA's rationale for concluding that evaluation of mechanistic data is not necessary for selection of critical effects for RfD development is needed. Discussion of the mode(s) and/or mechanism(s) of action for toxicity is normally an important component of toxicity assessments such as these draft EPA PFOA and PFOS assessments. As discussed in the draft ORD staff handbook for developing IRIS assessments (EPA, 2020), such an evaluation can provide information about the human relevance of effects observed in animal species, among other areas of potential uncertainty.

2.6 Evidence synthesis

The evidence synthesis/integration approach presented in this section of the documents is generally clear and appropriate. However, as discussed below, the Health Effects Evidence Synthesis and Integration sections on each health outcome in Section 3.3 do not appear to consistently follow the process presented in Section 2.6.

For example, it is not evident that "a summary discussion that addresses considerations regarding causation as adapted from Hill (1965)" is provided for each health outcome. Also, as mentioned above, there is frequently little or no discussion of mechanistic information, with only a reference to the section in the 2016 HESD on this topic and a statement that an updated evaluation will be completed after the SAB review.

As discussed in detail below, the content, format, and terminology are inconsistent among the evidence synthesis sections for different health endpoints. EPA stated at the December 16, 2021 SAB meeting that a structured approach was not used for evidence synthesis. EPA acknowledged the lack of consistency among evidence synthesis sections and stated that different sections were written by different scientists who used professional judgement as to the terms used and the way conclusions are presented.

These inconsistencies make it difficult to compare the conclusions of the different health effects sections.

Some specific examples of the general issues mentioned above are: The conclusions in different Evidence Integration sections do not use consistent terminology. For example, terms for which appear to have similar meanings, such as “*suggestive evidence*,” “*moderate evidence*,” and “*consistent evidence*” and “*inconsistent evidence*” and “*mixed evidence*,” but no definitions of these terms are provided. Furthermore, the overall nature of the conclusions for different health effects is not presented consistently (e.g., “*suggestive evidence for an association of PFOS with [the health outcome]*”, “*suggestive evidence that PFOA impacts [the health outcome]*”, or “*suggestive evidence for an effect of PFOA on [the health outcome]*”). It is unclear from the wording of these conclusions whether they are intended to apply to the evidence for association of the effect with PFOA or to the evidence that PFOA causes the effect; this is obviously an important distinction.

Additionally, information is not presented in a consistent manner in the sections on human and animal evidence for different health outcomes. As just one example (from the PFOA document), human studies considered in the 2016 HESD are discussed in detail for some endpoints (e.g., male reproductive, Section 3.3.2.1.1) but are not mentioned for other endpoints (e.g., birth weight), and no rationale is provided for why these studies are or are not discussed for each health outcome. As a second example (also from the PFOA document), the study selection criteria are presented for some endpoints (e.g., Developmental, Section 3.3.1.2) but not for other endpoints (e.g., Reproductive).

It is stated that: “The syntheses of human and animal health effects evidence focused on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of studies.” The meaning of “exposure context” here is unclear and should be clarified. It should be clarified whether this refers to the exposure levels that are relevant to environmental exposures, or to something else.

It is also stated that: “Low confidence studies were used [for evidence synthesis] if few or no studies with higher confidence were available to help evaluate consistency, or if the study designs of the low confidence studies addressed notable uncertainties in the set of high or medium confidence studies on a given health effect.” However, Section 2.4.1 says that low confidence animal studies were not considered. This inconsistency should be clarified.

To address these issues, it is suggested that a structured, consistent process and consistent terminology for analysis and synthesis of animal evidence, human evidence, and overall evidence, such as are presented in Chapters 9 and 11 of the draft ORD staff handbook for developing IRIS assessments (EPA, 2020), be used if possible. An example of the application of this approach in toxicity assessment of another PFAS is found in Sections

3.2 and 4.1 of the draft EPA IRIS assessment of perfluorobutanoic acid (PFBA) at https://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=543579. It is

recognized that it may not be possible to completely follow a systematic approach such as presented in Chapters 9 and 11 of EPA (2020), due to resources limitations and the "timeline to rule proposal." That being said, an approach that incorporates the general concepts for evidence integration presented in Chapter 11 (e.g., Figure 11-1) which include a more transparent strength of evidence evaluations using adapted Hill criteria and consistent criteria and terminology for determining strength of evidence is recommended. If it is not possible to use such an approach, the reason(s) should be provided.

Charge Question 1 (continued): Please identify additional peer-reviewed studies that the panel is aware of that could inform toxicity value derivation.

It is recommended that EPA consider the following additional studies:

Additional epidemiology studies on associations of PFAS and breastfeeding issues: Nielsen et al. (2021).

<https://www.sciencedirect.com/science/article/pii/S0013935121015073>

Timmerman et al. (2021). [https://academic.oup.com/jcem/advance-](https://academic.oup.com/jcem/advance-article-abstract/doi/10.1210/clinem/dgab638/6369501?redirectedFrom=fulltext)

[abstract/doi/10.1210/clinem/dgab638/6369501?redirectedFrom=fulltext](https://academic.oup.com/jcem/advance-article-abstract/doi/10.1210/clinem/dgab638/6369501?redirectedFrom=fulltext)

Additional epidemiology studies on associations of PFAS and vaccine antibody response: Shih et al. (2021).

<https://www.tandfonline.com/doi/epub/10.1080/1547691X.2021.1922957?needAccess=true>

Timmermann et al. (2022):

<https://www.sciencedirect.com/science/article/pii/S0013935121010069?via%3Dihub>

Additional epidemiology study on associations of PFAS with infectious disease: Bulka et al. (2021).

<https://www.sciencedirect.com/science/article/pii/S0269749121001974?via%3Dihub>
[1999e2016](https://www.sciencedirect.com/science/article/pii/S0269749121001974?via%3Dihub)

Additional epidemiology study on associations of PFAS with bone health: Buckley et al. (2021).

<https://ehp.niehs.nih.gov/doi/pdf/10.1289/EHP9424>

Banjabi et al. (2020). <https://pubmed.ncbi.nlm.nih.gov/32485360/>

Noncancer Hazard Identification

Charge question 1: Please comment on the health effect/outcome categories identified from the review of the available literature.

Response: The health effect/outcome categories included in the draft PFOA and PFOS documents appear to be complete and appropriate.

Charge question 1 (continued): Do you agree with the strong vs. suggestive evidencedesignations for the various health outcome categories?

Response: As a general comment, these assessments will provide the basis for the PFOA and PFOS MCLGs. They therefore have large practical impacts and will likely receive extensive attention and scrutiny. For this reason, it is especially important that a strong and transparent rationale for the conclusions about strength of evidence for health outcomes be provided.

The intent of this charge question was not clear regarding "strong vs. suggestive evidencedesignations for the various health outcome categories" because a conclusion of "strong evidence" was not made for any health outcome for PFOA and was only made for once (for altered serum lipid levels) for PFOS. At the December 16, 2021 meeting, EPA clarified the intent of the charge question. EPA stated that the question is not intended to ask about "strong vs. moderate evidence designations." It is intended to ask whether the evidence is strong enough to perform dose-response (develop PODs) for the endpoints selected for POD development and whether the evidence for any additional health endpoints is strong enough for POD development. As such, my response below discusses general issues about the strength of evidence designations and the selection of endpoints and studies for POD development. Some points in this response are related to my responses to the Systematic Review charge question.

The sections on Human Evidence and Animal Evidence for the specific health effects/outcomes are often difficult to follow due to the highly summarized presentation of the information and the inconsistent presentation of information and conclusions for different health outcomes (discussed in detail below). Although this may not be possible due to EPA's short timeline for MCL proposal, addition of summary tables that present basic information for each endpoint such as overall confidence rating, study population (e.g., general population, child, pregnant women, occupational, etc.) for epidemiology studies and lab animal species for toxicology studies, and results for endpoints evaluated (e.g., significant increase, significant decrease, or no effect) would be helpful to understanding the overall human and animal evidence for each effect.

Type of conclusion to which strength of evidence evaluation applies

There is inconsistency among the health outcomes sections in the draft PFOA and PFOS documents as to the specific type(s) of conclusion to which the strength of evidence terms apply. In some cases, it is stated that there is a certain level of evidence (e.g., suggestive) for "associations of PFOA with [the effect]", while in other cases, it is stated that there is a certain level of evidence (e.g., suggestive) that PFOA "impacts [the effect]" or a certain level of evidence "for [the effect]" (or similar language). It should be clarified whether or not these different terms are intended to distinguish between the level of evidence for an association (which is not necessarily causal)

versus the level of evidence supporting the conclusion that PFOA is actually causing the effect. Relevant to this point, Section 2.6 in the Systematic Review section states that "a summary discussion that addresses considerations regarding causation as adapted from Hill (1965)" is provided for each health outcome. Such a discussion would provide support for conclusions on strength of evidence for each health outcome, but it appears that such a discussion is not included for many health outcomes. Additionally, an evaluation of mechanistic evidence can be informative in determining weight of evidence for human health outcomes, especially when conclusions are based primarily on animal data. However, there is frequently little or no discussion of mechanistic information, with only a reference to the section in the 2016 HESD on this topic and a statement that an updated evaluation will be completed after the SAB review.

Terms used for weight of evidence conclusions

The level of evidence designations and the strength of evidence conclusions in the Evidence Integration sections are difficult to evaluate because a structured approach and consistent terminology were not used. At the December 16 meeting, EPA stated that consistent language and a structured approach were not used to describe the level of evidence for different health effects because the health endpoints sections were written by different individuals and the conclusions are based on professional judgment. EPA also acknowledged that terms such as "suggestive evidence," "moderate evidence," and other seemingly interchangeable terms are used in sections on different health outcomes, that these terms are not defined in the draft PFOA and PFOS documents, and that there is no intended difference among these seemingly similar terms. Additionally, no strength of evidence term is used in some cases, such as "...the evidence indicates an association between increased serum levels of PFOS and decreased antibody production following routine vaccinations in children," and "EPA concluded the impaired IgM response reported in [animal studies] supported the human results and this endpoint was considered for POD derivation [for PFOA]."

To address these issues, it is suggested that consistent descriptors be defined and used for human, animal, and overall strength of evidence conclusions for each endpoint. As an example, Table 11-5 in the draft ORD staff handbook for developing IRIS assessments (EPA, 2020) "Evidence integration judgments for characterizing potential human health hazards in the evidence integration narrative" provides the following descriptors: "evidence demonstrates", "evidence indicates [likely]", "evidence suggests but is not sufficient to infer", "evidence inadequate", and "strong evidence supports no effect".

Criteria and examples for applying these descriptors are also provided. Such an approach would be consistent with the use of consistent descriptors, with consistent criteria for selecting the descriptors, for the domains and overall confidence conclusions included in the evaluation of each individual study earlier in the overall process, as well as the descriptors for weight of evidence for carcinogenic potential in the EPA (2005) Guidelines for Carcinogen Risk Assessment.

Selection of health effects for POD development

The rationale and criteria for selection of endpoints for POD development are not

always clearly presented. Because a POD can potentially be used as the basis for a Reference Dose, the endpoints selected for POD development should be well established, sensitive, adverse or precursor to adverse, relevant to humans (for endpoints from animal studies), and have evidence supporting causality (for endpoints from human studies). It is suggested that it be clearly demonstrated that each endpoint selected for a POD meets these criteria.

For example, the draft PFOA and PFOS documents (PFOA - p. 317, first paragraph; PFOS – p. 290, final paragraph) state that “Well-conducted (i.e., high or medium confidence) human studies were prioritized for POD derivation and compared to PODs derived from animal data when possible when the human data provided an association between PFOA and an adverse effect. Such human studies were available for immunotoxicity, developmental, serum lipid, and hepatic effects. For other health effects where the epidemiological data were suggestive of adverse effects (i.e., reproductive and endocrine effects), dose response data from the animal studies were prioritized.” It is suggested that this statement be clarified to indicate that the weight of evidence for the human effects that were selected for POD development supports a conclusion of a causal relationship with exposure to PFOA, since human data described as showing an “association” for PFOA and an adverse effect does not appear to be sufficient to conclude that the data are appropriate for POD development.

Furthermore, the quoted text above indicates that human health effects with “suggestive” evidence were not used for POD development. However, the preceding Hazard Identification sections do not indicate that evidence for the human effects that were used for PODs (immune, developmental, serum lipids [for PFOA], hepatic) is stronger than “suggestive” or “moderate” (e.g., “strong” or “likely”). A conclusion of “strong” evidence is provided only for PFOS and increased serum lipids. For example, Section

3.3.1.4 on Evidence Integration for developmental effects states that there is “suggestive evidence that PFOA may impact fetal growth restriction across a variety of BWT [body weight]-related measures.” Uncertainties, including the potential impact of pregnancy hemodynamics and sample timing and the potential impact co-exposure to other PFAS, are mentioned, but no overall conclusion about whether or not these uncertainties preclude development of a POD for these effects is provided here. However, later in the document, PODs for decreased birthweight are developed for several human studies.

This issue is important because the draft ORD staff handbook for developing IRIS assessments (EPA, 2020) does not recommend PODs for health endpoints with “suggestive” evidence be used as the basis of toxicity values (i.e., Reference Doses). Because of the uncertainty associated with such PODs, EPA (2020) recommends that they be used only for range finding and prioritization. To address this issue, it is suggested that EPA reevaluate its strength of evidence conclusions to determine if some human endpoints, including (but not necessarily limited to) immune suppression, developmental effects, increased serum lipids (for PFOA), and decreased fetal growth, are better described as having “likely” or “strong” evidence rather than “suggestive” or “moderate” evidence. A conclusion of “likely” or “strong” evidence would provide support for development of a POD that can be used as the

basis for Reference Dose for the endpoint.

Based on the EPA (2020) criteria for “likely” evidence,¹ and the assumption that “strong evidence” mentioned in the charge question is equivalent to “evidence demonstrates” or “likely evidence” in EPA (2020), there appears to be sufficient evidence to classify additional endpoints as “strong” or “likely,” particularly if the studies included in the 2016 HESD are considered along with the more recent studies. As mentioned in my response to the systematic review charge question, consideration of these earlier studies is important for determining the overall weight of evidence for each health outcome, and the rationale for not considering them is not clear and does not appear to be supportable. As one example, the Evidence Integration section (3.3.4.4) for immune effects of PFOA states: “The evidence of an association between PFOA exposure and immunosuppressive effects in human studies is moderate based on largely consistent decreases in antibody response following vaccination ... in two medium confidence, overlapping birth cohorts.” Consideration of the older studies from the 2016 HESD that evaluated vaccine response populations in other locations could potentially support the conclusion that the evidence for this effect is stronger than “moderate.”

Selection of specific studies for POD development

A clearer explanation should be provided throughout as to why specific studies were selected for POD development when there are a number of possible choices. As one example, the BMDL presented in the Dong et al. publication was used by EPA as the POD for increased cholesterol, but it is unclear how it was decided that this BMDL is

¹ For “evidence indicates (likely)”, there should be “an evidence base that indicates that [PFOA] exposure likely causes [the health effect] in humans, although there may be outstanding questions or limitations that remain...” and “this conclusion level is used if there is robust animal evidence supporting an effect and slight-to-indeterminate human evidence, or with moderate human evidence when strong mechanistic evidence is lacking. This conclusion level could also be used with moderate human evidence supporting an effect and slight or indeterminate animal evidence, or with moderate animal evidence supporting an effect and slight or indeterminate human evidence. In these scenarios, any uncertainties in the moderate evidence are not sufficient to substantially reduce confidence in the reliability of the evidence, or mechanistic evidence in the slight or indeterminate evidence base (e.g., precursors) exists to increase confidence in the reliability of the moderate evidence.” valid as a POD. No information is provided in the PFOA document as to why Dong et al. (2019) was selected for dose-response for serum lipids from the many studies that are available. Furthermore, the BMD modeling section (Appendix B) states that few details are provided by the authors about several aspects of the BMD modeling presented in the Dong et al. (2019) publication; this lack of information does not appear to support use of this BMD as a POD.

Furthermore, the list of studies and endpoints considered for POD development in Table 15 is not totally consistent with the information in the text and the dose-response results in Appendix B. For example, some of the studies/endpoints (corpora lutea, body weight) stated in the text of the PFOA document to have been selected for dose-response/POD development were not modeled, while other endpoints (kidney weight) stated not to be selected for dose-response/POD development were modeled. Also, it is stated in the text that prenatal loss from Lau et al. (2006) was modeled, but the data from Wolf et al. (2007), not Lau et al. (2006), were actually modeled.

There is also inconsistency among the evidence integration sections for different health outcomes regarding information about whether any human and/or animal studies and, if so, which ones, were selected for POD development. For example, the human evidence integration discussions for developmental effects do not state whether any of the human studies were selected for POD development, although several birthweight studies were later selected (Table 15) for both PFOA and PFOS. As another example, the evidence integration sections for immune effects in both documents do not state that a human study of vaccine response (which eventually was used as the basis for the final RfD) was selected for POD development. In contrast, the evidence integration sections for some other endpoints specifically mention whether or not any studies were selected for POD development, and, if so, which studies were selected.

Finally, while it is stated (in the text quoted above - PFOA - p. 317, first paragraph; PFOS – p. 290, final paragraph) that PODs were developed from human data for hepatic effects, human data for hepatic effects was not used for POD development (as stated for PFOA on 148, for PFOS on p. 133, and in the charge question below).

Charge question 1 (continued): Do any other health systems or endpoints need to be considered for POD derivation?

I am not aware of any additional endpoints that should be considered for POD derivation.

Charge Questions 2 and 3: Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.

Charge Questions 2 and 3, Part A: Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a

POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.

EPA's rationale for not considering the ALT endpoint from human studies for derivation of a POD, particularly for PFOA, is not completely clear and does not appear to be totally consistent with the rationale for developing PODs for some other human health effects.

As stated in the charge question, increased ALT is indicative of liver damage. EPA (2002) guidelines for RfD development (<https://www.epa.gov/sites/default/files/2014-12/documents/rfd-final.pdf>) state that a Reference Dose should be based on an adverse effect or a precursor to an adverse effect. It was discussed at the December 16, 2016 SAB meeting that, although the magnitude of the effect on ALT may not be large, the same may also be true for the magnitude of the PFOA's effects on other human health endpoints such as increased cholesterol and decreased birth weight. As such, if a POD is not developed for the ALT endpoint, an explanation should be provided as to why the magnitude of the effect was not sufficient for ALT but was sufficient for other effects for which the magnitude is also small.

The draft EPA PFOA document discusses Darrow et al. (2016), which reported an association between modeled PFOA exposure and increased risk of clinically elevated ALT in a population with elevated PFOA exposure from contaminated drinking water. Gallo et al. (2012), which was not included in the draft PFOA document but was reviewed in the 2016 HESD, also found an association between measured serum PFOA levels and increased risk of clinically elevated ALT in the same study group.

Furthermore, as noted in a recent review of epidemiological evidence for health effects of PFOA by Steenland et al. (2020, <https://www.sciencedirect.com/science/article/pii/S0160412020320808>): "There is also evidence that effects on ALT are more pronounced among obese subjects, who are at higher risk of nonalcoholic fatty liver disease (Lin et al. 2010 https://journals.lww.com/ajg/Abstract/2010/06000/Investigation_of_the_Associations_Between_Low_Dose.24.aspx; Jain and Ducatman 2019, https://journals.lww.com/joem/Abstract/2019/04000/Selective_Associations_of_Recent_Low.5.aspx)."

Relevant to the potential significance of elevated ALT, Steenland et al. (2020) concluded that while "the limited existing evidence does not support a link between PFOA and diagnosed liver disease," there is a lack of "adequately powered epidemiologic studies of liver disease" and PFOA. They stated that "the established liver toxicity of PFOA in experimental animal studies ..., the storage of PFOA in liver tissue in humans, and extensive evidence that PFOA exposure is associated with markers of hepatocyte cell death, warrants additional research on PFAS and liver disease, particularly nonalcoholic fatty liver disease."

It should be noted that California EPA (2021; <https://oehha.ca.gov/media/downloads/crnrf/pfoapfosphgdraft061021.pdf>) selected

increased risk of clinically elevated serum ALT as the basis for its draft Reference Dose for PFOA. In their evaluation, California EPA (2021) considered the issues related to use of elevated ALT in humans as a critical effect that are discussed in the charge question above. It is suggested that EPA review the California EPA (2021) rationale for its decision to use elevated ALT as the critical effect for Reference Dose development. If it is decided to develop a POD for elevated ALT, EPA should review the modeling approach used by California EPA for this effect.

Charge Questions 2 and 3, Part B. *Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.*

As mentioned in my response to the Systematic Review charge question above, I recommend that the epidemiological studies included in the 2016 HESD be considered when evaluating the weight of evidence for epidemiological effects of PFOA and PFOS, including the studies reporting associations with increased ALT.

Additionally, as noted by Steenland et al. (2020), a recent study of adults from a community with elevated exposure to PFOA from contaminated drinking water showed that PFOA "was associated with cytokeratin 18 M30, a marker of hepatocyte apoptosis (Bassler et al., 2019), and a mechanism of disease progression in nonalcoholic fatty liver disease." Bassler et al. (2019, <https://www.sciencedirect.com/science/article/abs/pii/S0269749118341599>) provides further evidence that PFOA causes liver cell injury, and it is suggested that EPA consider this study.

c) Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

I am not aware of any other adverse liver endpoints that should be considered.

Cancer

Charge Question 1. *Cancer classification for PFOA/PFOS*

Charge Question 1, Part A. *PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a 'likely carcinogen' categorization which is a change from 'suggestive' in the 2016 HA. Does the panel agree with the 'likely' designation based on the new evidence?*

Based on the results of human and animal studies of PFOA presented in the draft document, I agree that it is appropriate to designate PFOA as "Likely to Be Carcinogenic to Humans."

As discussed in the draft EPA PFOA document, PFOA was associated with increased risk of testicular and kidney cancer in studies of a large population with elevated exposure from contaminated drinking water Barry et al., 2013; Vieira et al., 2013) and with increased risk of kidney cancer in a large general population study (Shearer et al., 2020).

PFOA also caused testicular Leydig cell, pancreatic acinar cell, and/or hepatocellular

tumors in male Sprague-Dawley rats in three chronic studies (Butenhoff et al., 2012 [also reported by Sibinski, 1987]; Biegel et al., 2001; NTP, 2020), In NTP (2020), the incidence of both malignant and benign tumors was increased, and the incidence of pancreatic tumors was very high in all dosed groups of males (2020). There was also a marginal increase in hepatocellular carcinomas and uterine adenocarcinomas, and non-significant increases in benign and malignant pancreatic acinar cell tumors, in females in NTP (2020). The lower response in females was stated by NTP (2020) to be consistent with the lower plasma PFOA levels due to the rapid excretion of PFOA in female rats.

Mode of action analyses (2016 PFOA HESD; New Jersey Drinking Water Quality Institute, 2017 <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>) have concluded that the rat liver tumors caused by PFOA may not be relevant to humans, although this is not a settled issue. However, the mode of action for other types of tumors caused by PFOA in rats has not been established, and, as specified in the EPA (2005 https://www.epa.gov/sites/default/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf) Guidelines for Carcinogen Risk Assessment, they are considered relevant to humans.

The EPA (2005) Guidelines for Carcinogen Risk Assessment provide several examples of data that support the descriptor "Likely to Be Carcinogenic to Humans." The data from human and animal studies of PFOA summarized above are consistent with the three examples below:

- "an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments."
- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans."
- "a positive tumor study that is strengthened by other lines of evidence, for example, ... plausible (but not definitively causal) association between human exposure and cancer."

In contrast, the data relevant to carcinogenic potential of PFOA are stronger than in the four examples below from the EPA (2005) guidelines for the descriptor "Suggestive Evidence of Carcinogenic Potential."

- "a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor 'Likely to Be Carcinogenic to Humans.' The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system."
- "a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due

to intrinsic factors that cause background tumors and not due to the agent being assessed."

- "evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence (such as structure-activity relationships)."
- "a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend."

Charge Question 1, Part A (continued). *If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.*

While the results of human and animal studies of PFOA support designation as a "Likely to Be Carcinogenic to Humans," the criteria from the EPA (2005) Guidelines for Carcinogen Risk Assessment for identification of a chemical as a "likely" or "suggestive" carcinogen are not presented in the draft PFOA document. It is strongly suggested that a discussion of these criteria and how they apply to the data for PFOA be added to the Weight of Evidence section.

Also, on p. 343-344 of the draft EPA PFOA document, the issue of genotoxic versus non-genotoxic MOA for carcinogenicity is discussed, followed by the conclusion that PFOA is considered "Likely to Be Carcinogenic to Humans." It should be made clear that the designation of "likely to be carcinogenic to humans" is independent of whether the MOA is genotoxic or non-genotoxic.

Finally, the weight of evidence evaluation (i.e., determination of the appropriate descriptor for carcinogenic potential, such as "suggestive" or "likely") is part of the Hazard Identification component of the risk assessment, not the Dose-Response component. As such, the Weight of Evidence section (section 4.2) should be moved from the Dose-Response section to the Hazard Identification (e.g., Evidence Integration) section on cancer.

Charge Question 1, Part B. *PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a 'suggestive' categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.*

The information on new cancer studies for PFOS identified since the 2016 HESD needs to be clarified in the final document. In the draft EPA PFOS document, the Hazard Identification section on cancer (Section 3.3.1.7) discusses Shearer et al. (2021), which showed an association of PFOS with kidney cancer. Regarding association of kidney cancer with PFOS in Shearer et al. (2021), the draft EPA PFOS document states (p. 286-287): "PFOS was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma) in a medium confidence study {Shearer, 2021, 7161466}. The study reported a statistically significant increase in risk in the highest exposure quartile and per doubling of

PFOS concentration. After adjusting for other PFAS the association remained elevated in the highest quartile (i.e., adjusted OR=1.14), but it was no longer statistically significant and was lower than the second quartile; additionally, there was no association when evaluated on a per doubling of PFOS."

As discussed above, the CSF for PFOA in the draft EPA PFOA document is based on increased incidence of kidney cancer in this study. Regarding association of kidney cancer with PFOA in Shearer et al. (2021), the draft EPA PFOA document states (p. 309): "PFOA was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma (RCC)) {Shearer, 2021, 7161466}. This large medium confidence case-control study nested within the NCI's Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO) reported a statistically significant increase in risk of kidney cancer in highest exposure quartile and per doubling of PFOA concentration. Even after adjusting for other PFAS the association remained significant in analyses on a per doubling increase in PFOA. The increase in the highest exposure quartile remained and the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS), but it was no longer statistically significant."

As indicated in the quotes from the draft EPA PFOA and PFOS documents above, Shearer et al. (2021) found associations of kidney cancer with both PFOA and PFOS, but some of specific analyses that were statistically significant differed for PFOA and PFOS. The draft EPA PFOS document does not mention that the Shearer et al. (2021) results for PFOA were used for CSF development, and it does not discuss why the PFOS results are not considered to be as definitive as the PFOA results. This information should be added to the final document.

Additionally, it is unclear why the cancer weight of evidence section (Section 4.2.1) of the draft PFOS document does not mention Shearer et al. (2021) in its discussion of new studies of PFOS and cancer identified since the publication of the 2016 HESD, especially since Shearer et al. (2021) is discussed in the Hazard Identification (3.3.1.7) section. Discussion of Shearer et al. (2021) should be added here.

Charge Question 2. Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends.

It was unclear what was meant by "peak exposure" in this charge question since this term is not mentioned and this topic is not discussed in the draft EPA PFOA document. At the December 16, 2021 SAB meeting, EPA clarified that the term "peak exposure" should be disregarded, and that input is requested on the development of the CSF in general.

The draft EPA PFOA document states that the CSF for Shearer et al (2021) is based on the modeling approach used by California EPA (2021, <https://oehha.ca.gov/media/downloads/cnr/pfoapfosphgdraft061021.pdf>). At the December 16, 2021 meeting, it was stated that EPA ORD independently replicated the modeling performed by California EPA. However, the details of the modeling and its

results are not shown in the draft PFOA document, and this should be included in the final EPA document.

Table 6.2.7 (p. 223) of California EPA (2021) provides the central tendency estimate of the slope factor from Shearer et al. (2021) as a serum PFOA level of $0.00178 \text{ (ng/ml)}^{-1}$ ($1.78 \times 10^{-3} \text{ ng/ml}$). However, the central tendency CSF shown in Table 25 of the draft EPA PFOA document as a PFOA serum levels of $1.78 \times 10^{-6} \text{ ng/ml}$, and this therefore appears to be a typographical error. The 95% upper confidence level of this CSF is also shown in Table 25 of the draft EPA PFOA document. However, the 95% upper confidence level CSF is not provided in Cal EPA (2021), and, as mentioned above, no information on the modeling results that provided this value are shown in the draft EPA document.

Table 15 (p. 345) of the EPA PFOA document also shows the central tendency and upper 95th percentile CSFs in terms of administered dose (ng/kg/day)^{-1} . However, the determination of the CSF in terms of administered dose (ng/kg/day)^{-1} from the CSF in terms of PFOS serum level (ng/ml)^{-1} (ng/ml) CSFs is not clear, as this is not even mentioned either in the main part of the document or in Appendix Section B.1.5.1 which briefly discusses the calculation of the cancer slope factor from Shearer et al. (2021). It appears that a clearance factor of 0.12 ml/kg/day , based on the half-life of 2.7 years and volume of distribution of 170 ml/kg (the values stated to have been selected by EPA in Sections 3.2.3 and 3.2.4 earlier in the document) was used, since applying this clearance factor to the central tendency serum level slope factor ($0.00178 \text{ (ng/ml)}^{-1}$) results in the administered dose slope factor shown (0.01483 day^{-1}). However, neither the clearance factor or its use in determination of the administered dose CSFs appear to be mentioned in the draft document. Additionally, application of the clearance factor of 0.12 ml/kg/day to the 95th percentile upper confidence level serum level slope factor does not result in the 95th percentile administered dose slope factor shown in Table 25. Finally, although the numerical value of the central tendency slope factor (0.00178) is shown correctly in Appendix B.1.5.1, the units shown, (ng/kg/day)^{-1} instead of (ng/ml)^{-1} , are incorrect. The development of the clearance factor and its use in determining that administered dose CSFs from the serum level CSFs should be clearly and completely described in the final document.

Charge Question 2 (continued). *Does SAB support the selection of this CSF in the derivation of a risk specific dose for PFOA (i.e., the concentration of PFOA in drinking water that would have a one-in-1-million chance of an increased cancer risk)? If not, please provide input on the strengths and weaknesses of the other candidate CSFs that EPA derived.**

I support the use of the CSF based on human epidemiological data from Shearer et al. (2021) to derive a risk specific dose for PFOA. The other CSFs derived by EPA are based on animal data, and it is preferable to base a CSF on human data when appropriate human data are available. However, it is not clear from the information presented in the draft PFOA document whether the central tendency CSF or the upper 95th percentile CSF would be used to develop a risk specific dose. Finally, I agree with EPA's decision not to apply age-dependent adjustment factors (ADAFs) to the PFOA CSFs. Although not mentioned in the draft EPA PFOA document, the EPA (2005)

Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (https://www.epa.gov/sites/default/files/2013-09/documents/childrens_supplement_final.pdf) recommend that application of ADAFs for carcinogens known to act through a mutagenic mode of action, and this is not the case for PFOA.

Toxicokinetic Models

Charge Question 1. Human model

Charge Question 1, Part A. *For endpoints observed in adults, EPA used a steady-state approach to calculate the HED, which assumes a relatively constant exposure and clearance during adulthood. Please comment on this method of HED calculation. Are there alternative approaches that EPA should consider? If so, please describe the rationale for recommending this approach(es).*

Consideration of prenatal and breastfeeding exposures in modeling of serum levels at age 5 that should be thoroughly described

In the draft PFOA document, it is not clear from the information provided in Section 4.1.3.2 (Toxicokinetic Model for Human Dosimetry) that prenatal and breastfeeding exposures were considered in development of the PODHED for decreased vaccine response from exposure at age 5. The excerpt from Section 4.1.3.2 of the draft PFOA document copied below indicates that the steady-state assumption was used to develop the PODHED for all of the human endpoints, including the PODHED based on exposure at age 5, and that exposures during early life stages were only considered in animal developmental studies.

“This updated [Verner et al., 2016] model was used to simulate the HED from the animal PODs that were obtained from BMD modeling of the animal studies (Section B.2). It was also used to simulate selected human studies to obtain a chronic dose that would result in the internal POD obtained from dose-response modeling. **For PODs resulting from chronic exposure, such as a long-term animal study or a human study, the steady state approximation was used** to calculate a HED that would result in the same dose metric after chronic exposure. **For PODs from exposure to developmental animals, the life-stage developmental model was used** to calculate a HED that results in the same dose metric during the developmental window selected by the Dose-Response team.”

In the draft PFOS document, it is indicated that prenatal and breastfeeding exposure was considered in development of the PODHED for decreased vaccine response from exposure at age 5, and EPA confirmed this at the December 16, 2021 SAB meeting. However, this topic is not discussed in the text; it is only mentioned in a footnote to the PODHED for decreased antibody response to tetanus and diphtheria vaccines in Table 21 as follows: "Calculated as the dose to mothers & children that results in the same serum concentration at 5 years of age. Note that the model predicted slightly different

serum concentrations for male and female children, so the lower HED was selected to be more health protective."

A complete discussion of how prenatal and breastfeeding exposures were considered in development of the PODHED for decreased vaccine response from exposure at age 5 should be provided, including clarification of what is meant by "the dose to mothers & children that results in the same serum concentration at 5 years of age." Information on this topic is especially important because these PODs are the basis for the final Reference Doses for PFOA and PFOS.

Unclear how model could be used to develop MCLG that considers both breastfeeding exposure and maternal and child drinking water ingestion rates

Although the draft documents develop Reference Doses, not MCLGs, EPA should consider how a Reference Dose based on serum PFOA levels at age 5 can be used to develop a drinking water concentration (MCLG) that is protective for all lifestages. It is unclear how a "dose to mothers & children that results in the same serum concentration at 5 years of age" can account for the different drinking water rates in adults (mothers) and children.

The draft documents discuss differences between the Verner et al. (2016) and Goeden et al. (2019) models for predicting serum PFAS levels in early life, and they state that the Verner et al. (2016) model was selected because "the Goeden et al. (2019...) model did not account for the decrease in concentration that occurs due to growth dilution which plays a substantial role in the PK of growing infants and children." However, the draft documents do not recognize that the two models have different purposes and provide different information. The Verner et al. model predicts infant and child serum PFOA or PFOS levels resulting from a constant daily PFOA or PFOS dose (ng/kg/day) to the mother and to the child after weaning. In contrast, the Goeden et al. (2019) model considers both age-specific toxicokinetic factors and the changing drinking water intake at different age periods. This model predicts the serum PFOA or PFOS levels at any age (including infancy, childhood, and adulthood) that result from maternal and child consumption of water with a certain concentration (ng/L) of PFOA or PFOS. Specifically, it considers the maternal drinking water intake rate which impacts PFOA or PFOS levels in breastmilk, and the varying drinking water intake rates in children of different ages after weaning. The daily water intake (L/kg/day) in young children is much higher than in older individuals, and it varies during different childhood age periods up to age 5.

As noted above, Goeden et al. (2019) did not consider growth dilution, which would decrease serum levels if the dose remains constant. However, when exposure to PFOA or PFOS comes from drinking water, the higher dose received by young children due to their higher water consumption would tend to counteract the growth dilution effect. For example, Goeden et al. (2019; Figure 6) predicts that, with a certain concentration of PFOA or PFOS in drinking water, the serum PFOA or PFOS level in a 5 year old child who was breastfed for 6 months is about 2.8-fold higher than at steady-state in adulthood. Serum levels at age 5 years would be even higher from breastfeeding for one year, as was assumed by EPA (p. 333, last paragraph of PFOA document). It is not

clear how a Reference Dose from the Verner et al. model, which predicts serum PFOA or PFOS levels at age 5 years from a constant daily dose to the mother and the child, can be used to develop an MCLG than considers both exposure through breastfeeding and post- weaning and the changing drinking water consumption rates up to age 5.

Charge Question 1, Part B. *Two key parameters are the half-life and volume of distribution, which were used to calculate clearance. Half-life and volume of distribution were assumed to be constant across sex and age groups because of a lack of strong quantitative data to parametrize changes across sex and age. Please comment on the strengths and weakness of the use of this assumption and the choice of these parameters by the EPA. Please describe the rationale for alternative recommended approaches.*

I have no comment on this specific question. However, Section 4.1.3.2 of the draft PFOA and PFOS documents discuss the parameters from the Verner et al. (2016) model that were modified by EPA, and these do not include the volume of distribution or half- life. For both PFOA and PFOS, the volumes of distribution used in Verner et al. are identical to those stated to have been selected by EPA in Section 3.2.4 of the draft documents (0.17 L/kg for PFOA; 0.23 L/kg for PFOS) are identical. However, the half- lives of 3.8 years for PFOA and 5.4 years for PFOS used by Verner et al. differ from the half-lives of 2.7 years for PFOA and 3.8 years for PFOS stated to have been selected by EPA in Sections 3.2.3 of the draft documents. It is unclear whether EPA used the half-life values that it selected or those selected by Verner et al. when applying the Verner et al. model. Also, if EPA did not use the half-lives it selected when applying the Verner et al. model, it is unclear where they were used in the EPA evaluation. This information should be clarified.

Charge Question 1, Part B (continued). *For endpoints observed in human neonates or children, EPA used a one-compartment TK model to simulate dosimetry during pregnancy and a two-compartment TK model (one-compartment models for the mother and the child) to simulate dosimetry during lactation, to calculate the HED for each POD. Please comment on the strengths and weaknesses of this choice of model structure for the task of predicting dosimetry in the human fetus and child compared to dosimetry in mice and rats in the similar lifestages.*

Please provide the rationale for any alternative recommended approaches.

I do not have a specific response to this charge question. However, please see my response to Part A regarding questions about how varying drinking water ingestion rates at different lifestages can be incorporated into the model that to predict dosimetry from exposure to PFOA and PFOS in drinking water in the human fetus and child.

Charge Question 1, Part C. *The key chemical-specific parameters that describe the transfer of the chemical from the mother to the child during gestation and lactation are the maternal to fetal serum ratio and the ratio of maternal serum to milk PFOA/S concentration. These ratios were assumed to be constant during gestation and lactation, respectively. Another important parameter is the rate of milk ingestion, which is chemical-independent and varies throughout lactation. Please comment on the strengths and weaknesses of the choice of parameters for*

fetal to maternal partitioning and partitioning into breastmilk, as well as the choice for lactation rate. Please also comment on the choice to assume that fetal to maternal partitioning and partitioning to breastmilk did not vary in time. Please describe whether there are other methods you would recommend to account for these changes over time and across development.

I do not have a response to this charge question.

Charge Question 2. Animal model

Charge Question 2, Part A. *After a review of the available toxicokinetic models for PFOA/S predictions in laboratory animals, EPA selected the Wambaugh et al. (2013) model because it was parametrized using all species of interest, demonstrated good agreement with training and test datasets, and used a single, biologically motivated, model structure across all species. Does the panel agree with selecting this model? If not, please describe the rationale for alternative recommended approaches for the calculation of the internal dose metrics in adult animals.*

The PFOA document (p. 330) states: “The purpose of the animal PBPK model is to make predictions of internal dose in lab animals used in toxicity studies or in humans. Therefore, to evaluate its predictive utility for risk assessment, a number of dose-metrics across life stages were selected for simulation in a mouse, rat, monkey, or human.” However, toxicokinetic parameters are presented only for animal species, but not humans, in Section 4.3.1, and application of the model to humans is not discussed. As such, it should be clarified whether or how this model was used to simulate human exposures.

Also, when appropriate serum or plasma PFOA/PFOS data are available from the study for which a POD is being developed, did EPA consider using the serum/plasma data from the study itself (e.g., from at the end of the dosing period) rather than the serum levels predicted by modeling? Use of data from the study itself could reduce uncertainty. The New Jersey Drinking Water Quality Institute (DWQ, 2017 <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>; DWQI, 2019 <https://www.state.nj.us/dep/watersupply/pdf/pfos-recommendation-appendix-a.pdf>) used an approach based on serum PFAS levels measured at the end of dosing in development of Reference Doses for PFOA and PFOS.

Charge Question 2, Part B. *The animal model parameters were obtained through a Bayesian inference parameterization which produced wide credible intervals for some parameter values, but relatively tight credible intervals for the predicted serum concentration. Does the panel agree with using the median values of the estimated animal parameter distributions for prediction of serum concentration and internal dose metrics?*

I do not have a response to this question.

Charge Question 2, Part C. *Based on visual inspection of model predictions to the calibration datasets, EPA utilized sex-independent parameters for PFOS. The male-specific parameters were used for all rat-specific PFOS predictions including predictions in pregnant and nursing*

dams and the female-specific parameters were used for all mouse-specific PFOS predictions because the parameter values obtained from fitting the female-specific rat data and male-specific mouse data were not consistent with the overall TK parameters for PFOS and produced poor fits to the training and test datasets. Does the panel agree with this approach and justification for this assumption for PFOS? If not, please describe other approaches that could be considered?

I do not have a response to this question.

Charge Question 2, Part D. *EPA assumed a one compartment model for the developing infant based on the lack of infant-specific toxicokinetic data from rats and mice. This model utilizes averages of half-life and volume of distribution from the literature coupled with physiologically relevant lactational parameters for pup nursing. Does the panel agree with the decision to use this model structure for infant animals? If not, please provide data on infant-specific changes during the animal lactational-period that could be used to account for toxicokinetic differences between the adult and infant rats and mice.*

I do not have a response to this question.

Charge Question 2, Part E. *Several parameters dictate the transfer of chemical from the mother to her pup. Does the panel agree with the selection of these parameters for the animal model? If not, please provide your justification and alternative parameters.*

I do not have a response to this question.

Charge Question 2, Part F. *For neonatal animals, EPA assumed no sex differences in clearance in neonatal animals based on the lack of identification of sex-dependent differences in PFOA/S toxicokinetics from the available data. Does the panel agree with this assumption? If not, please provide your justification and available data on sex differences in neonatal rats.*

I do not have a response to this question.

Epidemiological Study RfD Derivation

Charge Question 1. *EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only 'medium' and 'high' quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?*

Exposures to PFOA, PFOS, and other PFAS are often correlated, and the quantitative data used for dose-response modeling of the association of a health effect with a specific PFAS may be confounded by co-exposure to other PFAS that also cause the same effect. Therefore, information on potential confounding by co-exposure to other PFAS should be presented for each of the epidemiological studies selected for POD derivation. This should include whether the impact of other PFAS was evaluated, and, if so, the results of the evaluation.

The BMDs for decreased antibody response to vaccines that was published by Budtz-Jorgensen and Grandjean (2018) were selected as PODs for the final PFOA and PFOS RfDs. Budtz-Jorgensen and Grandjean (2018) addressed the issue of confounding due to co-exposure to PFOA and PFOS and stated that this was accounted for in development of BMDs for PFOA and PFOS.

EPA also selected five human studies for development of PODs for decreased birthweight caused by PFOA, and four of these five studies were also used for development of PODs for this effect for PFOS. Information on potential impact of PFOA and other PFAS on associations of PFOS with decreased birthweight, and vice versa for PFOA, in each of these studies should be discussed in the PFOA and PFOS documents. For example, Chu et al. (2020) evaluated the impact of adjustment for PFOA and PFOS on the effect of another PFAS (CIPFESA), but it appears that the impact of PFOA on PFOS and vice versa were not evaluated. Additionally, it appears that Sagiv et al. (2018) did not evaluate potential confounding by co-exposure to other PFAS; the HAWC evaluation states that "there is some minor concern over potential bias due to confounding by other PFAS." The sensitivity analysis conducted by Starling et al. (2017) does not appear to support an association with PFOS for birthweight after co-exposure to other PFAS is considered. This sensitivity analysis is not mentioned in the HAWC evaluation of this study, and it should be reviewed by EPA to determine if this study is appropriate for dose-response for the effects of PFOS on birthweight. Finally, as noted in the HAWC file, Wikstrom et al. (2020) did not consider confounding by co-exposure to other PFAS; the authors discuss this as a limitation of their study.

Additionally, BMDs from the same human study (Dong et al., 2019) were used as PODs for increased serum cholesterol for both PFOA and PFOS. As stated in the HAWC evaluation for Dong et al. (2019), there was "no discussion of potential confounding across PFAS."

Charge Question 2. *Studies of developmental immune health outcomes (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]) after PFOA/S exposure identified associations with very low doses of either PFOA or PFOS with developmental immune effects. The RfD for this outcome was selected as the critical effect because it was the lowest among the candidate RfDs for PFOA or PFOS and can result in severe illness. Does the panel agree with the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS?*

Charge Question 2, Part A. *If so, please explain your justification.*

I do not disagree with the selection of the critical study. However, please see my comments in Part C below.

Charge Question 2, Part B. *If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of chronic RfDs.*

I do not have a response as I am not suggesting an alternative critical study or effect.

Charge Question 2, Part C. *Are any additional analyses or rationales needed to increase the confidence in the chronic RfDs for PFOA and PFOS?*

My response to this question below addresses several issues: 1) the need for an expanded explanation for use of human data as the basis for RfDs; 2) the need to clarify the duration of exposure to which the RfDs apply; and 3) the need for EPA to provide additional support for the PODs from Grandjean et al. (2012) that are the basis of the PFOA and PFOS RfDs.

Need for expanded explanation for use of human data as basis for RfDs

I agree with the overall EPA conclusion that there is strong evidence that very low exposure to PFOA and PFOS (i.e., within the general population exposure range) increases the risk of several health effects, and I support the use of human epidemiology data as the basis for the Reference Doses. That being said, there is a need for an expanded explanation of the rationale for the preferential use of human studies for POD derivation, resulting in much lower PODs than if animal data were used. It is important that the rationale for this decision be thoroughly explained and supported because it is a major change from the approach used in the 2016 HESD, which concluded that human data were precluded from use for PODs and RfDs.

Duration(s) of exposure to which Reference Doses (RfDs) apply

It is important for EPA to clarify the duration(s) to which the PFOA and PFOS Reference Doses apply. At the December 16, 2021 SAB meeting, EPA stated that the RfDs apply to shorter-than-chronic as well as chronic exposure. However, the draft documents state that PFOA and PFOS RfDs are intended for chronic exposure, and the fact that they apply to shorter exposure durations is not mentioned. As discussed at the December 16, 2021 meeting, these RfDs are based on an effect in childhood (decreased antibody levels in response to tetanus or diphtheria vaccine at age 7) that results from exposure in childhood (serum PFOA or PFOS levels at age 5). Although the critical effect results from shorter- than-chronic exposure, these RfDs are considered to also be protective for chronic exposure because they are more sensitive (i.e., have lower PODs) than chronic effects. Relevant to this point, the PFOA document (p. 338) states that no adjustment for subchronic to chronic exposure is needed (i.e., UF of 1) because "...the developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure," and "... the developing immune system is recognized as a susceptible life stage; therefore, exposure during this time window can be considered more relevant than lifetime exposure."

As such, these RfDs are applicable to shorter (subchronic and/or short-term) exposures as well as chronic exposure, in contrast to many other contaminants (e.g., GenX) for which subchronic RfDs are higher than chronic RfDs (see EPA, 2021 final GenX toxicity evaluation, https://www.epa.gov/system/files/documents/2021-10/genx-chemicals-toxicity-assessment_tech-edited_oct-21-508.pdf).

Clarity from EPA regarding the exposure duration(s) to which the RfDs apply will be extremely important when addressing exposures to PFOA and PFOS. In

situations of drinking water contamination, the duration of exposure to which MCLGs and MCLs based on these RfDs apply has practical implications for the timeframe (e.g., acute, short term, longer term) in which exposure to contaminated drinking water should be stopped when the MCL is exceeded.

It should be noted that the EPA (2009) Provisional Drinking Water Health Advisories for PFOA and PFOS were stated to apply to short-term exposure and were based on the higher exposure to drinking water contaminants in children, although they did not consider the even higher exposures to breastfed infants. See

<https://www.epa.gov/sites/default/files/2015-09/documents/pfoa-pfos-provisional.pdf>.

Additionally, the EPA (2016) Lifetime Health Advisories, which were based on developmental effects were stated to apply to both short-term (weeks to months) and lifetime (chronic) exposure, as follows: "... short-term exposure to PFASs can result in a body burden that persists for years and can increase with additional exposures. Thus, EPA recommends that the lifetime HA for PFOA of 0.07 µg/L apply to both short-term (i.e., weeks to months) scenarios during pregnancy and lactation, as well as to lifetime-exposure scenarios."

Need for additional support for selection of BMDs from piecewise model for age 5 serum levels and age 7 vaccine response from Grandjean et al. (2012) as basis of the PFOA and PFOS RfDs

The PFOA and PFOS RfDs are based on data from Grandjean et al. (2012), a study that was included in the 2016 HESDs. Specifically, the RfDs are based on serum PFOA or PFOS levels at age 5 and tetanus or diphtheria vaccine antibody concentrations at age 7 in a cohort that was born in 1997-2000 from Grandjean et al. (2012). Subsequent studies (e.g., Mogensen et al., 2015; Grandjean et al., 2017) that evaluated exposure and effects at other age points and/or included an additional cohort also used the data from Grandjean et al. (2012) in some of their analyses, but the BMDL (from Budtz-Jorgensen and Grandjean, 2018) that is used as the POD for the final RfD comes only from data originally presented in Grandjean et al. (2012).

Presumably because it was included in the 2016 HESDs, Grandjean et al. (2012) is not included in the tables summarizing epidemiology studies (Table C-7) in the PFOA and PFOS documents, and there is no systematic review evaluation of Grandjean et al. (2012) in HAWC. As such, it is not clear whether or not the full systematic review evaluation of the various domains included in HAWC was performed for the Cohort 3 analysis from Grandjean et al. (2012) as part of the HAWC evaluation of Grandjean et al. (2017), a later study that used the same data in some of its analyses. Since the data used as the basis for the final RfDs come from Grandjean et al. (2012), it should be clarified that this study was systematically reviewed, if this is the case. If it was not systematically reviewed, it should be.

Budtz-Jorgensen and Grandjean (2018) developed BMDs and BMDLs for maternal serum PFOA and PFOS and antibodies to tetanus and diphtheria vaccines at age 5 years (pre-booster), and for serum PFOA and PFOS at age 5 and antibodies to tetanus and diphtheria vaccines at age 7. EPA selected the BMDLs for serum PFOA at age 5 and tetanus antibody response at age 5, and serum PFOS at age 5 and diphtheria antibody response at

age 7, as PODs, and ultimately as the basis for the RfDs. However, the fact that the PODs are based on age 5 serum data and age 7 antibody response does not appear to be mentioned in the text or tables in any of the subsections of Section 4.0 on dose-response for non-cancer endpoints. The only place this choice appears to be mentioned is in Appendix B.1.1 where the BMD modeling is discussed. Even in Appendix B.1.1, the fact that additional BMDs and BMDLs for maternal serum PFOA and PFOS and antibody response at age 5 were also developed by Budtz-Jorgensen and Grandjean (2018) is not mentioned. An explanation should be provided about why the BMDLs for age 5 serum PFAS and age 7 antibody response were selected rather than the BMDLs for maternal serum PFAS and age 5 antibody response. This is unclear because the BMDLs for maternal serum PFOA and response to tetanus and diphtheria vaccines for the piecewise model are lower, and the ratio between the BMDs and the BMDLs are smaller, than for the BMDLs for the piecewise model for serum PFOA at age 5 years and tetanus antibody response at age 7 years that was selected by EPA.

Also, the rationale in the first paragraph of section B.1.1 of the draft PFOA and PFOS documents for selection of the BMDLs for the piecewise model instead of the linear model is not clear and should be clarified. Also, in the last sentence of this paragraph in the draft PFOA document, "PFOS exposure" should be "PFOA exposure,"

Need to provide details of BMD modeling from Grandjean et al. (2012) and EPA's review of the modeling

EPA did not independently replicate of the BMD modeling conducted by Budtz-Jorgensen and Grandjean (2018), and EPA's review of this BMD modeling by EPA is not discussed in the draft PFOA and PFOS modeling. Details of the modeling and the modeling output are not included in the Budtz-Jorgensen and Grandjean (2018) publication. However, at the December 16, 2016 meeting, EPA stated that Budtz-Jorgensen and Grandjean provided a supplemental document with additional details of the BMD modeling to the EPA Office of Research and Development. The details and conclusions of EPA's review of this BMD modeling should be included in the final PFOA and PFOS documents.

At the December 16, 2021 meeting, EPA also stated that they would find out if the supplemental document can be shared with the SAB. It is important that EPA make all information on this BMD modeling publicly available, since the results of this modeling provide the basis of the final EPA RfDs for both PFOA and PFOS. It is therefore strongly recommended that the supplemental document provided by Budtz-Jorgensen and Grandjean be included as an Appendix to the final PFOA and PFOS documents.

Additionally, both California EPA (2021, <https://oehha.ca.gov/media/downloads/crn/pfoapfosphgdraft061021.pdf>) and EFSA (2020, <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6223>) considered using decreased antibody response to vaccination from Grandjean (2012) as the basis for their toxicity factors (California EPA RfDs; EFSA Tolerable Weekly Intakes) for PFOA and PFOS. Both agencies provide their rationales for not selecting the data from Grandjean et al. (2012) as the basis for their toxicity factors. It is recommended that EPA review the evaluations of Grandjean et al. (2012) provided by California EPA (2021) and EFSA (2020) to determine if any of the conclusions made by these agencies should be considered

by EPA.

Charge Question 3. *The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.*

I do not have a response to this question.

Charge Question 4. *EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for PFOA and PFOS.*

i. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

The application of an uncertainty factor of 10 for inter-individual human variability and 1 for the other four uncertainty factors (interspecies, duration of exposure, NOAEL-to-LOAEL, and database) is appropriate and sufficiently protective. As discussed in the draft EPA PFOA and PFOS documents, there is no basis to select a factor other than the default value of 10 for intra-human variability. An interspecies uncertainty factor of 1 (i.e., no adjustment is made) is appropriate because the RfDs are based on human data. A value of 1 is also appropriate for the uncertainty factor for exposure duration because the critical effects (decreased antibody response to tetanus or diphtheria vaccine from exposure at age 5) result from shorter-than-chronic exposure, and they are more sensitive than chronic effects of PFOA and PFOS. Therefore, the RfDs based on these effects are expected to also be protective for chronic effects. A NOAEL-to-LOAEL uncertainty factor of 1 is appropriate because the RfDs are based on BMDLs, and a database uncertainty factor of 1 is appropriate because it is not expected that effects that have not been adequately studied would be more sensitive than the critical effects.

ii. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

The rationale for the selection of uncertainty factors is clearly and thoroughly presented in both the text and Table 22 in Section 4.1.5 of the draft PFOA and PFOS documents.

Relative Source Contribution

Charge Question 1. *EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources. Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at: <https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS.*

Charge Question 1, Part A. *Are you aware of additional relevant exposure data that EPA should consider in developing the RSCs for PFOA and PFOS? If so, please provide citations.*

Please see information on daily dietary exposures to PFOA and PFOS and NHANES serum levels for PFOA and PFOS in my response to Part ii below.

Charge Question 1, Part B. *Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS is adequately supported and clearly described.*

For reasons discussed later in this response, I agree that the recommended RSC of 20% is appropriate and scientifically supportable for MCLGs that are based on the PFOA and PFOS RfDs developed in the draft documents. However, the rationale presented in the draft documents for selecting the 20% RSC is not consistent with the approach provided in the EPA (2000) guidance for RSC development that is cited. It is important that the rationale for selecting an RSC of 20% be revised to be consistent with the approach provided in the EPA (2000) guidance.

As stated at the beginning of the RSC sections of the draft PFOA and PFOS documents (Section 5)² and in the charge question, an RSC is applied to ensure that total exposure from all sources does not exceed the Reference Dose. The RSC is the portion of the Reference Dose that is allocated to drinking water, based on the portion of the Reference Dose that is known or assumed to come from non-drinking water sources. For example, if it is known that exposures from non-drinking water sources (food, consumer products, air, dust, etc.) are 40% of the Reference Dose, then 60% of the Reference Dose is allocated to drinking water and the RSC is 60%. The highest value recommended for the RSC is 20%, and if it is known that exposure to more than 80% of the Reference Dose comes from non-drinking water sources, the RSC is set at 20%. Additionally, the default RSC is 20% when there is insufficient information to determine a chemical-specific value. (See EPA, 2000, including Figure 4-1, Decision Tree used to determine RSC.)

Because the RSC is based on the portion of the RfD that comes from non-drinking water sources, the choice of the RSC depends on the numerical value of the RfD. The RSC decreases as the RfD decreases since the non-drinking water exposures represent a higher proportion of a lower RfD. Because the RfDs used in the 2016 EPA Health Advisories and in state drinking water guidelines are several orders of magnitude higher than the RfDs presented in the draft documents, the RSCs used in the 2016 HESD and by states discussed on p. 347-348 of the PFOA document) are not relevant to selection of the RSC in the current draft EPA PFOA and PFOS assessments.

As above, the relevant parameter for selection of the RSC is the percentage of the RfD that comes from non-drinking water sources. Therefore, actual PFOA or PFOS exposures from drinking water are not relevant to RSC selection. Specifically, the percentage of total PFOA/PFOS exposure that comes from drinking water and the concentrations of PFOA/PFOS in drinking water at locations where the MCL will be applied are not relevant to RSC selection.

The important concept that actual exposures from drinking water are irrelevant to RSC selection does not appear to be correctly presented in the draft documents. The documents (PFOA - p. 347; PFOS – p.314) state that: “For disproportionately affected subpopulations, such as the occupationally exposed or site-impacted (e.g., by a particular source or industry) where there may be higher average PFAS concentrations in drinking water, it may be appropriate to apply an RSC greater than 20 percent if there is sufficient information to quantitatively characterize sources other than drinking water. This is a less conservative approach from a public health perspective and would result in a higher MCLG for those disproportionately affected subpopulations.”

Additionally, Section 5.1.4 of the draft PFOA document and Section 5.4 of the draft PFOS document on Recommended RSC discuss several studies (for both PFOA and PFOS - Hu et al., 2019; East et al., 2021; Gebbink et al., 2015; also for PFOS - Jogsten et al., 2012), that estimate that the percentage of total exposure to PFOA or PFOS that comes from drinking water is less than 20%. The draft documents (PFOA - p. 360; PFOS – p. 327) state that these “*estimates support a 20% RSC for drinking water.*” However, these data on percentage of total exposure that comes from drinking water are not relevant to selection of the RSC since, as discussed above, the actual percentage of exposure from drinking water does not affect the choice of the RSC.

Although the rationale for a 20% RSC in the draft document needs clarification, available data on non-drinking water exposures to PFOA and PFOS definitely support the choice of an RSC of 20% for MCLGs based on the RfDs presented in the draft document. Estimates of daily dietary exposure to PFOA in studies from North America and Europe reviewed by the New Jersey Drinking Water Quality Institute (2017, <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>) range from 0.16 ng/kg/day to 6.2 ng/kg/day, and the lower bound estimate of median daily dietary exposure for adults from EFSA (2020, <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6223>) is 0.18 ng/kg/day, with higher values for younger age groups, and the recommended RfD of 1.5×10^{-9} mg/kg/day (0.0015 ng/kg/day) is two orders of magnitude below the lowest of these dietary estimates. Similarly, PFOS RfD of 1.9×10^{-9} mg/kg/day (0.0079 ng/kg/day)

is almost two orders of magnitude lower than the EFSA (2020) lower bound estimate of median daily dietary exposure to PFOS in adults is 0.58 ng/kg/day. Additionally, there are non-drinking water exposures from other sources such as consumer products and house dust. Therefore, it is clearly evident that exposures from non-drinking water sources far exceed the RfD, indicating the choice of the default RSC of 20%.

An RSC of 20% is also supported by data on serum PFOA and PFOS levels from the U.S. general population. The serum PFOA level associated with the RfD can be determined by applying an Uncertainty Factor of 10 to the POD (Internal Dose (e.g., human serum level at the POD) of 1.7×10^{-4} mg/L from Table 21 of the draft PFOA document and 5.4×10^{-4} mg/L from Table 21 of the draft PFOS document. The serum levels associated with the RfDs, 1.7×10^{-5} mg/L (0.017 ng/ml) for PFOA and 5.4×10^{-5} mg/L (0.054 ng/L), are far below even the lower percentiles for serum PFOA in the U.S. general population in the most recent (2017-18) NHANES data (obtained from <https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Laboratory&Cycle=2017>). In the 2017-18 NHANES, the 5th percentile serum PFOA level, presumably representing those with little or no drinking water exposure, is 0.48 ng/ml, which is 28-fold higher than the serum level associated with the RfD of 0.017 ng/ml. Similarly, the 5th percentile serum PFOS level, also presumably representing those with little or no drinking water exposure, is 1.08 ng/ml, which is 20-fold higher than the serum level associated with the RfD, 0.054 ng/ml. Therefore, serum PFOA and level in the lowest 5th percentile of the general population (presumably without exposure to contaminated drinking water) far exceeds 100% of the RfD, supporting the default RSC of 20%.

² The draft EPA PFOA and PFOS documents state that: “EPA applies an RSC when calculating the MCLG to provide a margin of safety that an individual’s total exposure from a contaminant (i.e., PFOA/PFOS) does not

exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water...; the remainder of the exposure equal to the RfD is allocated to other potential sources.”

PRELIMINARY RESPONSES to Charge Questions on EPA’s Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Introduction

Per- and polyfluoroalkyl substances (PFAS) present many unique challenges from a risk management perspective due to the large number (1000’s) and structural diversity of members in this chemical class, limitations in available human health and exposure information, and the

spatial and temporal variability of their presence in drinking water and other environmental media. To inform various decision contexts in addressing PFAS contamination, EPA has developed a draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of PFAS to illustrate the practical application of EPA chemical mixtures approaches and methods^{1,2} for two or more PFAS co-occurring in environmental media. Specifically, this document describes an approach for providing a tiered, flexible, data-driven framework that facilitates practical component-based mixtures evaluation of two or more PFAS under an assumption of dose additivity. While this framework is being developed to inform the National Primary Drinking Water Regulation for PFAS, it is not intended to be media-specific in practical applications.

Overall charge: EPA is seeking SAB comment on whether the framework and illustrative examples provided in the document are scientifically supported, clearly described, and informative for assessing potential health risk(s) associated with exposure to mixtures of PFAS.

Charge questions

- 1. The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, an assumption of dose addition for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA's supplementary guidance (EPA, 2000) states: "The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which "similarity" can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.*
- A. Please comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.*

Assumption of dose additivity

All of the component-based approaches for assessing the risks of PFAS mixtures (HI, TOSHI, RPF, Mixture BMD) presented in the draft EPA mixtures framework document are based on the assumption of dose additivity. Sections 2 and 3 of the draft EPA document present information supporting the assumption of dose additivity for chemical mixtures in general, including mixtures of PFAS. The information included in these sections supports the conclusion that toxicological interactions of chemical mixtures are usually additive or close to additive. It also supports the conclusion that dose additivity is a public health protective assumption that usually does not underestimate the toxicity of a mixture. Based on the information presented by EPA, I agree with the assumption of dose additivity for evaluation of the toxicity of PFAS

mixtures, in the absence of chemical-specific information indicating that another type of toxicological interaction should be assumed.

Section 3.4 discusses data indicating a common mode of action and dose additivity for PFAS. For example, the draft EPA document discusses that Wolf et al. (2014) reported additivity for PPAR-alpha activation in binary mixtures of PFOA and four other PFAS in cultured cells transfected with the mouse or human PPAR-alpha receptor. While I support the dose additivity assumption for reasons discussed above, it is suggested that the discussion of studies of toxicological interactions in PFAS mixtures in the EPA mixtures framework document be expanded to also include studies that do not indicate dose- additivity and/or a common mode of action for PFAS. Some of these studies are summarized below. Acknowledging and including this information will increase transparency and characterization of the uncertainties associated with the assumption of dose additivity.

A recent study, Nielsen et al. (2021, <https://pubmed.ncbi.nlm.nih.gov/34743024/>), that was not included in the draft EPA document did not find dose additivity for activation of PPAR-alpha by PFAS mixtures in cultured cells transfected with a full length human PPAR-alpha construct. It is suggested that discussion of Nielsen et al (2021) be included in the final EPA mixtures framework document. As previously reported by others, Nielsen et al. (2021) found that the potency (EC50) for PPAR-alpha activation varied among the seven PFAS tested. They also reported that the efficacy (maximal PPAR- alpha activation compared to positive control) was lower for PFASs than for PFCAs, and that a general concentration addition (GCA) model that considers differences in both potency and efficacy among PFAS predicts the PFAS interactions better than a RPF approach that considers only differences in potency. They further conclude that an effect summation model can also likely predict the interactions at low concentrations.

Additional studies that report non-additive interactions of PFAS included Kjeldsen and Bonefeld-Jorgensen (2013, <https://pubmed.ncbi.nlm.nih.gov/23764977/>) who studied PFAS activation of the estrogen and androgen receptor in a cultured cell line transfected with these receptors; Ojo et al. (2020, <https://pubmed.ncbi.nlm.nih.gov/32247900/>) who studied effects of binary and ternary PFAS mixtures on cell viability of a human liver cell line, HepG2; Ding et al. (2013) who studied interactions of PFOA and PFOS for lethality in zebrafish; and Menger et al. (2020, <https://pubmed.ncbi.nlm.nih.gov/31877453/>) who studied behavioral effects in zebrafish of nine PFAS individually and a mixture of equal concentrations of all nine PFAS;

Surprisingly, no published mammalian studies of defined mixtures of PFAS were available to EPA for the draft mixtures framework document. As discussed in the draft EPA document, a recent EPA study of rat developmental toxicity of mixtures of PFOA and PFOS (Conley et al. - Appendix A of draft EPA document) indicates additive toxicity for developmental effects of these two PFAS.

However, a recent paper not cited in the draft EPA document, Marques et al. (2021,

<https://www.sciencedirect.com/science/article/abs/pii/S0300483X21002444>) indicates that toxicological interactions of a mixture of PFOA, PFOS, and PFHxS in mice can be additive, synergistic, or antagonistic for specific hepatic and metabolic effects after perinatal exposure. As stated by Marques et al. (2021): "The PFAS mixture had very distinct effects when compared to single compound treatment. With regard to liver weights and liver to body weight ratios increases, the PFAS mixture data were analogous to the effects seen with PFOA treatment. However, unlike PFOA, the serum ALT level, did not increase in the PFAS mixture. In the case of liver lipids, only the PFAS mixture in combination with HFD [high fat diet] feeding decreased total cholesterol in the pups and increased total lipid in the pups. However, liver triglycerides were increased with all three single PFAS treatments with the SD [standard diet], and in treatment with the PFASmixture with SD, there was no change compared to control... These results suggest that there are multiple pathways in which PFAS could add, synergize, or antagonize specific effects, and warrants further investigation of dose response data with model predictions of additivity." These results also suggest that co-exposure to other PFAS may impact the toxicokinetics of individual PFAS, as follows: "PFOS levels in pup and dam serum were lower in the PFAS mixture compared to PFOS treatment alone." It is suggested that discussion of this paper be added to the EPA mixtures document.

Assumption of similarity of toxicity endpoint rather than MOA in mixtures evaluation

The charge question states: "For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA," and "As an emerging chemical class, MOA data is limited or not available for many PFAS." The draft EPA mixtures framework document emphasizes that the EPA (2000) mixtures risk assessment guidance states: "The common mode of action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." However, it is not completely clear how "duration" is incorporated into the approaches based on similar toxic endpoint that are proposed in the draft EPA mixtures framework document, and this should be clarified.

Furthermore, while it is undoubtedly true that there are little or no MOA data for many PFAS, information from *in vivo* studies indicates that the mode(s) of action for several key toxicological effects differ among several well-studied PFAS. It is suggested that EPA include this information in its discussion of approaches based on a common apical endpoint, rather than a common mode of action. For example, PFOA, PFNA and PFOS cause the same general types of hepatic toxicity. However, as reviewed by Post et al. (2017, <https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.2002855>) and the New Jersey Drinking Water Quality Institute (DWQI), the hepatic effects of PFOS in rodents are primarily PPAR- α independent, while hepatic effects of PFOA (DWQI, 2017, <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>) and PFNA (DWQI, 2015, <https://www.state.nj.us/dep/watersupply/pdf/pfna-health-effects.pdf>) involve substantial contributions from both PPAR- α dependent and independent processes. Likewise, while the developmental effects of PFOA, PFOS, and PFNA are

generally similar, most (but not all) developmental effects of PFOA (reviewed in DWQI, 2017) and PFNA (reviewed DWQI, 2015) in mice are PPAR-alpha dependent, while the developmental effects of PFOS (reviewed in DWQI, 2019, <https://www.state.nj.us/dep/watersupply/pdf/pfos-recommendation-appendix-a.pdf>) appear to be independent of PPAR-alpha.

Consideration of human data

The examples of mixtures assessments provided in the draft EPA mixtures framework document are based on the four PFAS that currently have final EPA Reference Doses (PFOA, PFOS, PFBS, GenX); all these Reference Doses are based on animal data.

However, the Reference Doses (and cancer slope factor for PFOA in the draft EPA PFOA and PFOS assessments are based on human data, and additional toxicity factors based on human data may be developed in the future for other PFAS. It is suggested that EPA consider how toxicity factors based on human data could be used in evaluations of PFAS mixtures, including mixtures in which toxicity factors for some PFAS are based on animal data and toxicity factors for other PFAS are based on human data.

Development of toxicity factors for PFAS for which final EPA toxicity factors are not available

The draft EPA mixtures framework document (p. 33, last paragraph) discusses that toxicity values are needed to address PFAS (and other contaminants) for which final EPA toxicity factors have not been developed. The draft EPA document also discusses that several states have developed toxicity factors for several PFAS for which there are no EPA toxicity factors (see Post, 2021, <https://setac.onlinelibrary.wiley.com/doi/epdf/10.1002/etc.4863>). As noted in the draft EPA document, EPA has developed guidance for development of subchronic and chronic oral RfDs, and most or all states follow this EPA guidance.

While I agree with EPA recommendations that toxicity values for PFAS should be developed by scientists with appropriate expertise and that their basis be transparent, the recommendation that such values "undergo independent peer review" does not appear to be appropriate for inclusions in the EPA mixtures framework. This recommendation is not specific to toxicity values used in mixtures assessments and would apply equally to toxicity values used in chemical-by-chemical approaches to address PFAS in drinking water or other media. It is important to recognize that each state has its own processes (established in legislation, regulation, or by policy) for development of such toxicity values, and that these processes may or may not include formal peer review. In fact, the Minnesota Department of Health oral toxicity values mentioned in the draft EPA mixtures framework document for potential use in HI calculations (p. 33, first paragraph) do not undergo external peer review. In some states, advisory bodies consisting of scientific experts develop and recommend toxicity factors to state environmental agencies. These toxicity factors recommendations be revised in response to the public comments before finalization. While such a process may not be considered to be a formal "independent peer review", it can be a rigorous

process that considers extensive scientific input from outside of the agency that will use the toxicity factor. A recommendation in the EPA PFAS mixture framework for "external peer review" of toxicity values developed by states could potentially be used as the basis for challenges to the validity of state processes that do not include formal "external peer review." If such a recommendation is to be included in the EPA mixtures framework document, it is suggested that it be broadened to recommend the opportunity for scientific input and review in general, rather than only "external peer review."

Use of NAMs data in component-based mixtures approaches for PFAS

The potential use of NAMs (e.g., high throughput assays, read-across) data for hazard identification and dose-response evaluation in assessment of PFAS mixtures is mentioned in several places in the draft EPA mixtures framework document (p. 12, 27, 34, 37, 52). I agree with the draft EPA document's statement (p. 41) that using NAMs data to develop screening level toxicity Reference Values (RfVs) will allow for consideration of toxicity of "data-poor PFAS" detected in environmental media that would not otherwise be considered.

However, it should be recognized that the potential use of NAMs data to address environmental contaminants without sufficient human or animal data for traditional toxicity factor (e.g., Reference Dose) development is not specific to PFAS mixtures assessment. This is a key issue for both chemical-by-chemical and mixtures assessment of PFAS and other contaminants. This issue has become especially important because toxicity factors cannot be developed for several PFAS (e.g., perfluoropentanoic acid; perfluoroheptanoic acid) that commonly occur in drinking water, because there are no or virtually no data on their toxicity in animals or humans. Compounding this problem, EPA has adopted a policy of minimizing animal studies in toxicology research, despite the obvious high impact of even the small number of recent animal studies from ORD which have provided key information on developmental effects of several PFAS with a high level of current concern. For example, for GenX - Conley et al. (2019, <https://pubmed.ncbi.nlm.nih.gov/30920876/>) and Conley et al. (2021, <https://pubmed.ncbi.nlm.nih.gov/33126064/>), for Nafion Byproduct 2 - Conley et al., (2021; <https://www.sciencedirect.com/science/article/pii/S0160412021006814>), and for mixtures of PFOA and PFOS - the recent studies highlighted in the draft EPA mixtures framework.

Current EPA risk assessment guidance does not provide for the use of NAMs data as the basis for toxicity factors such as Reference Doses. As a rule, state environmental agencies follow EPA risk assessment guidance in developing health-based standards and guidance values for environmental contaminants. Therefore, states would face difficulties in justifying and implementing either a chemical-specific or mixture-based standard or guidance value based on NAMs data for contaminants (PFAS and others) in drinking water or other environmental media. Regarding this issue, EPA stated at the December 16, 2021 SAB meeting that it does not plan to develop guidance for development of toxicity factors from NAMs data in the near future. EPA also stated

that the use of NAMs in mixtures assessment is currently "quite abstract," and that it is not expected that NAMs data will currently be used as the basis for standards or guidance values. EPA clarified that that an approach based on NAMs data might be used to get a sense of whether PFAS detected in drinking water pose a risk in the absence of traditional toxicity data and that EPA hopes to develop case studies using NAMs data to evaluate the potential risk of PFAS mixtures. This information on EPA's vision for the use of NAMs data in PFAS mixtures assessments is not included in the draft EPA mixtures framework document, and it should be added to the final document.

B. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).

I do not recommend an alternative methodology. As above, I agree that an approach based on dose additivity and a common toxicity endpoint/health effect can be used as a default approach for PFAS mixtures and that this approach is public health protective. As above, the uncertainties associated with this approach should be more thoroughly and clearly presented along with the information that supports this approach.

2. *Section 4.3 (Hazard Index; HI) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and hypothetical exposure information. The example calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as "GenX chemicals").*

A. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.

In general, the screening level Hazard Index (HI) approach, in which Reference Values (RfV) for the mixture components are used regardless of the effect on which the RfVs are based, is appropriate for initial screening of whether exposure to a mixture of PFAS poses a potential risk that should be further evaluated.

The Target Organ Specific Hazard Index (TOSHI) approach, in which RfVs based on a common effect are used, provides a more refined estimate of whether exposure to a mixture of PFAS poses a potential risk. However, as stated in the draft EPA mixtures framework document (p. 42), some PFAS present in a PFAS mixture may not have been tested for some of the health effects of concern.

B. Please provide specific feedback on whether the proposed HI methodologies in

the framework are scientifically supported for PFAS mixture risk assessment.

The proposed HI methodology (to be used for both the screening HI and the TOSHI approaches) uses Health-based Water Concentrations (HBWCs). As such, chemical-specific HBWCs, not just chemical toxicity factors (e.g., Reference Doses, Minimal Risk Levels), are needed for each PFAS included in the HI or TOSHI evaluation. Development of such HBWCs would require additional effort beyond what is needed for toxicity factor development. Additionally, as shown in Table 4-3 (p. 39) of the draft EPA mixtures framework document, development of HBWCs requires chemical-specific toxicity factors (e.g., Reference Doses) and chemical-specific exposure assumptions (ingestion rates, Relative Source Contribution factors). Additionally, HBWCs may apply to different exposure durations (short-term, subchronic, chronic). The EPA mixtures framework document should consider whether it is appropriate to use HBWCs based on different exposure assumptions and/or different exposure durations in HI evaluation of PFAS mixtures.

For example, the HBWCs used in the examples of the HI approach (Section 4 of the draft EPA mixtures framework document) are the EPA (2016) Health Advisories (HAs) for PFOA and PFOS. As shown in Table 4-3, the PFOA and PFOS HAs are based on the drinking water ingestion rate for lactating women which is higher than the default adult ingestion rate. The ingestion rate for lactating women was selected because PFOA and PFOS are transferred to breastmilk, and exposure to PFOA and PFOS in breastfed infants (via maternal consumption of PFOA/PFOS-contaminated drinking water) is higher than in infants who consume formula prepared with the contaminated water or older individuals. However, ingestion rates for subgroups other than lactating women (e.g., infants, children, default adults) may be appropriate for HBWCs for other PFAS. For example, the ingestion rate for lactating women is not likely to be appropriate for HBWCs for PFBS or GenX, since there is no information to indicate that GenX or PFBS are present in breastmilk. Additionally, the EPA (2016) Health Advisories are stated to apply to both short-term (weeks to months) and chronic exposures, while HBWCs for other PFAS may apply to different exposure duration(s). As above, EPA should consider these issues in developing the HI methodologies for PFAS mixtures that uses HBWCs.

An additional comment on the presentation of the HI approach in the draft document relates to the examples provided in Tables 4-4 and 4-5. In the example in Table 4-4, the individual concentrations of 20 ng/L for PFOA and PFOS are below the HBWCs of 70 ng/L for these chemicals and the combined concentration of PFOA and PFOS is also below 70 ng/L. It is therefore not unexpected that the HI is below 1 for the combined concentration. In the example in Table 4-5, individual concentrations of PFOA and PFOS of 400 ng/L exceed the HA of 70 ng/L, so it is not unexpected that the HI for the combined concentration (and for each individual PFAS) exceeds 1. It would be useful to provide an additional example in which the concentration of each individual PFAS is below its HBWC (e.g., the HA), yet the HI that considers both PFAS exceeds 1. For example, 40 ng/L for PFOA and 50 ng/L for PFOS.

3. *Section 4.4 (Relative Potency Factor; RPF) of the framework document demonstrates the*

application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and HFPO dimer acid and GenX chemicals.

A. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.

It appears that the RPF approach is a reasonable methodology for estimating risks associated with PFAS mixtures. However, it would be helpful if additional explanation is provided to clarify the conceptual differences between the TOSHI approach and the RPF approach, since both are based on health effect-specific values (RfVs or RPFs) for the individual PFAS in the PFAS mixture. Although the relevant information is provided in the draft EPA document, it should be more clearly explained and indicated that the RPF approach is based on a specific effect (e.g., decreased offspring body weight) while the TOSHI approach is based on a general category of effects (e.g., developmental effects).

For example, Table 4-6 shows a PODHED (LOAEL) for “Developmental Effect: Decreased Pup Body Weight” for PFOA of 0.0109 mg/kg/day. However, Tables 4-7 and 4-8 refer only to “Developmental Effect” RPFs, without mentioning that the values are specific to decreased offspring body weight. It is important that this point is clarified because, for example, the 2016 HESD for PFOA provides a lower PODHED (LOAEL) of 0.0053 mg/kg/day for a different developmental effect, reduced ossification and accelerated male puberty in mouse offspring.

B. Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.

The RPF approach is based on the assumptions of dose additivity and use of a common health effect/toxicity endpoint as a surrogate for a common MOA, as discussed in Charge Question 1 above. As such, the comments on the scientific basis of these assumptions from Charge Question 1 apply here as well.

The scientific basis for the RPF approach presented in the draft EPA mixtures framework document is strengthened by the use of PODs from animal studies that are based on human equivalent doses (HEDs) rather than administered doses. In contrast, the PFAS RPFs based on BMDs for a 5% increase in relative liver weight from subchronic exposure to male rats developed by Bil et al. (2020, <https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.4835>), which are being used to address PFAS mixtures by some European environmental authorities, are based on administered dose and do not consider differences among PFAS regarding animal-to-human toxicokinetic extrapolation.

4. Section 4.5 (Mixture BMD) of the framework document demonstrates the application of a

component-based mixture approach using established EPA dose-response modeling (i.e., benchmark dose; BMD) of hypothetical PFAS dose-response data, and hypothetical exposure information.

A. Please provide specific feedback on whether the Mixture BMD approach is a reasonable methodology for estimating what is in essence a mixture-based point-of-departure. If not, please provide an alternative.

This methodology appears to be reasonable. It is based on the same assumptions (dose additivity and use of a common health endpoint, in the absence of knowledge of a common MOA, as the TOSHI and RPF approaches. However, the practical utility of this approach is unclear and difficult to envision. The output of this approach is a BMD in units of mg/kg/day for the total concentration of a mixture of specific PFAS in specific proportions. At the December 16, 2021 SAB meeting, EPA stated that a Mixture BMD could be used to characterize a unique site or exposure and that it is applicable when it is fairly certain that the composition of the mixture is relatively stable. However, it is unclear what benchmark the Mixture BMD could be compared to in order to determine whether or not there is a potential risk from a mixture of PFAS in drinking water or other environmental media.

B. Please provide specific feedback on whether the proposed Mixture BMD methodology in the framework is scientifically supported for PFAS mixture risk assessment.

It is stated that an advantage of this approach is that only PODs (NOAELs, LOAELs, BMDs) rather than RfVs (RfDs, HBWCs) are needed. However, the RPF approach is also based on PODs, rather than HBWCs or RfDs. In the RPF approach, the PODs are based on human equivalent doses (HEDs) rather than administered doses. However, the use of HEDs does not appear to be shown in the Mixture BMD approach. The use of PODs based on HEDs is recommended, and it should be clarified that PODs based on HEDs should be used in the Mixture BMD approach.

REVISED COMMENTS

Noncancer Hazard Identification #2

Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

- It is unclear to me where in the documents the “strong” and “suggestive” language occurs, so it is difficult to evaluate this explicitly
- Assuming that this is a discussion of confidence designations, I am supportive of the Office of Water designation of “high confidence” for the immunotox endpoint. This evaluation is consistent with that of other entities (e.g., OEHHA) and is supported by the evidence base.

Cancer #4a

i. Cancer classification for PFOA/PFOS

PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a ‘likely carcinogen’ categorization which is a change from ‘suggestive’ in the 2016 HA. Does the panel agree with the ‘likely’ designation based on the new evidence? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

- Yes – I agree with this finding. It is consistent with the review done by OEHHA for the setting of public health goals for PFOA and PFOS⁷. OEHHA found that PFOA was a carcinogen based on strong evidence from both human and animal studies.
- An additional study that supports the setting of PFOA as likely carcinogen that did not seem to be in the EPA review is a recent paper by Bartell and Vieira⁸

i. PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a ‘suggestive’ categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

- No. I do not agree with this find. It is inconsistent with the OEHHA PHG classification, which utilized human, animal (including a finding of hepatocarcinogenicity in rainbow

⁷ [Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water- First Public Review Draft](#)

⁸ [Critical review on PFOA, kidney cancer, and testicular cancer: Journal of the Air & Waste Management Association: Vol 71, No 6 \(tandfonline.com\)](#)

trout⁹), and mechanistic information in its weight of evidence determination. The mechanistic data were used to identify the shared characteristics between PFOS and other known carcinogens. Of particular importance in the OEHHHA document was the finding of suggestive evidence that PFOS (and PFOA) is genotoxic – leaving the potential for a genotoxic MOA that differs from the 2016 HESD for PFOS. Given that the Office of Water has not completed the mechanistic studies for the cancer endpoint, it seems premature to have kept the 2016 determination without further evaluation of the mechanistic evidence.

- In addition to the lack of inclusion of mechanistic data in the WOE for PFOS carcinogenicity, the interpretation of the hepatocellular carcinoma data from the Butenhoff, 2012¹⁰ in the 2016 HESD is overly conservative in dismissing the appearance of a dose-response relationship for this endpoint. Given that multiple MOAs may be operative in this outcome, a reevaluation of the 2012 Butenhoff study is warranted.
- One endpoint that seems to be in need of additional consideration is mammary tumor development associated with PFOS. Human¹¹, animal¹², and mechanistic¹³ studies support this as an endpoint of concern, warranting a POD derivation.
 - For mammary tumor development, there are some recent studies that have shown epidemiologic and mechanistic evidence linking PFOS with breast cancer outcomes. On the epi side, Mancini et al ([Perfluorinated alkylated substances serum concentration and breast cancer risk: Evidence from a nested case-control study in the French E3N cohort - Mancini - 2020 - International Journal of Cancer](https://doi.org/10.1093/toxsci/kfr267))

⁹ Benninghoff, Abby D., Gayle A. Orner, Clarissa H. Buchner, Jerry D. Hendricks, Aaron M. Duffy, and David E. Williams. “Promotion of Hepatocarcinogenesis by Perfluoroalkyl Acids in Rainbow Trout.” *Toxicological Sciences* 125, no. 1 (January 2012): 69–78. <https://doi.org/10.1093/toxsci/kfr267>.

¹⁰ Butenhoff, John L., Shu-Ching Chang, Geary W. Olsen, and Peter J. Thomford. “Chronic Dietary Toxicity and Carcinogenicity Study with Potassium Perfluorooctanesulfonate in Sprague Dawley Rats.” *Toxicology* 293, no. 1–3 (March 2012): 1–15. <https://doi.org/10.1016/j.tox.2012.01.003>.

¹¹ See, for example:

Bonefeld-Jorgensen, Eva C et al. “Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case control study.” *Environmental Health* 10 (2011): 88. [doi:10.1186/1476-069X-10-88](https://doi.org/10.1186/1476-069X-10-88);
Wielsøe, Maria et al. “Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study.” *Environmental Health* 16,1 (2017): 56. [doi:10.1186/s12940-017-0269-6](https://doi.org/10.1186/s12940-017-0269-6);
Cohn, Barbara A. et al. “In utero exposure to poly- and perfluoroalkyl substances (PFASs) and subsequent breast cancer.” *Reproductive Toxicology* 92 (2020): 112-119. [doi:10.1016/j.reprotox.2019.06.012](https://doi.org/10.1016/j.reprotox.2019.06.012);
Mancini FR, Cano-Sancho G, Gambaretti J, Marchand P, Boutron-Ruault MC, Severi G, Arveux P, Antignac JP, Kvaskoff M. Perfluorinated alkylated substances serum concentration and breast cancer risk: Evidence from a nested case-control study in the French E3N cohort. *Int J Cancer*. 2020 Feb 15;146(4):917-928. doi: 10.1002/ijc.32357. Epub 2019 May 3. PMID: 31008526.

¹² See, for example:

Butenhoff JL, Kennedy GL Jr, Chang SC, et al. Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. *Toxicology* 2012;298:1–13.

¹³ See, for example:

Pierozan P, Karlsson O. PFOS induces proliferation, cell-cycle progression, and malignant phenotype in human breast epithelial cells. *Arch Toxicol* 2018;92:705–16.

- [Wiley Online Library](#)) found a linear dose response for PFOS and receptor positive tumors. (there was also a potential association with ER- tumors). On the mechanistic side, Pierozan et al ([Perfluorooctane sulfonate \(PFOS\) and perfluorooctanoic acid \(PFOA\) induce epigenetic alterations and promote human breast cell carcinogenesis in vitro | SpringerLink](#)) showed that exposure to PFOS (at 10 microM) induced malignant transformation of MCF-10A cells. Though the evidence on PFOA and tumors is more robust, it seemed to me that the agency should also consider tumor development for PFOS as well. I tend to be of the precautionary approach camp on things like this, though.

PRELIMINARY COMMENTS

Study Identification #1

EPA used systematic review methods consistent with the current ORD systematic review practice to ensure transparency and completeness of literature identification, sorting, and study quality evaluation. Is the process clearly described? Please identify additional peer-reviewed studies that the panel is aware of that could inform toxicity value derivation.

- The process followed for systematic review is hard to follow and, at times, seemingly inconsistent with established systematic review methodology both within EPA (e.g. IRIS) and at sister agencies (e.g., NTP OHAT). There appeared to be some missing steps (e.g., publishing of the a priori protocol), some inconsistent/confusing use of terms (e.g., study quality, study validity, study risk of bias), and a lack of clarity as to how some steps were performed (e.g., evidence synthesis and integration). The way that the agency handled mechanistic data is unclear/confusing – with language in the document suggesting that the analysis of these data points is still occurring and is likely to occur after SAB review (p. 289 of PFOS review and page 99 of PFOA review)
- Though there are some issues with the systematic review protocol that cannot be fixed (e.g., the a priori protocol development/publishing), prior to the publication of the proposed final rule, the document would benefit from streamlining and clarification of both process and outcomes. As the Office of Water moves forward in creating systematic reviews for chemicals with potential for regulation under the SDWA, it should carefully review and follow existing systematic review protocols – including those in the ORD systematic review handbook – taking into account the recent 2021 review of the handbook by the NASEM¹⁴.
- The Office of Water should also use/refer to the California OEHHA¹⁵ First Public Review Draft of the Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane

¹⁴ Committee to Review EPA'S IRIS Assessment Handbook, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, and National Academies of Sciences, Engineering, and Medicine. *Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version*. Washington, D.C.: National Academies Press, 2021. <https://doi.org/10.17226/26289>.

¹⁵ [Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water-First Public Review Draft](#)

Sulfonic Acid in Drinking Water for its protocol, study inclusion/interpretation, and integration of mechanistic data – especially via the use of the key characteristics approach – in developing its toxicity values and cancer classification.

Noncancer Hazard Identification #2

Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

- It is unclear to me where in the documents the “strong” and “suggestive” language occurs, so it is difficult to evaluate this explicitly
- Assuming that this is a discussion of confidence designations, I am supportive of the Office of Water designation of “high confidence” for the immunotox endpoint. This evaluation is consistent with that of other entities (e.g., OEHHA) and is supported by the evidence base.
- One endpoint that seems to be in need of additional consideration is mammary tumor development associated with PFOS. Human¹⁶, animal¹⁷, and mechanistic¹⁸ studies support this as an endpoint of concern, warranting a POD derivation.
- Mammary gland development should also have been considered for a POD derivation for both PFOA and PFOS¹⁹.

Noncancer #3

Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in

¹⁶ See, for example:

Bonefeld-Jorgensen, Eva C et al. “Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case control study.” *Environmental Health* 10 (2011): 88. [doi:10.1186/1476-069X-10-88](https://doi.org/10.1186/1476-069X-10-88);

Wielsøe, Maria et al. “Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study.” *Environmental Health* 16,1 (2017): 56. [doi:10.1186/s12940-017-0269-6](https://doi.org/10.1186/s12940-017-0269-6);

Cohn, Barbara A. et al. “In utero exposure to poly- and perfluoroalkyl substances (PFASs) and subsequent breast cancer.” *Reproductive Toxicology* 92 (2020): 112-119. [doi:10.1016/j.reprotox.2019.06.012](https://doi.org/10.1016/j.reprotox.2019.06.012);

Mancini FR, Cano-Sancho G, Gambaretti J, Marchand P, Boutron-Ruault MC, Severi G, Arveux P, Antignac JP, Kvaskoff M. Perfluorinated alkylated substances serum concentration and breast cancer risk: Evidence from a nested case-control study in the French E3N cohort. *Int J Cancer*. 2020 Feb 15;146(4):917-928. doi: 10.1002/ijc.32357. Epub 2019 May 3. PMID: 31008526.

¹⁷ See, for example:

Butenhoff JL, Kennedy GL Jr, Chang SC, et al. Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. *Toxicology* 2012;298:1–13.

¹⁸ See, for example:

Pierozan P, Karlsson O. PFOS induces proliferation, cell-cycle progression, and malignant phenotype in human breast epithelial cells. *Arch Toxicol* 2018;92:705–16.

¹⁹ For review, see: EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel), Schrenk D, Bignami M, et al. Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA J*. 2020;18(9):e06223. Published 2020 Sep 17. doi:10.2903/j.efsa.2020.6223

alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.

- Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.
- Magnitude of effect should not be used as a rationale to exclude an endpoint for dose response modeling, as both a general principle and in this specific case.
- A lack of specificity of biomarkers should also not prevent an endpoint from being considered for dose response modeling. While ALT is not as specific to liver disease as more invasive (e.g., biopsy) or emerging methods (e.g., miRNA analysis), the lack of specificity for this marker does not exclude it from being relevant to the evaluation of the potential for liver injury.

Cancer #4a

ii. Cancer classification for PFOA/PFOS

PFOA: Based on new cancer studies identified since the 2016 PFOA Health Advisory (HA), EPA concludes that the available cancer data for PFOA indicate a 'likely carcinogen' categorization which is a change from 'suggestive' in the 2016 HA. Does the panel agree with the 'likely' designation based on the new evidence? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

- Yes – I agree with this finding. It is consistent with the review done by OEHHHA for the setting of public health goals for PFOA and PFOS²⁰. OEHHHA found that PFOA was a carcinogen based on strong evidence from both human and animal studies.
- An additional study that supports the setting of PFOA as likely carcinogen that did not seem to be in the EPA review is a recent paper by Bartell and Vieira²¹

i. PFOS: Based on a small number of new cancer studies identified since the 2016 PFOS HA, EPA concludes that the available cancer data for PFOS indicate a 'suggestive' categorization which is unchanged from the categorization identified in the 2016 HA. Does the panel agree that the new studies do not change the designation? If yes, is the rationale clearly described? If no, please provide an explanation for arriving at a different conclusion.

²⁰ [Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water-First Public Review Draft](#)

²¹ [Critical review on PFOA, kidney cancer, and testicular cancer: Journal of the Air & Waste Management Association: Vol 71, No 6 \(tandfonline.com\)](#)

- No. I do not agree with this find. It is inconsistent with the OEHHA PHG classification, which utilized human, animal (including a finding of hepatocarcinogenicity in rainbow trout²²), and mechanistic information in its weight of evidence determination. The mechanistic data were used to identify the shared characteristics between PFOS and other known carcinogens. Of particular importance in the OEHHA document was the finding of suggestive evidence that PFOS (and PFOA) is genotoxic – leaving the potential for a genotoxic MOA that differs from the 2016 HESD for PFOS. Given that the Office of Water has not completed the mechanistic studies for the cancer endpoint, it seems premature to have kept the 2016 determination without further evaluation of the mechanistic evidence.
- In addition to the lack of inclusion of mechanistic data in the WOE for PFOS carcinogenicity, the interpretation of the hepatocellular carcinoma data from the Butenhoff, 2012²³ in the 2016 HESD is overly conservative in dismissing the appearance of a dose-response relationship for this endpoint. Given that multiple MOAs may be operative in this outcome, a reevaluation of the 2012 Butenhoff study is warranted.

- **Cancer #4b**

Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor using peak exposure for PFOA. Has EPA adequately justified the use of this study and peak exposure for the quantification of a cancer slope factor for PFOA? If no, please describe alternate approaches that SAB recommends. Does SAB support the selection of this CSF in the derivation of a risk specific dose for PFOA (i.e., the concentration of PFOA in drinking water that would have a one-in-1-million chance of an increased cancer risk)? If not, please provide input on the strengths and weaknesses of the other candidate CSFs that EPA derived.

No comments at this time

Human Toxicokinetic #5 & Animal Toxicokinetic #6

No comments at this time

Epidemiological #7a

Epidemiological Study RfD Derivation

EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only ‘medium’ and ‘high’ quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling

²² Benninghoff, Abby D., Gayle A. Orner, Clarissa H. Buchner, Jerry D. Hendricks, Aaron M. Duffy, and David E. Williams. “Promotion of Hepatocarcinogenesis by Perfluoroalkyl Acids in Rainbow Trout.” *Toxicological Sciences* 125, no. 1 (January 2012): 69–78. <https://doi.org/10.1093/toxsci/kfr267>.

²³ Butenhoff, John L., Shu-Ching Chang, Geary W. Olsen, and Peter J. Thomford. “Chronic Dietary Toxicity and Carcinogenicity Study with Potassium Perfluorooctanesulfonate in Sprague Dawley Rats.” *Toxicology* 293, no. 1–3 (March 2012): 1–15. <https://doi.org/10.1016/j.tox.2012.01.003>.

sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?

I disagree with what appears to be the exclusion of Abraham et al 2020 study – this document was evaluated by OEHHA and found to be useful in developing PODs for PFOA.

Epidemiological #7b

Studies of developmental immune health outcomes (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]) after PFOA/S exposure identified associations with very low doses of either PFOA or PFOS with developmental immune effects. The RfD for this outcome was selected as the critical effect because it was the lowest among the candidate RfDs for PFOA or PFOS and can result in severe illness. Does the panel agree with the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS?

i. If so, please explain your justification.

- I agree with the finding that developmental immunotox is the most sensitive endpoint for PFOA and PFOS. As noted in the MCLG document, the effects observed in the Grandjean studies are large in magnitude and are consistent with the literature – particularly animal literature – demonstrating associations between PFOA and PFOS exposure and suppression of the antibody response (NTP, 2016)²⁴.
- *If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of chronic RfDs.*
- *Are any additional analyses or rationales needed to increase the confidence in the chronic RfDs for PFOA and PFOS?*

Epidemiological #7c

The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive

²⁴ NTP. “Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate.” Research Triangle Park, NC: National Toxicology Program, September 2016. https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf.

subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.

Yes – I am supportive of taking a more protective approach to the BMR, i.e., a 5% BMR, given that the population with highest risk is children.

Epidemiological #7d

EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for PFOA and PFOS.

i. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

Given the agency acknowledgement that PFAS tend to co-occur with each other, particularly in drinking water, the current UFs do not take into account the potential uncertainties associated with the risk posed by PFOA and PFOS occurring in mixtures. As noted in the approach to mixtures document (EPA 2021²⁵), there is a broad landscape of over 150 PFAS (which is a small fraction of the total number of PFAS in the chemical family) for which testing is still ongoing. Though the Office of Water is taking an additive approach to mixtures, the database is not sufficiently populated with information to help the Office of Water determine the impact that co-occurring with other PFAS has on the individual toxicity of PFOS and PFOA.

Relative Source Contribution #8

EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources. Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at: <https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS

- i. Are you aware of additional relevant exposure data that EPA should consider in developing the RSCs for PFOA and PFOS? If so, please provide citations.*
- ii. Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS is adequately supported and clearly described.*

²⁵ Conley, Justin, Colleen Flaherty, Earl Gray, Brittany Jacobs, Jason C Lambert, Alex Lan, Casey Lindberg, and Kathleen Raffaele. "Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)," n.d., 29.

I agree with the use of the most protective RSC allowable for PFOA and PFOS, which in this case is 0.20.

Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Question #1

The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, an assumption of dose addition for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However, EPA's supplementary guidance (EPA, 2000) states: "The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which "similarity" can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.

- a. *Please comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.*
 - This approach seems consistent with the data available on the biological activity of PFAS mixtures. The database is quite small on this, though, so the Office of Water should re-evaluate the appropriateness of this method as additional information is obtained about the behavior of PFAS mixtures.
- b. *If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).*
 - I agree with the use of a common endpoint rather than specific MOA for PFOA and PFOS (and for other PFAS) given the limited and diverse mechanisms by which these chemicals (and the chemical class) can cause adverse health outcomes.

Question #2

Section 4.3 (Hazard Index; HI) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available oral reference doses from completed EPA human health assessments, and hypothetical exposure information. The example calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorobutane sulfonic acid (PFBS), and hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt (referred to as "GenX chemicals").

a. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. If not, please provide an alternative.

- The HI approach can be a reasonable approach but should be adjusted to account for the large universe of PFAS chemicals that may co-occur with one another and can result in a wide range of adverse outcomes – particularly in developing children. Component based approaches like the HI also carry inherent uncertainty because they are not necessarily true representatives of a whole mixture²⁶. To protect against this uncertainty, variability, and vulnerability of the most impacted populations, the result indicating concern should be reduced from >1 to >0.1. Additional reductions of the threshold for risk indication in the HI could be applied if/when additional uncertainties are introduced via the incorporation of NAMs and other emerging methods.
- Comparing the TOSHI to the HI, the HI approach is both more practical and provides more flexibility to account for the number and variety of PFAS within the chemical family. The TOSHI approach would require a level of information about the chemical substance that would likely prohibit its use for a class of chemicals with significant gaps in the database.
- In addition to the HI, another approach that should be explored to lessen the need for this approach would be to apply an uncertainty factor accounting for mixtures in the calculation of the RfD.

b. Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment

With the adjustments to the value for which concern is raised, and with the use of the most sensitive endpoint rather than a common MOA as the mechanism for evaluating the mixture, this seems like a sound, easy to use approach to evaluate mixtures.

Question #3

Section 4.4 (Relative Potency Factor; RPF) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and GenX chemicals.

a. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.

²⁶ Risk Assessment Forum. “Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures.” Washington DC: USEPA, August 2000.

- It is unclear to me why this approach utilized a narrower view of shared endpoint than that taken for the HI. Could RPFs and resultant ICECs be calculated for the most sensitive endpoint of each component in the mixture, regardless of target organ or outcome? If not, why not?

b. Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.

- The method seems relatively straightforward when existing PODs are known, but the scientific soundness of the approach for this application is unclear at the present. Given that considerable space was given to describing the use of the method when obtaining information from high-throughput bioassays, it would be helpful to see a case study that demonstrates what this would look like for a real-world mixture. It would be particularly helpful to see a comparison between HT derived PODs and those obtained through more traditional methods. While the exploration of the method with well-understood PFAS is helpful, it doesn't allow for a deeper understanding of the potential limitations of applying the method for mixtures with data poor members and/or with data derived from non-traditional means.

Question #4

Section 4.5 (Mixture BMD) of the framework document demonstrates the application of a component-based mixture approach using established EPA dose- response modeling (i.e., benchmark dose; BMD) of hypothetical PFAS dose- response data, and hypothetical exposure information.

a. Please provide specific feedback on whether the Mixture BMD approach is a reasonable methodology for estimating what is in essence a mixture-based point-of-departure. If not, please provide an alternative.

- This approach is interesting in that it provides a POD for the mixture that can be compared against the RfD set for individual chemicals or for a chemical class. The method seems consistent with the approaches described in 2008 by the NAS²⁷.
- The method seems quite complicated, though, and as described in the document is based on endpoints that, while critical, may prove difficult to obtain for many environmental chemicals – especially the thousands of PFAS known to exist. While I appreciate the inclusion of the possibility of NAMs filling data gaps, for thyroid and developmental endpoints, current NAMs are quite limited. I am concerned that data gaps may be an extraordinary limiting factor in the use of this method, which could make the approach ultimately non-viable and/or lead to a bottleneck through which many PFAS mixtures will never be able to emerge.
- How this method will be applied in practice is also unclear. It appears to be created for risk assessment of specific mixtures, i.e., those that are found in a specific location or water

²⁷ National Research Council (U.S.) and Committee on the Health Risks of Phthalates. *Phthalates and Cumulative Risk Assessment: The Tasks Ahead*. Washington, DC: National Academies Press, 2008. <http://site.ebrary.com/id/10274055>.

system. If this is the case, I do not know that the method is fit-for-purpose enough to help water system operators or regulators determine if a system is in excess of the MCLG (and eventual MCL).

b. Please provide specific feedback on whether the proposed Mixture BMD methodology in the framework is scientifically supported for PFAS mixture risk assessment.

- At present, it is unclear whether the mixture BMD approach is scientifically supported. Development of an additional case studies that highlight how this method would work for a real world sample (rather than a hypothetical case) and how it would work with data poor chemicals would be helpful in establishing both scientific confidence in the method and evaluation of whether it is fit for its intended purpose.

SAB Panel Charge Questions CVD Risk Reduction Analysis

Question #1

Section 4.2 presents EPA's meta-analysis for the total cholesterol dose-response function.

i. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature. To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water would add additional strengths for the CVD risk reduction application.

No comments at this time

Question #2

Section 5.1 presents EPA's life table approach methodology.

i. Please comment on the extent to which this analysis is scientifically supported and clearly described. To the extent improvements are suggested, please provide specific changes that are implementable in a U.S. national-level benefits analysis with readily available data.

No comments at this time

Question #3

Section 5.2 presents EPA's application of the atherosclerotic cardiovascular disease (ASCVD) risk model used to estimate the probability of hard CVD events corresponding to total cholesterol changes.

i. Please comment on the scientific validity of the ASCVD model application for estimating the probability of first time CVD events in various sub-populations and the extent to which it is clearly described.

- ii. Please comment on whether EPA's approach and assumption of a uniform first CVD event hazard distribution over the 10-year period is sufficiently robust given current data sources and literature. If additional distributional sources of information are suggested, please provide specific citations/sources for EPA's consideration.*
- iii. Please comment on the scientific validity of using the ASCVD risk model for estimating reduced CVD risk stemming from changes in total cholesterol in response to reducing exposure to PFOA and PFOS in drinking water.*

No comments at this time

Question #4

Section 7 and Appendix A describe the limitations and uncertainties of the CVD risk reduction analysis. Has EPA clearly described the individual contributions of the sources of uncertainty?

No comments at this time

REVISED COMMENTS

MCLG 5B

Among the most consistently documented associations of PFOA and PFOS with health outcomes, the volume and quality of evidence is fairly similar for vaccine response in children (the one chosen), serum cholesterol, liver enzymes, and birthweight. Arguments can be made for any one of these but there's not a compelling basis for arguing that one is "best" among them. Given that menu, a stronger strategy would be to consider perhaps an initial evaluation of all of them with the strongest inferences where there is consistency among them. Regardless, for interpretation of any chosen one, how it compares to other reasonable options would be informative. Each has merits and each is fallible as well.

The argument that antibody response to vaccine is more directly tied to health outcomes than the other clinical biomarkers is not valid. All are relevant to clinical health outcomes and likely to be predictive of risk of disease, but none have strong empirical support for a direct link between PFAS exposure and the disease of concern, specifically infectious disease with respect to antibody response. The evidence linking small variations in vaccine response to risk of infection is weak at best, and this is also the case for linking serum cholesterol to heart disease, liver enzymes to liver disease, and birthweight to perinatal mortality or other outcomes. Perhaps among these, there is the most compelling case for serum cholesterol since it has been associated with cardiovascular disease across a wide range of levels and more is almost certain to be worse. For the others, clinical consequences of shifts are largely within what would be considered a normal range though probabilistically, some individuals may be shifted into a more high-risk situation.

Within any one of the primary endpoint options, there are an array of reasonably good studies to draw upon, and this is true for the vaccine response papers. There is some concern that the same research team led by Professor Grandjean has generated essentially all of the reports and a number of them base their assessment on peculiar populations in the Faroe Islands, in contrast to the other endpoints such as cholesterol where findings have been replicated by many different investigators across many different populations. While there is no obvious flaw in the vaccine response studies, they are more susceptible to some peculiarities in methodology since the researchers, designs, and interpretations are so homogeneous.

The choice of the study or studies that show effects at the lowest levels is both understandable and worrisome. Seeking the most sensitive endpoint is the priority as it should be and there is certain to be variability across endpoints and across studies of a specific endpoint in lowest adverse effect levels. On the other hand, random error and other poorly understood biases are certain to be operative, and choosing the study that generates the lowest adverse effect level is certain to be most deviant in the spectrum of studies and deviant in a particular direction. It is not likely to be the most accurate simply because it identifies the lowest effect levels, just as the one that identifies the highest effect levels would not be. By at least exploring alternatives, there would be the ability to see if the chosen study is a true outlier or simply in the lower range of other studies generating similar results. The latter would be more reassuring than the former.

PRELIMINARY COMMENTS

MCLG

1. Please comment on the health effect/outcome categories identified from the review of the available literature. Do you agree with the strong vs. suggestive evidence designations for the various health outcome categories? Do any other health systems or endpoints need to be considered for POD derivation?

a. The examination of consistency between human and animal studies, where possible, is appropriate and helps to triangulate evidence from different sources to increase confidence in the validity of the results.

b. In the range of non-cancer endpoints, I fully agree that the most informative human studies pertain to immunologic effects, developmental effects, serum lipids, and liver enzymes. There are multiple studies for each of these outcomes and reasonable if not perfect consistency across studies in different populations and settings. There are no single definitive studies for any of these (which is not a realistic goal) but when there is a series of studies of adequate quality that all point in the same direction, it is reasonable to infer that the effects are present.

Other outcomes have been associated with PFOA or PFOS in isolated studies, including thyroid disease, ulcerative colitis, neurodevelopmental effects, and others, but in my view, none are close to the consistency that exists for the 4 that were chosen.

I would question the characterization of any of the human health effects as “large” since all four are based on small changes in clinical biomarkers. The biomarkers are of health relevance, but in fact none have been shown to mediate an effect on the clinical disease that would be expected to follow. That is, PFOA has been associated with immunologic effects but not consistently with infectious disease, with developmental effects but not clinically meaningful endpoints, with serum lipids but not heart disease, and with liver enzymes but not liver disease. Attaining statistically precise results for small change in biomarkers is one of their strengths but it is important to be careful not to overstate the clinical relevance for any individual even if a shift in the population values is of public health concern.

7a. EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only ‘medium’ and ‘high’ quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?

The potential for confounding is dependent on the presence and magnitude of association between other causes of the health outcome and PFAS levels. More specifically, the question becomes whether there are other causes of disease that are correlated with PFAS biomarker levels since all the studies of interest were reliant on exposure *biomarkers* rather than exogenous

exposures based on location, behaviors, etc. Essentially all the studies took into account the usual sociodemographic and behavioral predictors of disease, i.e., age, social class, smoking. A number of studies also considered determinants of blood levels of PFAS such as BMI, and parity and breastfeeding history for women.

Substantial confounding is unlikely since exposure to PFAS is not strongly associated with any particular determinants such as social class or lifestyle, so that an argument can be made that a reasonable effort to adjust for “the usual suspects” is sufficient. The one major concern is the potential impact of shared physiology that drives both PFAS biomarker levels and clinical biomarkers of interest. The pitfalls in reliance on biomarkers was carefully and thoughtfully examined by Weisskopf and Webster (Weisskopf MG, Webster TF. Trade-offs of Personal Versus More Proxy Exposure Measures in Environmental Epidemiology. *Epidemiology*. 2017 Sep;28(5):635-643. doi: 10.1097/EDE.0000000000000686. PMID: 28520644; PMCID: PMC5543716). The concern here is that measured PFAS exposure in blood is determined not just or even primarily by the exogenous exposure to PFAS in water, food, air, etc. but rather by physiologic variation in uptake and excretion. Studies do not measure exogenous exposure but variation across individuals in largely similar environments with differing biomarker levels. To the extent that these reflect physiologic differences, there is a risk of confounding that applies to essentially all the studies considered.

The susceptibility to “physiologic confounding” is greatest when there is no clear environmental basis for differing levels among study participants, so that the few studies that are based on differing water contaminant levels (e.g., C8 Science Panel reports, studies in Ronneby, Sweden) are less susceptible to this bias even when they also rely on biomarker levels. There is some possibility that more highly exposed people have differing diets or varying exposures to indoor environments or drink different amounts of water, but these are likely to be subtle and modest influences, leaving a great deal of room for physiologic confounding.

Studies that use clinical biomarkers are more susceptible to this than studies of clinical disease. When the product of the study is a correlation of blood levels of PFAS with blood levels of antibody, cholesterol, or liver enzymes, anything that affects metabolism may jointly affect PFAS and clinical outcome measures. Birthweight studies are clearly susceptible since it is well-established that greater plasma volume expansion is associated with greater birthweight and likely also associated with lower (diluted) PFAS levels, which would create a spurious correlation of PFAS and birthweight. A recent examination of the literature provided indirect support for this hypothesized bias, with studies that measured PFAS exposure later in pregnancy when this would have the greatest effect showing the strongest association with reduced birthweight (Steenland K, Barry V, Savitz D. Serum Perfluorooctanoic Acid and Birthweight: An Updated Meta-analysis With Bias Analysis. *Epidemiology*. 2018 Nov;29(6):765-776. doi: 10.1097/EDE.0000000000000903. PMID: 30063543).

There is no direct way to overcome this since too few studies have included populations with clear exogenous sources that drive differences in PFAS levels. To the extent that some studies seem to have clearer environmental as opposed to physiologic determinants of blood PFAS levels, these would be preferred. For example, studies of birthweight that measure PFAS before or early in pregnancy are more informative than those that measure exposure later in pregnancy.

If there are studies that provide some temporal separation of the measures of PFAS and clinical biomarkers, those would be preferred to those that measure them simultaneously. Because susceptibility to confounding of the sort described would likely differ across the endpoints of interest, this provides a strong justification to consider multiple endpoints rather than just one – that is, consider antibody response to vaccines, serum lipids, liver enzymes, and birthweight, not just one arbitrarily chosen endpoint or one arbitrarily chosen study from among an array of broadly similar ones.

7b. Studies of developmental immune health outcomes (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]) after PFOA/S exposure identified associations with very low doses of either PFOA or PFOS with developmental immune effects. The RfD for this outcome was selected as the critical effect because it was the lowest among the candidate RfDs for PFOA or PFOS and can result in severe illness. Does the panel agree with the selection of the critical study and critical effect for the derivation of chronic RfDs for PFOA and PFOS?

- i. If so, please explain your justification.
- ii. If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of chronic RfDs.
- iii. Are any additional analyses or rationales needed to increase the confidence in the chronic RfDs for PFOA and PFOS?

Among the most consistently documented associations of PFOA and PFOS with health outcomes, the volume and quality of evidence is fairly similar for vaccine response in children (the one chosen), serum cholesterol, liver enzymes, and birthweight. Arguments can be made for any one of these but there's not a compelling basis for arguing that one is "best" among them. Given that menu, a stronger strategy would be to consider perhaps a cursory look at all of them with the strongest inferences where there is consistency among them. Regardless, for interpretation of any chosen one, how it compares to other reasonable options would be informative. Each has merits and each is fallible as well.

The argument that antibody response to vaccine is more directly tied to health outcomes than the other clinical biomarkers is not valid. The evidence linking small variations in vaccine response to risk of infection is weak at best, and this is also the case for linking serum cholesterol to heart disease, liver enzymes to liver disease, and birthweight to perinatal mortality or other outcomes. Perhaps among these, there is the most compelling case for serum cholesterol since it has been associated with cardiovascular disease across a wide range of levels and more is almost certain to be worse. For the others, clinical consequences of shifts within the normal range, which is what the studies have demonstrated, is less convincingly of clinical relevance.

Within any one of the primary endpoint options, there are an array of reasonably good studies to draw upon, and this is true for the vaccine response papers. There is some concern that the same research team led by Professor Grandjean has generated essentially all of the reports and a number of them base their assessment on peculiar populations in the Faroe Islands, in contrast to the other endpoints such as cholesterol where findings have been replicated by many different investigators across many different populations. While there is no obvious flaw in the vaccine

response studies, they are more susceptible to some peculiarities in methodology since the researchers, designs, and interpretations are so homogeneous.

The choice of the study or studies that show effects at the lowest levels is both understandable and worrisome. Seeking the most sensitive endpoint is the priority as it should be and there is certain to be variability across endpoints and across studies of a specific endpoint in lowest adverse effect levels. On the other hand, random error and other poorly understood biases are certain to be operative, and choosing the study that generates the lowest adverse effect level is certain to be most deviant in the spectrum of studies and deviant in a particular direction. It is not likely to be the most accurate simply because it identifies the lowest effect levels, just as the one that identifies the highest effect levels would not be. By at least exploring alternatives, there would be the ability to see if the chosen study is a true outlier or simply in the lower range of other studies generating similar results. The latter would be more reassuring than the former.

CVD

Overall Charge Question

EPA is seeking SAB evaluation on the extent to which the approach to estimating reductions in CVD risk associated with reductions in exposure to PFOA and PFOS in drinking water is scientifically supported and clearly described.

The approach is quite reasonable overall given that perhaps the most well-established effect of PFAS exposure is elevated serum cholesterol. The threshold for assessing the benefits of reducing PFAS exposure levels is indicated to only require “a meaningful opportunity for health risk reduction” and that does seem to be the case here. Therefore examining the sequence from regulation and reduction of PFAS in drinking water to changes in serum PFAS to changes in serum cholesterol to changes in rate of cardiovascular events provides a reasonable basis for estimating the benefits of the regulation. In the description of their work, it would be helpful at the outset to provide a clearer rationale and main assumptions before launching into the considerable detail that follows. They should specify additional assumptions and explanations, perhaps by expanding Section 2 of the report:

- 1) The assumption is that a shift in cholesterol resulting from PFAS exposure will have the same impact on clinical disease that cholesterol levels based on natural levels or use of cholesterol lowering medications have had. However, even though the epidemiologic literature that provides strong support for an effect of PFAS on cholesterol does not provide support for an effect on the risk of cardiovascular disease. In fact, the research from the C8 Science Panel shows essentially no relationship between elevated PFOA levels and risk of clinical cardiovascular disease. This does not negate the value of the exercise but does call for some acknowledgement that the exercise follows the pathway that links cholesterol to cardiovascular events rather than looking at the reported effects of PFAS directly on cardiovascular disease.
- 2) The temporal sequence of events is addressed at many points in the technical details, but it would be helpful to start with a more general explanation. Presumably the regulation would be expected to take effect at a specific point in time and produce an immediate change in the levels of PFAS in drinking water. Over some period of time, the altered PFAS in drinking water would

alter the serum PFAS levels (but the half-life data indicate that this would not be instantaneous). With that altered serum PFAS level, serum cholesterol would also shift and they do note that this is presumed to be instantaneous. With that altered serum cholesterol level, going forward there would be changes in the risk of cardiovascular events, the number of such events occurring per year for example. It may or may not be necessary to address the complexity of the transition time and go right to a steady state lower cholesterol level, but in reality, the benefits in reducing disease risk would be realized over time.

3) The value of sensitivity analysis from beginning to end steps is worth considering while recognizing the need to manage the complexity and volume of results. Perhaps in addition to the “most likely” approach that was used, a chain of assumptions that lead to “best case” (maximum benefit) and “worst case” (minimum benefit) would be worth considering.

4) The introduction of the demographic factors seems unnecessary. Even though these have a substantial impact on risk of cardiovascular events, there is no basis for supposing that the proportionate change in risk related to reduced PFAS would affect these groups differentially. That is, if it were predicted to reduce risk by X% in one group, it would be reasonable to assume it reduces risk by X% in all groups. There is not evidence in the literature to my knowledge that the relationship between PFAS and serum cholesterol differs across demographic groups, and while there may be evidence that the cholesterol—cardiovascular event relationship differs, this level of refinement seems unnecessary for these purposes as opposed to assuming a similar proportionate effect across all groups.

Section 4.2 presents EPA’s meta-analysis for the total cholesterol dose-response function.

Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

To inform the CVD risk reduction analysis for those ages 40-89 using the ASCVD risk model, EPA used a meta-analysis approach for the total cholesterol dose-response function. Please provide specific feedback on the extent to which this approach is reasonable for this application, or whether using a single dose-response study (e.g. Dong et al., 2019) selected in the analysis of cholesterol impacts in the *Proposed Approaches for Deriving Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water* would add additional strengths for the CVD risk reduction application.

1A. The approach to identifying and managing the literature on PFAS and cholesterol is reasonable for this purpose, with little basis for more esoteric or complex approaches. What is needed, however, is some sense of the impact had other approaches been used, setting the stage for a sensitivity analysis. If, in fact, a variety of reasonable alternative options would all generate roughly the same predictions, then this would be sufficient to put aside concerns with the specific approach used. If reasonable alternatives would lead to meaningfully different estimates, it would be useful to carry through one or more alternatives to the final results in a sensitivity analysis. The literature is fairly homogeneous in design – large, cross-sectional

studies measuring the association between PFAS and serum cholesterol. Given that similarity, it is likely that there would be a good amount of agreement among studies and it is unlikely to make a notable difference if other ways of summarizing the association had been chosen.

One issue that should be addressed is the exclusion of those on cholesterol-lowering medications (statins or other drugs). Obviously, if there is treatment for hypercholesterolemia, the association between PFAS and cholesterol is uninformative. Studies that failed to make this restriction or those that fail to isolate the untreated should not be part of the basis for estimating the effect of PFAS on cholesterol.

1B. The approach to estimating the dose-response function using the ASCVD risk assessment tool seems like a wise choice. It was developed by leading cardiovascular disease researchers, it is widely used, and there is no reason to invent a new tool for the purposes of this exercise. In fact, it seems the instrument was developed for exactly this purpose, to estimate the impact of modifying cholesterol levels, and the reduction of PFAS in drinking water is one of the ways this might be accomplished. For conducting sensitivity analysis, I would not expect that other models would yield notably different results, or even if they would, that the others are somehow more accurate.

As above, starting with a simple, intuitive overview of how this was done would be helpful before launching into the details that follow. For many readers, understanding the overall approach used and the reasoning behind it would be sufficient, and those who wish to delve into the details could do so based on the material that follows.

Section 5.1 presents EPA's life table approach methodology.

Please comment on the extent to which this analysis is scientifically supported and clearly described. To the extent improvements are suggested, please provide specific changes that are implementable in a U.S. national-level benefits analysis with readily available data.

This seems like a reasonable approach to estimating CVD risk reductions. It would seem that a simpler approach would be to estimate the shift in cholesterol resulting from reduced PFAS, then to provide some global estimate of the proportionate impact of this lowered cholesterol on risk of cardiovascular events. The life table approach takes the timing into account as people age and the changes to drinking water contaminant levels are shifted. It seems that if the ultimate effect is a reduction of a given amount of cholesterol, and that shift is expected to reduce cardiovascular events by a given amount, then this would generate the total benefit. But if there is a need to develop these models that account for the transition from one steady-state to the new steady-state, this is the appropriate method to use. The choice of this particular model, the ASCVD model, is well-justified.

REVISED COMMENTS

Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Noncancer Hazard Identification

2. Elevation of liver serum biomarkers in humans is frequently used an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.
- A. Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.

Response: This panelist does not agree with the rationale and suggests that this endpoint be reconsidered, as there are numerous studies that point to less than 2-fold elevation in ALT being associated with certain types of liver disease, liver-disease mortality, and potentially other health outcomes such as cardiovascular disease. In addition, there are examples of <2-fold increase in serum ALT being associated with pathology confirmed liver disease, such as NAFLD. Importantly, the AASLD has a position paper about serum ALT being considered a predictor for overall health and mortality. To summarize, we cannot conclude that less than a 2-fold change in serum ALT is innocuous and without risks to human health.

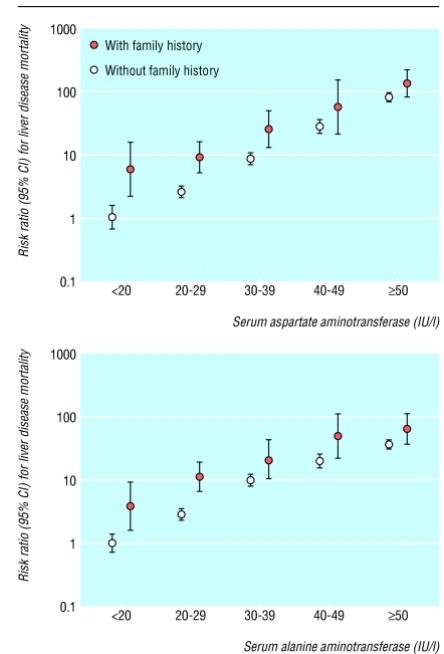
Aside from this point, there are numerous observations in human epidemiological studies that associate serum PFOA and PFOS concentrations with serum ALT. There are multiple rat and mouse studies that also demonstrate PFOA and PFOS administration can raise serum ALT. This observation has been made by numerous research groups in more than one species, so this panelist's view is that the use of ALT represents a reproducible and rigorous endpoint that is predictive of adverse health effects.

- B. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.

Response: The citations below support the argument that there is clinical relevance to a less than two-fold elevation in ALT with regard to liver disease and the slight elevations in ALT associated with PFOA and PFOS exposure could have human health relevance.

Kim et al., Hepatology, 2008;

<https://doi.org/10.1002/hep.22109>. The American Association for the Study of Liver Diseases (AASLD) guidance regarding serum ALT levels as an indicator of health and disease, which should be considered. Basically, this special article argues that serum ALT is more than a marker for liver injury, but argues that ALT “may also be a good indicator of overall health, particularly in the context of obesity, the metabolic syndrome, and presence of cardiovascular disease, as many patients affected by these conditions also are at risk of having non-alcoholic fatty liver disease” (Kim et al., Hepatology, 2008; <https://doi.org/10.1002/hep.22109>). This review article argues that abnormal ALT activity is often ignored or minimized by practitioners as most patients are asymptomatic, but that ALT also actually has a relationship to overall health, liver disease, and cardiovascular disease. The basis for this argument is that some studies indicate that mild to moderate ALT elevation is associated with asymptomatic liver disease, but also as a measure of overall health and mortality.



Kim et al., BMJ, 328(7446):983, 2004

Mathiesen et al., Scandinavian Journal of Gastroenterology, 34, 1999. With regard to asymptomatic liver disease, a Scandinavian study of 151 consecutive patients with mild to moderate elevations of serum aminotransferase levels for at least 6 months in combination with liver biopsy revealed that non-alcoholic steatohepatitis and hepatic steatosis (noted in 42%), chronic HCV (15%), alcoholic liver disease (8%) and autoimmune hepatitis, primary biliary cirrhosis and alpha1 antitrypsin deficiency was associated with only slight to moderate elevation in serum ALT. With regard to overall health, a population-based study that included 142,055 individuals of ages between 35 and 59 years in whom baseline demographic and laboratory data obtained between 1990 and 1992 were available looked at the association between elevated ALT values and subsequent mortality risk in a cohort of participants of a large health insurance program in Korea. The objective of the study was examine the relation between the normal range of serum aminotransferase concentration and mortality from liver disease. The authors observed a positive association between the aminotransferase concentration and mortality from liver disease in serum ALT values in normal range (35-40 IU/l). The risk ratio for liver disease mortality also correspondingly increased as serum ALT increased. The findings from Kim et al., 2004 is that slight elevations in ALT are associated with increased risk of mortality and that patients with even slight elevations in ALT should be monitored for liver disease.

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Park et al., Liver Int. 2012 Jul;32(6):937-44. Another cross-sectional study using Korean NHANES data saw an association with the upper limit of the normal range for ALT with liver disease. They defined the 95th percentile levels for ALT in healthy weight, metabolically normal, liver disease-free KNHANES participants as 34 IU/L for men and 25 IU/L for women. The sensitivity for detecting chronic liver disease was significantly improved when the threshold for normal ALT was lowered.

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C. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

Response: Non-alcoholic steatohepatitis. Jin et al., Environ International, 2020; <https://pubmed.ncbi.nlm.nih.gov/31744629/>. The odds of having steatosis increased with increased PFOS exposure in 7-19 year-old patients. The mean serum PFOS concentration was 3.59 ng/ml. This observation of Pfos-induced steatosis has been observed in rodent models (Marques et al., 2021; Wang et al., 2014; Das et al., 2017).

EPA’s Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Charge Question #1

The component-based mixtures approaches presented in the framework are based on dose addition. Traditionally, **an assumption of dose addition** for a mixture is based on components sharing a common mode of action (MOA) for a given health effect. However,

EPA's supplementary guidance (EPA, 2000) states: "The common mode-of-action (MOA) assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)." This suggests that although the common MOA metric for application of dose addition is optimal, there is flexibility in the level of biological organization at which "similarity" can be determined among mixture components. As an emerging chemical class, MOA data is limited or not available for many PFAS. For purposes of a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity at the level of toxicity endpoint/health effect rather than MOA.

Cory-Slechta, Kamendulis, Post and Slitt

- E. Please comment on the appropriateness of this approach for a component-based mixture evaluation of PFAS under an assumption of dose additivity.
 - F. If common toxicity endpoint/health effect is not considered an optimal similarity domain for those PFAS with limited or no available MOA-type data, please provide specific alternative methodologies for integrating such chemicals into a component-based mixture evaluation(s).
- A. Response: Dose Additivity** – I agree with use of the default assumption of dose additivity when evaluating PFAS mixtures that have similar effects because with little known regarding PFAS-PFAS interaction in complex biological systems (i.e. *in vivo* models), this approach will use an assumption that is health protective. Data included in the document illustrating PFOA + PFOS interactions for pup body weight, liver weight, and Dam liver weight suggest the *potential* for additivity. Marques et al., 2021 (PMID 34464680), described the toxicological interactions of a mixture of PFOA, PFOS, and PFHxS (1 mg/kg) to pregnant dams and demonstrated that the outcomes can be additive, synergistic, or antagonistic depending on which health outcome is assessed. Because there is a lack of *in vivo* studies, and unclear outcomes, an assumption of dose additivity would be the most cautious approach.

However, it is important for the document to address that PFAS-PFAS interactions are poorly understood, and more comprehensive data sets are needed. The unique properties of PFAS make them unlike other EPA-regulated chemicals and this is a limitation to the assumption. While mechanisms governing toxicodynamics for numerous PFAS are better understood and are largely overlapping for Peroxisome proliferator-activated receptor- α activation and can justify dose addition, this additivity is based on the assumption that PFAS do not compete with each other to gain entry into sensitive tissues and their entry into cells is mutually exclusive from each other. This could be the case for transporter-independent penetration of cells, but might not be the case for transporter-mediated interactions. The latter could also be confounded by the concentration or dose used of one PFAS versus another. The interactions of PFAS in combination in cells or rodent models is largely unknown, so to assume dose additivity without solid empirical evidence or understanding of interactions at exposure-relevant concentrations is premature. There is cell-based evidence to suggest that at high concentrations PFAS can compete with each other and not have additive effects in cells. A recent study by Bjork et al., *Toxicology* 2021 using combinations of PFAS in FaO cells demonstrated that combined exposures to PFOS and either PFBS or PFHxS at low noncytotoxic concentrations produced a transcriptional effect that was significantly less than the sum of the individual effects. Another recent study by Ojo et al., *J. Hazard Mater.*, 2022 demonstrated differing effects on additivity based on PFAS assessed,

showing that combinations with PFOS showed synergistic interactive effects, whereas PFOA combinations produced additive effects. Our recent work published by Marques et al., 2021 in mice also points to mixtures eliciting complicated adverse effects and toxicodynamic interactions when administered in combination. A limitation to our work is that the dose of 1 mg/kg is relatively high and might induce PFAS-PFAS interactions that would not occur at lower, much exposure relevant concentrations.

- B. Response: Again, to re-iterate what has been stated above, at the moment, this is the most conservative and health protective approach because there is not enough scientific literature to support another method or approach. It is possible as more information is generated regarding PFAS-PFAS interactions, the approach to dose additivity could change, as PFAS have unique physiochemical properties compared to the other regulated chemicals that have been modeled previously.

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Charge Question #3

Section 4.4 (**Relative Potency Factor; RPF**) of the framework document demonstrates the application of a component-based mixture approach, based on dose addition, using available dose-response information (i.e., points-of-departure) from completed EPA human health assessments, and hypothetical exposure information. The example RPFs and corresponding Index Chemical Equivalent Concentration (ICEC) calculations presented are primarily focused on four PFAS with finalized EPA Human Health Assessments: PFOA, PFOS, PFBS, and GenX chemicals. **Burman, DeWitt, Fisher, and Slitt**

- A. Please provide specific feedback on whether the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS. If not, please provide an alternative.
- B. Please provide specific feedback on whether the proposed RPF methodology in the framework is scientifically supported for PFAS mixture risk assessment.
- A. Selection of the RPF approach is a reasonable methodology for assessing mixtures toxicology. The document does a good job of justifying the use of RPF and the supporting literature that has been used to create RPFs using *in vitro* models. There may be more novel approach methods to include for some classes of chemicals, such as PFAS, that might be able to better enable the modeling. A change to the document (Figure 4-1) is suggested to allow for more flexibility with HI, TOSHI and use of RPFs without having a tiered approach and more guidance on which approach should be used depending on the data in hand.
- B. The RPF framework could be useful for PFAS mixture assessment, but there are some assumptions, such as dose additivity, that seem to be uncertain based on recent literature. For example, Neilson et al., 2022, Toxicology, examined PPAR-alpha additivity and compared different models, such as summation, generalized concentration addition, versus Relative Potency Factor. They demonstrated that GCA and RPF performed equally well at predicting the effects of mixtures with three PFCAs, but only GCA predicted experimental

activity with mixtures of PFASs and a mixture of PFCAs and PFASs at ratios found in the general population. They concluded that of the three approaches, GCA most accurately models the effect of PFAS mixtures on hPPAR α activity *in vitro*.

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Toxicokinetic Models

2. Animal Model –

A. After a review of the available toxicokinetic models for PFOA/S predictions in laboratory animals, EPA selected the Wambaugh et al. (2013) model because it was parametrized using all species of interest, demonstrated good agreement with training and test datasets, and used a single, biologically motivated, model structure across all species. Does the panel agree with selecting this model? If not, please describe the rationale for alternative recommended approaches for the calculation of the internal dose metrics in adult animals.

Response: Yes, this panelist agrees with this approach. Wambaugh et al., 2013 is a comprehensive toxicokinetic model – using 22 studies that include in vitro and in vivo dosimetry data. The in vivo studies include datasets for both PFOA and PFOA from mouse, rat, and monkey. The in vivo studies include single and repeat dose studies. Most of the rodent studies used are well powered with n= 5-25/group. Three in vitro assays were also included in the analysis. No other model is suggested.

B. The animal model parameters were obtained through a Bayesian inference parameterization which produced wide credible intervals for some parameter values, but relatively tight credible intervals for the predicted serum concentration. Does the panel agree with using the median values of the estimated animal parameter distributions for prediction of serum concentration and internal dose metrics?

Response: Yes, this approach is reasonable.

C. Based on visual inspection of model predictions to the calibration datasets, EPA utilized sex-independent parameters for PFOS. The male-specific parameters were used for all rat-specific PFOS predictions including predictions in pregnant and nursing dams and the female-specific parameters were used for all mouse-specific PFOS predictions because the parameter values obtained from fitting the female-specific rat data and male-specific mouse data were not consistent with the overall TK parameters for PFOS and produced poor fits to the training and test datasets. Does the panel agree with this approach and justification for this assumption for PFOS? If not, please describe other approaches that could be considered?

Response: Yes, there are insufficient studies for proper modeling. There are no other suggested approaches.

D. EPA assumed a one compartment model for the developing infant based on the lack of infant-specific toxicokinetic data from rats and mice. This model utilizes averages of half-life and volume of distribution from the literature coupled with physiologically relevant lactational parameters for pup nursing. Does the panel agree with the decision to use this model structure for

infant animals? If not, please provide data on infant-specific changes during the animal lactational-period that could be used to account for toxicokinetic differences between the adult and infant rats and mice.

*Response: Yes. This model is reasonable because little is known regarding transfer of PFOA from placenta or PFAS to breastmilk with active transport. While this is likely and feasibly, this has yet to be demonstrated. However, some consideration could be given to the fact that neonatal elimination of PFOA could likely be lower than adults because renal expression of transporters is low after birth. With regard to PFOA clearance in the neonate during lactation, the assumption is that clearance would be similar to that of adults at low doses. This might be the case, but it should be considered that in rats, renal Organic Anion Transporter (OAT1 and 3), and *Mate1* mRNA expression are substantially lower in fetal kidney and then expression increases during the neonatal period, with increases at weaning and adulthood (Xu et al., Mol Med Rep. 2017 Jan;15(1):474-482).*

E. Several parameters dictate the transfer of chemical from the mother to her pup. Does the panel agree with the selection of these parameters for the animal model? If not, please provide your justification and alternative parameters.

Response: Yes, the modeling includes key works in the area of lactational transfer (e.g. Loccisano et al., 2013). The parameters that dictated transfer of chemical from mother to pup were Maternal Milk: Blood Partition Coefficient (P_{milk}), Fetus:Mother Concentration Ratio (R_{fm}), species-specific in vivo determined half-life ($t_{1/2}$), V_d for PFOA and the species-specific milk consumption rate during lactation. The parameters and assumptions used are up to date with regard to publications and our knowledge of PFOA transfer from mother to pup.

F. For neonatal animals, EPA assumed no sex differences in clearance in neonatal animals based on the lack of identification of sex-dependent differences in PFOA/S toxicokinetics from the available data. Does the panel agree with this assumption? If not, please provide your justification and available data on sex differences in neonatal rats.

Response: Yes, I agree with this assumption. There is little to no information information to argue that sex-dependent differences in PFOA/S toxicokinetics exist in neonatal animals. There is minimal to no evidence to suggest sex-specific mechanism are present that could affect toxicokinetics (i.e. renal expression of transporters, renal reabsorption). There are a few publications that examine sex-specific OAT expression in neonatal mouse and rat kidney (Buist SC, Klaassen CD., Drug Metab Dispos. 2004 Jun;32(6):620-5, Buist et al., J Pharmacol Exp Ther. 2002 Apr;301(1):145-51). But these have not evaluated PFOA or PFOS toxicokinetics in neonatal animals.

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Epidemiological Study RfD Derivation

1. EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only 'medium' and 'high' quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling

sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?

Response: This is out of my area of expertise to weigh in on the statistical design of the various epidemiological studies and the potential for confounding. Overall strengths of the endpoint include findings by several groups in multiple, large cohorts.

3. The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.

Response: Given the importance of vaccination and a robust immune response in children, a 5% BMR is health protective, however the document must better justify the relevance of a 5% reduction in antibody response and how that relates to the developing immune system. It will need to reconcile the varying research studies that have been presented and how a reduced antibody response to toxoids will related to health protection against infection. This reviewer has searched the literature to find studies that can demonstrate the amount of decrease in antibody response to vaccination confers a measurable increase in risk. For the sake of transparency and consideration of other relevant health endpoints, it's important to justify this point.

The document should include additional new findings:

Timmermann CAG, Pedersen HS, Weihe P, Bjerregaard P, Nielsen F, Heilmann C, Grandjean P. Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7-12 years exposed to marine pollutants, a cross sectional study. Environ Res. 2022 Jan;203:111712. doi: 10.1016/j.envres.2021.111712. Epub 2021 Jul 31. PMID: 34343554.

Shih YH, Blomberg AJ, Bind MA, Holm D, Nielsen F, Heilmann C, Weihe P, Grandjean P. Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substances: A birth cohort in the Faroe Islands. J Immunotoxicol. 2021 Dec;18(1):85-92. doi: 10.1080/1547691X.2021.1922957. PMID: 34143710.

PRELIMINARY COMMENTS

EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)

Noncancer Hazard Identification

- 3. Elevation of liver serum biomarkers in humans is frequently used as an indication of liver injury, although it has not been shown to be as specific as functional tests, such as histology findings and liver disease (Boone, 2005, HERO ID: 782862). However, greater than 2-fold increases in alanine aminotransferase (ALT) activity, the most sensitive test of hepatocellular injury in humans, above the upper limit of normal are considered indicative of hepatocellular injury. EPA concluded that the available data in adults show a consistent positive association between PFOA and/or PFOS exposure and increased serum ALT levels in the epidemiological literature. However, this response was not selected for dose response modeling because 1) the magnitude of the effect was not large compared to control levels; and 2) concerns about the clinical relevance of the findings and non-specificity of the biomarkers relationship to adverse liver injury and disease.**
 - a. Does the SAB panel agree with EPA's rationale for not considering the ALT endpoint reported in the epidemiological studies for the derivation of a POD for the liver health effects? Please provide your justification and if you suggest that EPA consider this endpoint for POD derivation, please provide your recommendations for a modeling approach.**

Response: This panelist does not agree with the rationale, as there are numerous studies that point to less than 2-fold elevation in ALT being associated with certain types of liver disease, liver-disease mortality, and potentially other health outcomes such as cardiovascular disease. In addition, there are examples of <2-fold increase in serum ALT being associated with pathology confirmed liver disease, such as NAFLD. Importantly, the AASLD has a position paper about serum ALT being considered a predictor for overall health and mortality. To summarize, we cannot conclude that less than a 2-fold change in serum ALT is innocuous and without risks to human health.

Aside from this point, there are numerous observations in human epidemiological studies that associate serum PFOA and PFOS concentrations with serum ALT. There are multiple rat and mouse studies that also demonstrate PFOA and PFOS administration can raise serum ALT. This observation has been made by numerous research groups in more than one species, so this panelist's view is that the use of ALT represents a reproducible and rigorous endpoint that is predictive of adverse health effects.

- b. Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.**

Response: The citations below support the argument that there is clinical relevance to a less than two-fold elevation in ALT with regard to liver disease and the slight elevations in ALT associated with PFOA and PFOS exposure could have human health relevance.

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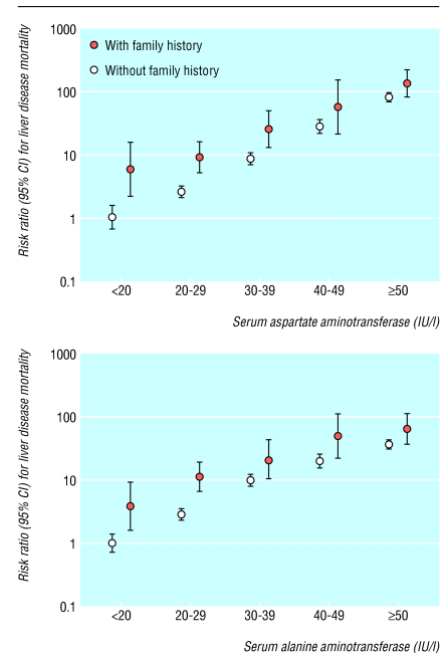
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Ruhl C.E., Everhart J.E. The Association of Low Serum Alanine Aminotransferase Activity With Mortality in the US Population. *American Journal of Epidemiology*, 12:2013, p1702–1711, 178. <https://doi.org/10.1093/aje/kwt209>. This study used NHANES 1988–1994 data and the mortality risk of persons in ALT deciles 1, 2, 3, and 10 was compared with that of persons in deciles 4–9 (mortality was relatively flat across these deciles) over an 18-year period (through 2006) among 14,950 viral-hepatitis-negative adults. Low ALT was associated with increased mortality risk.

c. Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?

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Response: Yes, this panelist agrees with this approach. Wambaugh et al., 2013 is a comprehensive toxicokinetic model – using 22 studies that include in vitro and in vivo dosimetry data. The in vivo studies include datasets for both PFOA and PFOA from mouse, rat, and monkey. The in vivo studies include single and repeat dose studies. Most of the rodent studies used are well powered with n= 5-25/group. Three in vitro assays were also included in the analysis. No other model is suggested.

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Does the panel agree with this approach and justification for this assumption for PFOS? If not, please describe other approaches that could be considered?

Response: Yes, there are insufficient studies for proper modeling. There are no other suggested approaches.

D. EPA assumed a one compartment model for the developing infant based on the lack of infant-specific toxicokinetic data from rats and mice. This model utilizes averages of half-life and volume of distribution from the literature coupled with physiologically relevant lactational parameters for pup nursing. Does the panel agree with the decision to use this model structure for infant animals? If not, please provide data on infant-specific changes during the animal lactational-period that could be used to account for toxicokinetic differences between the adult and infant rats and mice.

Response: Yes. This model is reasonable because little is known regarding transfer of PFOA from placenta or PFAS to breastmilk with active transport. While this is likely and feasibly, this has yet to be demonstrated. However, some consideration could be given to the fact that neonatal elimination of PFOA could likely be lower than adults because renal expression of transporters is low after birth. With regard to PFOA clearance in the neonate during lactation, the assumption is that clearance would be similar to that of adults at low doses. This might be the case, but it should be considered that in rats, renal Organic Anion Transporter (OAT1 and 3), and Mate1 mRNA expression are substantially lower in fetal kidney and then expression increases during the neonatal period, with increases at weaning and adulthood (Xu et al., Mol Med Rep. 2017 Jan;15(1):474-482).

E. Several parameters dictate the transfer of chemical from the mother to her pup. Does the panel agree with the selection of these parameters for the animal model? If not, please provide your justification and alternative parameters.

Response: Yes, the modeling includes key works in the area of lactational transfer (e.g. Loccisano et al., 2013). The parameters that dictated transfer of chemical from mother to pup were Maternal Milk: Blood Partition Coefficient (P_{milk}), Fetus:Mother Concentration Ratio (R_{fm}), species-specific in vivo determined half-life ($t_{1/2}$), V_d for PFOA and the species-specific milk consumption rate during lactation. The parameters and assumptions used are up to date with regard to publications and our knowledge of PFOA transfer from mother to pup.

F. For neonatal animals, EPA assumed no sex differences in clearance in neonatal animals based on the lack of identification of sex-dependent differences in PFOA/S toxicokinetics from the available data. Does the panel agree with this assumption? If not, please provide your justification and available data on sex differences in neonatal rats.

Response: Yes, I agree with this assumption. There is little to no information to argue that sex-dependent differences in PFOA/S toxicokinetics exist in neonatal animals. There is minimal to no evidence to suggest sex-specific mechanism are present that could affect toxicokinetics (i.e. renal expression of transporters, renal reabsorption). There are a few publications that examine sex-specific OAT expression in neonatal mouse and rat kidney (Buist SC, Klaassen CD., Drug Metab Dispos. 2004 Jun;32(6):620-5, Buist et al., J Pharmacol Exp Ther. 2002 Apr;301(1):145-51). But these have not evaluated PFOA or PFOS toxicokinetics in neonatal animals.

Epidemiological Study RfD Derivation

1. EPA evaluated potential confounding as part of their study quality evaluation of the epidemiological studies and selected only ‘medium’ and ‘high’ quality studies for POD derivation. Have the epidemiological studies that were selected for dose-response modeling sufficiently addressed confounding? If not, are there key additional analyses that could be performed to further address the potential confounding of PFAS exposures in these studies?

Response: This is out of my area of expertise to weigh in on the statistical design of the various epidemiological studies and the potential for confounding. Overall strengths of the endpoint include findings by several groups in multiple, large cohorts.

4. The health outcomes identified in the critical studies were decreased antibody response, specifically in serum anti-tetanus and anti-diphtheria, in children after vaccination (Grandjean et al., 2012 [HERO ID: 1248827]; Grandjean et al. 2017 [HERO ID: 3858518]; Grandjean et al., 2017 [HERO ID: 4239492]; and Budtz-Jorgensen and Grandjean, 2018 [HERO ID: 5083631]). This health outcome represents an increased susceptibility to a disease that can cause very severe symptoms, including lethality. Furthermore, children who are immunocompromised may mount a lower antibody response and in turn, be more susceptible to contracting the disease, if exposed than healthy children. Because this health outcome has the potential for severe illness and was assessed in children (i.e., EPA guidelines [US EPA, 1991] support a 5% BMR for developmental effects), a benchmark response (BMR) of 5% was selected for benchmark dose modeling. While some clinical findings are available, the clinical relevance of a 5% decrease in antibody response is not clear. Given the need to protect sensitive subpopulations (e.g., children, individuals with pre-existing conditions) and the available clinical data (i.e., antibody response clinical level), does the SAB support the 5% BMR selection for modeling to identify the POD? If not, please recommend the BMR level and a scientific rationale for an alternative selection.

Response: Given the importance of vaccination and a robust immune response in children, a 5% BMR is acceptable. This reviewer has searched the literature to find studies that can demonstrate the amount of decrease in antibody response to vaccination confers a measurable increase in risk. A 5% reduction is an extremely conservative and health protective for to include sensitive populations.