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EPA Response to Final Science Advisory Board Recommendations (August 2022) on Four Draft Support Documents for the EPA's Proposed PFAS National Primary Drinking Water Regulation

Four Draft Support Documents for the EPA's PFAS Rule:

1. ***Draft Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water;***

2. ***Draft Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water;***

3. ***Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS); and***

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

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ACRONYMS AND ABBREVIATIONS

ALT	alanine aminotransferase
ASCVD	atherosclerotic cardiovascular disease
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower bound limit
BMDS	Benchmark Dose Software
BMR	benchmark response
CI	confidence interval
CSF	cancer slope factor
CVD	cardiovascular disease
DA	dose additivity
DWI-BW	Body weight-adjusted drinking water intake
ED_{50}	dose response effective dose 50%
EFSA	European Food Safety Authority
ELG	Effluent Limitation Guideline
EPA	U.S. Environmental Protection Agency
GCA	general concentration addition
GenX	hexafluoropropylene oxide (HFPO) dimer acid and HFPO dimer acid ammonium salt
glst	Generalized Least Squares for Trend Estimation
HA	Health Advisory
HAWC	Health Assessment Workspace Collaborative
HBWC	health-based water concentration
HDLC	high-density lipoprotein cholesterol
HED	human equivalent dose
HESD	health effects support document
HFD	high fat diet
HFPO	hexafluoropropylene oxide
HI	hazard index
HQ	hazard quotient
IC	index chemical
IC_{50}	dose response inhibitory concentration 50%
ICEC	Index Chemical Equivalent Concentration
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
LOAEC	lowest observed adverse effect concentration
LOAEL	lowest observed adverse effect level
M-BMD	mixture benchmark dose
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MDH	Minnesota Department of Health
MOA	mode of action/mechanism of action
MSLE	mean squared log error
NAM	new approach methodology

NASEM	National Academies of Sciences, Engineering, and Medicine
NOAEC	no observed adverse effect concentration
NPDWR	National Primary Drinking Water Regulation
NTP	National Toxicology Program
OHAT	Office of Health Assessment and Translation
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PECO	population, exposure, comparator, and outcome
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonic acid
PFCA	perfluorocarboxylic acid
PFHxS	perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFSA	perfluorosulfonic acid
PK	pharmacokinetic
POD	point of departure
PPAR-α	peroxisome proliferator-activated receptor-alpha
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QAPP	quality assurance project plan
RfD	reference dose
RfV	reference value
ROB	risk of bias
RPF	relative potency factor
RSL	regional screening level
SAB	Science Advisory Board
SD	standard deviation
SD	standard diet
SDWA	Safe Drinking Water Act
TC	total cholesterol
TK	toxicokinetic
TOSHI	target-organ-specific hazard index
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	lowest observed adverse effect level-to-no observed adverse effect level extrapolation uncertainty factor
UF_S	duration uncertainty factor
WHO	World Health Organization

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency (EPA) policy and approved for publication. It addresses all the recommendations in the final SAB report. The SAB also provided extensive comments that were not characterized as recommendations and therefore are not addressed in this document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) has initiated the process to develop a Maximum Contaminant Level Goal (MCLG) and National Primary Drinking Water Regulation (NPDWR) for per- and polyfluoroalkyl substances (PFAS) under the Safe Drinking Water Act (SDWA). As part of the proposed rulemaking, EPA prepared four draft support documents:

1. *EPA's Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water,*
2. *EPA's Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water,*
3. *EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS),* and
4. *EPA's Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water.*

The agency sought independent advice and peer review from the EPA Science Advisory Board (SAB)[1] on key scientific issues related to the development of these four draft support documents. The SAB PFAS Review Panel initiated its review on December 16, 2021 and provided final recommendations on August 22, 2022 in its report entitled *Review of EPA's Analyses to Support EPA's National Primary Drinking Water Rulemaking for PFAS* (U.S. EPA, 2022a). EPA addressed SAB's recommendations by considering the feedback and suggestions, conducting analyses, revising the draft PFAS rule support documents, and transparently describing EPA's responses to SAB recommendations in this Response to Comments document.

EPA appreciates the thoughtful advice and thorough recommendations that the SAB provided as part of its review process. The SAB recommendations on the four draft support documents have greatly improved the scientific quality, clarity, and transparency of the materials supporting rule proposal. EPA has developed this Response to Comments document to transparently and publicly document how EPA addressed the recommendations made by SAB in its final report (U.S. EPA, 2022a). The responses herein describe the actions EPA took to address those recommendations, including conducting additional analyses and providing more complete descriptions and a systematic review protocol. In the very few instances where EPA did not follow the recommendations of SAB, EPA described the rationale for these decisions. This Response to Comments document addresses all the recommendations in the final SAB report. The SAB also provided extensive comments that were not characterized as recommendations. EPA considered every comment included in the final report when revising the four draft support documents. In this Response to Comments document, the verbatim recommendations of the SAB are organized by section of the final SAB report. The updated versions of the four support documents can be found in the PFAS NPDWR docket (# EPA-HQ-OW-2022-0114) at www.regulations.gov.

[1] The SAB is a scientific/technical advisory committee, the objective of which is to provide independent advice and peer review to EPA's Administrator on the scientific and technical aspects of environmental issues. The SAB charter can be found here: https://sab.epa.gov/ords/sab/f?p=114:2:19191344437428.

SECTION I - MCLG derivation

Proposed Approaches to the Derivation of Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water

Charge Question #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.

Problem Formulation and Criteria

I.S1.1 SAB Recommendations:

Although it is not possible at this point to establish a protocol for the existing review process, the Panel recommends that EPA provide additional clarification and corrections to the existing systematic reviews to fill in gaps about how specific tasks were completed. Furthermore, when designing additional reviews for sensitive endpoints identified as having the strongest evidence in the draft MCLG documents (see Panel recommendations for charge question #2- noncancer hazard identification), EPA should establish protocols prior to beginning any new systematic review process for these endpoints. Protocol development will not only help increase transparency of the subsequent reviews but may also assist in the coordination of multiple teams working across various endpoints.

I.E1.1 EPA Response:

EPA established internal protocols for the systematic review steps of literature search, population, exposure, comparator, and outcome (PECO) development, literature screening, study quality evaluation, and data extraction prior to initiating the systematic review for PFOA and PFOS. However, EPA recognizes that while components of the protocols were included in the November 2021 draft *Proposed Approaches* documents, the protocols were incomplete. EPA has since incorporated detailed, transparent, and complete protocols for all steps of the systematic review process into the updated versions of the *Proposed Approaches* documents, now named the *Proposed MCLG* documents (see Appendix A of both documents). Additionally, the protocols and methods have been updated and expanded based on SAB recommendations to improve the clarity and transparency of the process used to derive the MCLGs for PFOA and PFOS (see responses to the SAB recommendations for this charge question below). The updated methods can be found in Section 2 of the *Proposed MCLG* documents, and the detailed protocols can be found in the appendices.

Literature Search Strategy and Screening Process

I.S1.2 SAB Recommendations:

The Panel recommends several changes to the evidence identification step of the PFOA and PFOS systematic reviews.

- Inclusion and exclusion criteria need to be more clearly described.
- A list of excluded evidence after the full-text review should be developed and made publicly accessible. This may help provide clarity about why specific studies were excluded.

- Earlier literature used for the 2016 HESDs must be included in the literature search and considered for both strength of evidence evaluation and dose-response.
- The PECO statements should be updated to include salts of PFOA and PFOS so that they are included in future literature searches in support of PFOA and PFOS MCLG development.
- In accordance with the EPA Office of Research and Development (ORD) handbook (U.S. EPA, 2022b), the literature search should be updated, with an established protocol, throughout the draft development such that the full literature search update is less than one year from the final review.

I.E1.2 EPA Response:

EPA added an assessment protocol to more clearly define inclusion and exclusion criteria at each stage of the systematic literature review for PFOA, PFOS, and their related salts (see Section 2 and appendices of the *Proposed MCLG* documents). To provide clarity about why specific studies were excluded at the title-abstract and full-text review steps, EPA developed a publicly accessible interactive flow diagram (available here) based on a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) template (see Page et al., 2020). The diagram lists studies included and excluded, as well as brief rationales for exclusion, and thus provides the clarity and transparency the SAB recommended. Additionally, when multiple epidemiological studies included overlapping cohorts, EPA added text to the syntheses sections to describe which epidemiological studies were selected and the reasons why.

EPA expanded the assessment to include epidemiological studies identified in EPA's 2016 Health Effects Support Documents (HESDs) for PFOA and PFOS and considered these studies for both strength-of-evidence evaluation and dose-response analysis as recommended by SAB. Note that EPA included key animal toxicological studies from the 2016 HESDs in the strength-of-evidence evaluation and dose-response assessment in the *Proposed Approaches* documents. Additionally, to ensure studies published before 2016 were fully captured in the assessment, EPA performed a cross-check of the references from the 2020 ATSDR PFAS toxicological profile and included studies relevant to the five main priority health outcomes that were not cited in the 2016 HESDs.

The systematic review work conducted and described in the draft *Proposed Approaches* documents considered studies using salts of PFOA and PFOS, though the PECO statement did not explicitly state that the salts of PFOA and PFOS were included. The search strings used for this effort captured salts of PFOA and PFOS and therefore were not revised. However, EPA has since updated the PECO statements to explicitly list salts of PFOA and PFOS to indicate that studies using various salts were considered for inclusion (see Table A-1 in the PFOA/PFOS appendices).

EPA performed an updated literature search in February 2022 (covering September 2020 through February 3, 2022) with an established protocol (see PFOA/PFOS appendices) consistent with EPA's Integrated Risk Information System (IRIS) Handbook (U.S. EPA, 2022b). EPA also considered studies recommended in the SAB's draft report dated June 3, 2022. EPA is monitoring the literature for studies published after the 2022 literature search update cut-off date that were not proposed by SAB in its June 3, 2022 draft report. These studies are not included as part of the evidence base for these assessments but are provided in a publicly available

repository, and the appendices of the *Proposed MCLG* documents contain a section detailing the results and potential impacts of these studies on the assessments.

Study Evaluation

I.S1.3 SAB Recommendations:

The Panel recommends that the EPA clearly explain the protocols used in its evidence evaluation process. It is critically important to clearly define how each domain in the evaluation protocol is used and to ensure that terms (e.g., "study quality"; "study validity"; and "study risk of bias") are defined and used consistently.

To enhance the transparency of the study evaluations, the Panel recommends that the domains evaluated should be identified in the draft MCLG documents. While they are available in the Health Assessment Workspace Collaborative (HAWC) database, they are not easily located which hinders the ability of readers to review this information.

I.E1.3 EPA Response:

EPA added a protocol that describes the study quality evaluation procedures for epidemiological and animal toxicological studies (see Section 2 of the *Proposed MCLG* documents and their appendices). To enhance the transparency of the study quality evaluations, EPA has added clear descriptions of each study quality evaluation domain in the *Proposed MCLG* documents. The PFOA and PFOS appendices include additional details about study quality evaluation, including prompting questions and suggested considerations used to evaluate each domain. The study quality evaluation domains are also publicly available in the HAWC database, along with study quality evaluation results for each PECO-relevant study identified as part of this assessment.

Data Extraction

I.S1.4 SAB Recommendations:

The Panel recommends that the EPA clearly and transparently articulate the processes and final products of data extraction efforts as they revise the draft MCLG documents. If data extracted are not publicly available, this should also be stated in the revised documentation.

The Panel recommends that EPA include mechanistic evaluations for key non-cancer and cancer weight of evidence evaluations.

I.E1.4 EPA Response:

EPA added a protocol to more clearly and transparently describe how data extraction was conducted for all relevant human epidemiological and animal toxicological studies (see Section 2 of the *Proposed MCLG* documents and their appendices). Extractions were conducted using DistillerSR for epidemiological studies and HAWC for animal toxicological studies. EPA has provided links to data extraction results in Section 3.2 of the *Proposed MCLG* documents. The data extracted from animal toxicological studies are publicly available in HAWC, and they can be accessed using links provided as footnotes to the relevant figures and tables in the *Proposed MCLG* documents. Data extracted from epidemiological studies are publicly available as figures in the *Proposed MCLG* documents and as tables in the PFOA/PFOS appendices. A link to the Tableau site containing all of the data extracted from epidemiological studies is provided in Section 3.2 of each *Proposed MCLG* document.

EPA also evaluated and transparently described and integrated the mechanistic information for the five priority health outcomes (hepatic, immune, developmental, cardiovascular, and cancer) into the *Proposed MCLG* documents. Mechanistic syntheses for these five health outcomes are in Section 3 of the *Proposed MCLG* documents and were considered together with the health effects syntheses to evaluate the weight of evidence for carcinogenicity and to determine the weight-of-evidence judgments for noncancer health outcomes.

Evidence Synthesis

I.S1.5 SAB Recommendations:

The Panel urges the EPA to implement a structured, consistent process with consistent terminology for analyzing and synthesizing animal evidence, human evidence, and overall evidence. One example of such an approach is presented in Chapters 9 and 11 of the IRIS Handbook (U.S. EPA, 2022b), and an example of the application of this approach can be found in Sections 3.2 and 4.1 of the draft EPA IRIS assessment of perfluorobutanoic acid (PFBA) (U.S. EPA, 2021a). Alternatively, the National Toxicology Program (NTP) Office of Health Assessment and Translation (OHAT) recommends a tier system to characterize the overall risk of bias for each study as a way of comparing the internal validity across the evidence base (NTP OHAT, 2015). As stated earlier, 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.

I.E1.5 EPA Response:

For PFOA and PFOS, EPA expanded the systematic review steps beyond study quality evaluation and data extraction to include evidence integration consistent with the IRIS Handbook (U.S. EPA, 2022b). This ensures that EPA implemented a structured, consistent process with consistent terminology for analyzing and synthesizing animal evidence, human evidence, and overall evidence (see Section 2 of the *Proposed MCLG* documents). Specifically, evidence integration was performed by discerning weight-of-evidence judgments for each health outcome category based on the available evidence within each evidence type (i.e., human or animal) using standard terminology (i.e., robust, moderate, slight, indeterminate) and definitions according to the framework described in the IRIS Handbook and outlined in the appendices for PFOA and PFOS (see tables A-30 and A-40). The evidence integration was conducted following the guidance outlined in the "Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments" (U.S. EPA, 2020). A detailed description of the methods for evidence synthesis and integration was added to the PFOA/PFOS appendices. This structured evidence integration framework was used for the five prioritized health outcomes (i.e., those with the strongest evidence). A similar framework was used for evidence integration of the nonpriority health outcomes; however, following recommendations by the SAB, mechanistic evidence was not synthesized for these outcomes as these effects were not "considered as the potential basis for the reference doses (RfDs) and cancer slope factors (CSFs)" (U.S. EPA, 2022a).

Additional Peer-Reviewed Studies that Could Inform Hazard Identification and Toxicity Value Derivation

I.S1.6 SAB Recommendations:

The Panel identified the following key study of immunotoxicity that may be useful:

Dong GH, Liu MM, Wang D, Jin YH, Zheng L, Liang ZF. 2011. Sub-Chronic Effect of Perfluorooctanesulfonate (PFOS) on the Balance of Type 1 And Type 2 Cytokine in Adult C57BL6 Mice. *Arch Toxicol* 85, 1235–1244. https://doi.org/10.1007/s00204-011-0661-x

The Panel also identified the following additional epidemiology studies on associations of PFAS and breastfeeding issues:

Nielsen C, Li Y, Lewandowski M, Fletcher T, Jakobsson K, 2022. Breastfeeding Initiation and Duration After High Exposure to Perfluoroalkyl Substances Through Contaminated Drinking Water: A Cohort Study from Ronneby, Sweden, *Environmental Research*, Volume 207, 112206, ISSN 0013-9351, https://doi.org/10.1016/j.envres.2021.112206.

Timmermann CAG, Andersen MS, Budtz-Jørgensen E, Boye H, Nielsen F, Jensen RC, Bruun S, Husby S, Grandjean P, Jensen TK, 2022. Pregnancy Exposure to Perfluoroalkyl Substances and Associations with Prolactin Concentrations and Breastfeeding in the Odense Child Cohort, *The Journal of Clinical Endocrinology & Metabolism*, Volume 107, Issue 2, Pages e631–e642, https://doi.org/10.1210/clinem/dgab638

The Panel identified the following additional epidemiology studies on associations of PFAS with infectious disease:

Timmermann CAG, Jensen KJ, Nielsen F, Budtz-Jorgensen, van der Klis F, Benn CS, Grandjean P, Fisker AB. 2020. Serum Perfluoroalkyl Substances, Vaccine Responses, and Morbidity in a Cohort of Guinea-Bissau Children. *Environmental Health Perspectives*. 128(8):87002.

Dalsager L, Christensen N, Halekoh U, Timmermann CAG, Nielsen F, Kyhl HB, Husby S, Grandjean P, Jensen TK, Andersen HR. 2021. Exposure To Perfluoroalkyl Substances During Fetal Life and Hospitalization for Infectious Disease in Childhood: A Study Among 1,503 Children from the Odense Child Cohort. *Environ Int*. 149:106395. doi: 10.1016/j.envint.2021.106395. Epub 2021 Jan 25. PMID: 33508532

Bulka CM, Avula V, Fry RC. 2021. Associations of Exposure to Perfluoroalkyl Substances Individually and in Mixtures with Persistent Infections: Recent Findings From NHANES 1999–2016, *Environmental Pollution*, Volume 275, 116619, ISSN 0269-7491. https://doi.org/10.1016/j.envpol.2021.116619.

The Panel identified the following additional epidemiology studies on associations of PFAS with bone health:

Buckley JP, Kuiper JR, Lanphear BP, Calafat AM, Cecil KM, Chen A, Xu Y, Yolton K, Kalkwarf HJ, Braun JM, 2021. Associations of Maternal Serum Perfluoroalkyl Substances Concentrations with Early Adolescent Bone Mineral Content and Density: The Health Outcomes and Measures of the Environment (HOME) Study. *Environmental Health Perspectives*, 129(9):097011-1

Banjabi AA, Li AJ, Kumosani TA, Yousef JM, Kannan K. 2020. Serum Concentrations of Perfluoroalkyl Substances and Their Association with Osteoporosis in a Population in Jeddah, Saudi Arabia. *Environ Res*. 187:109676. doi: 10.1016/j.envres.2020.109676. Epub 2020 May 16. PMID: 32485360.

The Panel identified the following studies on PFAS exposure and reduced vaccine response:

Shih YH, Blomberg AJ, Bind MA, Holm D, Nielsen F, Heilmann C, Weihe P, Grandjean P, 2021. Serum Vaccine Antibody Concentrations in Adults Exposed to Per- and Polyfluoroalkyl Substances: A Birth Cohort in The Faroe Islands. *Journal of Immunotoxicology*, 18(1):85-92 (Hepatitis A antibody) https://pubmed.ncbi.nlm.nih.gov/34143710/

Timmermann CAG, Pedersen HS, Weihe P, Bjerregaard P, Nielsen F, Heilmann C, Grandjean P. 2022. Concentrations of Tetanus and Diphtheria Antibodies in Vaccinated Greenlandic Children Aged 7-12 Years Exposed to Marine Pollutants, A Cross Sectional Study. *Environmental Research.* 203:111712. (Cross-sectional in Greenlandic children at ages 7-12 years) https://pubmed.ncbi.nlm.nih.gov/34343554/

von Holst H, Nayak P, Dembek Z, Buehler S, Echeverria D, Fallacara D, John L. 2021. Perfluoroalkyl Substances Exposure and Immunity, Allergic Response, Infection, and Asthma in Children: Review of Epidemiologic Studies. *Heliyon*, 7:e08160 (review article) https://pubmed.ncbi.nlm.nih.gov/34712855

I.E1.6 EPA Response:

EPA appreciates the identification of these peer-reviewed publications by the SAB panel members. As recommended by the SAB, EPA conducted an updated literature search in February 2022, and the majority of the listed references were captured through that literature search (Banjabi et al., 2020; Buckley et al., 2021; Bulka et al., 2021; Dalsager et al., 2021, Shih et al., 2021; Timmerman et al., 2020, 2022a, 2022b; von Holst et al., 2021). EPA also compared the reference list of the *Proposed MCLG* documents with the reference list of the 2021 Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for Perfluoroalkyls to ensure that all relevant literature, especially literature published before 2016, was considered for this assessment; Dong et al. (2011) was captured through this comparison.

EPA has focused its efforts on examining studies that provide quantitative analyses of endpoints related to the five priority health outcomes having the strongest evidence (i.e., hepatic, immune, cardiovascular, developmental, and cancer). EPA incorporated the studies relevant to immunotoxicity, infectious disease, and vaccine response into the systematic review process for the assessments, and study quality evaluations for these studies can be found in Section 3.4.2 (Bulka et al., 2021; Dalsager et al., 2021; Dong et al., 2011; Shih et al., 2021; Timmermann et al., 2020, 2022a (*Concentrations of Tetanus and Diphtheria Antibodies in Vaccinated Greenlandic Children Aged 7–12 Years Exposed to Marine Pollutants, A Cross Sectional Study*)). While Timmerman et al. (2022b; *Pregnancy Exposure to Perfluoroalkyl Substances and Associations with Prolactin Concentrations and Breastfeeding in the Odense Child Cohort*), Buckley et al. (2021), and Banjabi et al. (2020) were identified in the literature search, these studies did not undergo further evaluation (e.g., study quality evaluation, incorporation into syntheses) because they did not investigate effects in the prioritized health outcomes. Similarly,

Nielsen et al. (2022b) post-dated the updated literature search and was not further considered because this study did not investigate effects in the prioritized health outcomes. Von Holst et al. (2021) is a review paper and was therefore considered supplemental information. This study was used in a supplemental capacity to characterize the uncertainties associated with deriving an RfD based on decreased antibody response to vaccination in Section 6 of the assessments.

EPA evaluated all the studies recommended by the SAB and incorporated the studies relevant to the five prioritized health outcomes into the assessment. To track these and other studies through the systematic review process, please see the publicly accessible interactive flow diagram tool EPA developed (available here).

Charge Question #2A - 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?

I.S2A.1 SAB Recommendations:

In the short-term and in consideration of the agency's time constraints, the Panel recommends that EPA initially focus on health outcomes having the strongest evidence. Additional health outcomes should be evaluated using the recommendations below, over a longer timeframe if necessary.

I.E2A.1 EPA Response:

As recommended by the SAB and supported by the conclusions presented in the *Proposed Approaches* documents, EPA focused its toxicity value derivation efforts on five health outcomes with the strongest evidence. EPA prioritized health outcomes and endpoints with the strongest overall weight of evidence (i.e., evidence integration judgments of "evidence demonstrates" or "evidence indicates," outlined in the IRIS Handbook) based on human, animal, and mechanistic evidence for point of departure (POD) derivation using the systematic review methods described in Section 2 of the *Proposed MCLG* documents and the PFOA/PFOS appendices. For both PFOA and PFOS, these five priority health outcomes are immunological, developmental, cardiovascular (serum lipids), hepatic, and cancer. EPA did not quantitatively assess the dose-response data for other health outcomes (e.g., reproductive, endocrine, nervous, hematological, musculoskeletal) but did follow the structured evidence integration framework to determine the strength of evidence for these health outcomes using systematic review. Evidence integration judgments for the nonpriority health outcomes are included in the appendices of the *Proposed MCLG* documents.

Need for a Consistent Approach and Terminology

I.S2A.2 SAB Recommendations:

The Panel recommends that a consistent framework and descriptors be used for evidence synthesis and integration for each health outcome. A format or template should be developed so that the information is presented consistently for each endpoint, and consistent descriptors should be defined and used for human, animal, and overall evidence.

The Panel recommends that studies, particularly human studies, that were included in the 2016 HESDs be considered in the same manner as the more recent studies. 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 human health effects, which are generally observational rather than experimental, are based on the overall weight of evidence and should include all relevant data.

The Panel recommends 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(s).

I.E2A.2 EPA Response:
Following SAB recommendations, EPA revised the noncancer health effects synthesis and integration sections to provide a more detailed and consistent framework for study quality evaluation, evidence synthesis, and evidence integration for each health outcome following the IRIS Handbook (U.S. EPA, 2022b). The updated sections present information in a manner that is consistent across endpoints and health outcomes, and they use consistent descriptors for human, animal, and overall evidence. Please see EPA's response to SAB comments under Charge Question #1 (Evidence Synthesis) above for additional information.

EPA also updated the assessments to include epidemiological studies that were analyzed in EPA's 2016 HESDs for PFOA and PFOS relevant to the five priority health outcomes. These studies underwent study quality evaluation according to study quality domains consistent with the process for studies identified in the updated literature searches, and they were considered for both strength-of-evidence evaluation and dose-response analyses. Note that EPA had previously included key animal toxicological studies identified in the 2016 HESDs in the 2021 *Proposed Approaches* documents that underwent SAB review.

Furthermore, EPA evaluated and integrated mechanistic information for the five priority health outcomes (hepatic, immune, developmental, cardiovascular, and cancer). Mechanistic syntheses for these five health outcomes are presented in Section 3 of the *Proposed MCLG* documents and were used as the basis for evaluating the weight of evidence for carcinogenicity and determining the weight-of-evidence judgments for noncancer health outcomes.

Selection of Endpoints and Studies for POD Development

I.S2A.3 SAB Recommendations:
The Panel recommends that the process of hazard identification be separated from the process of dose-response assessment. A conclusion about evidence of hazard should not depend on whether or not the data can provide PODs. Instead, sufficient evidence for hazard is needed before dose-response assessment for a health outcome can be considered.

The Panel recommends that the rationale and criteria for selection of endpoints and specific studies for POD development be more clearly presented. 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. Internal inconsistencies in the criteria used for selection of endpoints for POD development should be

addressed. It is also important to explain why a specific study of a health endpoint was selected when there are several possible choices.

I.E2A.3 EPA Response:
EPA separated the hazard identification and dose-response assessment processes into different sections in each of the two *Proposed MCLG* documents (now located in sections 3 and 4, respectively). In the health effects evidence synthesis and integration sections (sections 3.4 and 3.5 of the *Proposed MCLG* documents for noncancer and cancer effects, respectively), EPA presents evidence integration judgments for each health outcome. EPA uses these judgments to determine which health outcomes and endpoints should be considered for quantitative analyses (i.e., only health outcomes with databases meeting criteria for *evidence demonstrates* or *evidence indicates* integration judgments were considered for dose-response assessment). In the dose-response assessment section of the documents (Section 4 of the *Proposed MCLG* documents), EPA presents a description of each endpoint selected for quantitative analysis and the reasons for selection, the strength of the database in support of each endpoint, consistency of findings in the database, and the relevance of each endpoint to human health. EPA lists the studies considered for POD derivation and provides confidence ratings for each study discussed. In cases where more than one study was considered for POD derivation, EPA provided a rationale for the selection of a particular study for POD derivation. EPA updated this discussion to ensure that consistent criteria were used to select endpoints for POD derivation.

Strength of Evidence Designations for Specific Health Outcomes

I.S2A.4 SAB Recommendations:
The Panel recommends 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 of an association with exposure to PFOA/PFOS. Such a reevaluation should consider studies included in the 2016 HESD and more recent studies published after the end date of the literature search for the current draft.

The Panel specifically recommends that issues related to the strength of evidence for PFOA and PFOS exposure and increased serum cholesterol be discussed clearly and thoroughly, including but not limited to the specific issues discussed in this response. This is particularly important because this effect is a major part of the basis for the separate evaluation of cardiovascular disease risk.

I.E2A.4 EPA Response:
After synthesizing and updating the weight-of-evidence narratives for epidemiological data following the evidence integration framework described in Section 2 of the *Proposed MCLG* documents and the PFOA/PFOS appendices, EPA determined that there is *moderate* epidemiological evidence for associations between PFOA or PFOS exposure and adverse immune, hepatic, cardiovascular, and developmental effects in humans. The updated syntheses include evidence from studies identified in the 2016 HESDs and studies identified from the recently performed February 2022 literature search. The rationale for the evidence synthesis judgments for each of these health outcomes is provided in the evidence integration descriptions

in Section 3 of the *Proposed MCLG* documents. EPA revised these sections to more thoroughly discuss and describe the strength of evidence for each endpoint, including increases in serum lipids.

Charge Question #2B - Elevation of ALT

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.

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. *Are you aware of additional studies that support the ALT levels as markers of adverse liver effects? Please provide citations.*
ii. *Are there other adverse liver endpoints identified in the epidemiological literature that need to be considered?*

Consideration of ALT as an Endpoint for PFOA and/or PFOS

I.S2B.1 SAB Recommendations:

Accordingly, the Panel recommends that Gallo et al. (2012) and other epidemiological studies of liver enzymes that were included in the 2016 HESD, as well as any new studies identified in the literature review, be considered when evaluating the weight of evidence for epidemiological effects of PFOA and PFOS as well as for POD derivation. The agency should consider the modeling approach used by California EPA for the epidemiologic studies of this effect.

I.E2B.1 EPA Response:

Following SAB recommendations, EPA broadened the scope of its literature review to include epidemiological studies from the 2016 HESDs, literature identified in three literature searches that covered the period from January 2013 through February 3, 2022, and studies included in the SAB draft report dated June 3, 2022. Gallo et al. (2012) and other epidemiological studies of liver enzymes were considered when evaluating the weight of evidence for the hepatic effects of PFOA and PFOS. Additionally, EPA considered epidemiological studies reporting alterations in serum liver enzymes, including Gallo et al. (2012), Darrow et al. (2016), and Nian et al. (2019), for POD and candidate RfD derivation.

As recommended by SAB, EPA considered California EPA's modeling approaches to derive PODs for the elevated ALT endpoint for PFOA. California EPA used several approaches, including a no observed adverse effect concentration/lowest observed adverse effect concentration (NOAEC/LOAEC) method and a benchmark dose (BMD) method with both EPA's Benchmark Dose Software (BMDS) and a Generalized Least Squares for Trend Estimation (glst) method (CalEPA, 2021). Though EPA incorporated multiple modeling approaches for serum ALT in the Appendix, EPA selected the hybrid approach for POD derivation (Crump, 1995). The hybrid approach defines a benchmark response (BMR) for continuous outcomes, where the BMD corresponds to the dose yielding a specific increase in the probability of an adverse response, compared with zero background exposure. The more commonly used standard deviation (SD)-definition of the BMR for continuous data is simply one specific application of the hybrid approach (U.S. EPA, 2012). The use of this approach for the ALT endpoint is consistent with the modeling that EPA performed for the other epidemiological effects data, such as birth weight and serum lipids. The hybrid approach offers several advantages, including using the estimated regression coefficients derived from the study-specific data and the ability to derive a BMD for continuous outcomes without dichotomization of outcomes and categorization of exposures to avoid inadequacies of information. Please see the modeling discussion in PFOA/PFOS Appendix E for further details.

Studies that Support the ALT Levels as Markers of Adverse Liver Effects

I.S2B.2 SAB Recommendations:

The Panel recommends the use of ALT as endpoint in light of the numerous studies in the literature support an association between slight elevations in ALT and increased risk of morbidity and/or mortality. Moreover, these studies suggest that patients with even slight elevations in ALT should be monitored for liver disease. The Panel additionally identified the following citations that appear to be relevant to the issues of the clinical relevance of ALT elevations and of the association of elevated ALT with morbidity and mortality:

Abdalgwad R, Rafey MF, Murphy C, Ioana I, O'Shea PM, Slattery E, Davenport C, O'Keeffe DT, Finucane FM. 2020. Changes in alanine aminotransferase in adults with severe and complicated obesity during a milk-based meal replacement programme. *Nutri Metab* (Lond). 17:87. Doi: 10.1186/s12986-020-00512-5.

Chen J, Liu S, Wang C, Zhang C, Cai H, Zhang M, Si L, Zhang S, Xu Y, Zhu J, Yu Y. 2021. Associations of Serum Liver Function Markers with Brain Structure, Function, and Perfusion in Healthy Young Adults. *Front Neurol*.12:606094. Doi: 10.3389/fneur.2021.606094.

Ji L, Cai X, Bai Y, Li T. 2021. 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. *Int J Gen Med*.14:2909-2922

Kim HR, Han MA. 2018. Association between Serum Liver Enzymes and Metabolic Syndrome in Korean Adults. *Int J Environ Res Public Health*. 15(8):1658.

Kim, WR, Flamm, SL, Di Bisceglie, AM, and Henry C. Bodenheimer Jr. 2008. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease, *Hepatology*, 47(4): 1363-1370; https://doi.org/10.1002/hep.22109.

Lee TH, Kim WR, Benson JT, Therneau TM, Burritt MF, Melton LJ. 2008. Serum aminotransferase activity and risk of mortality in a U. S. community population. *Hepatology*; 47. DOI: 10.1002/hep.22090.

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. 2020. Revision of serum ALT upper limits of normal facilitates assessment of mild liver injury in obese children with non-alcoholic fatty liver disease. *Clin Lab Anal*. 34(7):e23285. Doi: 10.1002/jcla.23285.

Newton KP, Lavine JE, Wilson L, Behling C, Vos MB, Molleston JP, Rosenthal P, Miloh T, Fishbein MH, Jain AK, Murray KF, Schwimmer JB. 2021. Alanine Aminotransferase and Gamma-Glutamyl Transpeptidase Predict Histologic Improvement in Pediatric Non-alcoholic Steatohepatitis. Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN). *Hepatology*.73(3):937-951

Oh TK, Jang ES, Song IA. 2021. Long-term mortality due to infection associated with elevated liver enzymes: a population-based cohort study. *Sci Rep*.11(1):12490.

Park JH, Choi J, Jun DW, Han SW, Yeo YH, Nguyen MH.J. 2019. Low Alanine Aminotransferase Cut-Off for Predicting Liver Outcomes; A Nationwide Population-Based Longitudinal Cohort Study. *Clin Med*. 8(9):1445.

Ruhl C.E., Everhart J.E. (2013). 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.

Schmilovitz-Weiss H, Gingold-Belfer R, Grossman A, Issa N, Boltin D, Beloosesky Y, Morag Koren N, Meyerovitch J, Weiss A. 2019. Lowering the upper limit of serum alanine aminotransferase levels may reveal significant liver disease in the elderly. *PloS One*. 14(4):e0212737. Doi: 10.1371/journal.pone.0212737.

Wahlang B, Appana S, Falkner KC, McClain CJ, Brock G, Cave MC. 2020. 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. *Environ Sci Pollut Res Int.* 27(6):6476-6487.

I.E2B.2 EPA Response:

EPA appreciates the identification of peer-reviewed publications supporting the association between PFOA/PFOS exposure and hepatic effects. Based on evidence integration of the available studies, including the studies recommended by the SAB (above), EPA has determined that the *evidence indicates* an association between PFOA/PFOS exposure and hepatic effects, particularly elevated ALT in adults. EPA has focused on examining studies providing quantitative analyses of associations between PFOA/PFOS exposure and serum ALT. EPA appreciates the recommended studies (above) that are nonspecific to PFOA or PFOS and has

included descriptions of these studies, in part, to strengthen the rationale regarding the clinical relevance of ALT as an endpoint for POD derivation as described in Section 4.1 of the *Proposed MCLG* documents.

Charge Question #3A - Cancer Designation

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.

I.S3A.1 SAB Recommendations:

While the Panel agrees with the "likely" designation for PFOA carcinogenicity based on new evidence and prior evidence included in the 2016 HESD, there is a need for a more structured and transparent "weight of evidence" discussion to support the rationale behind this designation, including:

- explicit description of how the available data for PFOA are consistent with one or more of the criteria in the EPA *Guidelines for Carcinogen Risk Assessment* (2005) for designation as a "likely" carcinogen and
- explicit description of how the available data for PFOA do not meet the criteria for the higher designation as "carcinogenic."

I.E3A.1 EPA Response:

EPA has updated the weight of evidence section of the *Proposed MCLG* document for PFOA (Section 3.5) to provide a more explicit, transparent description of how the available data for PFOA meet all of the criteria in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) for the designation of a "likely" carcinogen. Updates to this section also include more transparent and detailed descriptions of how the available data for PFOA do not meet the criteria for the higher designation of "carcinogenic." This information is also summarized in Table 3-7, which lists the consistencies of the PFOA carcinogenicity database with cancer descriptors as described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005).

Charge Question #3A - Cancer Designation (continued)

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.

I.S3A.2 SAB Recommendations:

The Panel recommends that a more structured and transparent "weight of evidence" discussion be added. Specific areas that should be addressed include:

- explicit description of why the available data for PFOS do not meet the EPA *Guidelines for Carcinogen Risk Assessment* (2005) criterion for the higher designation as "likely carcinogenic" and

- Inclusion and discussion of mechanistic data.

The Panel also recommends that the findings of the Shearer et al. (2021) study for PFOS be presented clearly including a discussion of why they were judged to be less definitive for PFOS than for PFOA and not considered sufficient to support a higher designation of "likely carcinogenic."

I.E3A.2 EPA Response:
Upon further evaluation of the carcinogenicity database for PFOS, including mechanistic data and potential modes of action as suggested by the SAB, EPA has updated the weight of evidence section for PFOS and has determined that PFOS meets the designation of "likely to be carcinogenic to humans." The rationale for this decision, including transparent and detailed descriptions of why EPA has determined that the database for PFOS exceeds the designation of "suggestive evidence of carcinogenicity," is in Section 3.5 of the *Proposed MCLG for PFOS* document.

A discussion about the evidence for kidney cancer provided by Shearer et al. (2021) can be found in Section 3.5.1.4. This study, among others described in Section 3.5.4, provides support for the determination that PFOS is "likely to be carcinogenic to humans."

Charge Question #3B - Cancer Slope Quantification

Cancer Slope Quantification: EPA used the Shearer et al., 2021 epidemiological study to quantify a cancer slope factor ~~using peak exposure~~[2] 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.

I.S3B.1 SAB Recommendations:
The Panel recommends that multiple candidate CSFs be developed, including those based on additional epidemiologic studies of sufficient quality as well as animal cancer bioassays. Strengths and limitations for each study should be discussed along with judgment made to obtain the overall slope factor.

The Panel recommends that a MOA evaluation for kidney cancer be included if this endpoint is used by EPA.

The Panel recommends that the details of the modeling and its results for the derivation of a CSF, and the conclusions of the EPA review of this information that are not shown in the draft PFOA document be included in the final EPA document.

[2] Wording was revised (strikeout) before Panel deliberations.

The Panel recommends that the development of the clearance factor and its use in determining that administered dose CSFs from the serum level CSFs be clearly and completely described in the final document.

I.E3B.1 EPA Response:
EPA developed multiple candidate CSFs for PFOA based on animal cancer bioassay studies and epidemiologic studies of sufficient quality (i.e., medium or high). PFOA CSFs were derived for kidney cancer (human), Leydig cell adenomas in testes (rat), hepatocellular adenomas or carcinoma (rat), and pancreatic acinar cell adenoma (rat). In Section 3.5 of the *Proposed MCLG* document for PFOA, EPA has added a detailed description and discussion of the strengths and limitations of each study that investigated the association between PFOA exposure and cancer, as well as the judgment made to obtain the overall slope factor in Section 4.2.3.

EPA provides a mode of action (MOA) evaluation for kidney cancer in Section 3.5.4.2.1, which discusses several potential modes of action, based on the available data, by which PFOA exposure may result in renal tumorigenesis.

Details of the cancer modeling and results for the derivation of each CSF have been added to section 4.2.1.2 and the PFOA Appendix.

Descriptions of the development of the clearance factor and how it is used to derive the candidate CSFs were added in sections 4.1.3 and 4.2, respectively.

Charge Question #4 - Toxicokinetic Modeling

Toxicokinetic Model- General[3]

I.S4.1 SAB Recommendations:
The Panel recommends that model performance, along with a statement on acceptable performance metrics, should be documented for every model (including those for different life stages). For instance, plotting predicted and observed concentrations as scatter plot can be helpful to evaluate overall bias and precision (e.g., including lines at 1, 2x, and 0.5x to put performance in context, with portion of samples outside these bounds giving some indication of the acceptability of the model). Comparisons of data and time-course simulations can be helpful as well. If no data are available for evaluating performance, this can be stated for a particular life stage.

The Panel recommends that when a model is used in dose-response analyses, the details and assumptions need to be documented sufficiently so that someone can reproduce the simulations, as noted above. Specifically, for every human or animal simulation there should be information stating which model was employed and what model parameter and input values were used to simulate the specific study or scenario, with the code made available so someone can reproduce the work. It may be helpful to develop a "big picture" workflow schematic for the TK model, how they fit into the BMD and human equivalent dose (HED) calculations.

[3] The SAB PFAS Review Panel provided general recommendations on the pharmacokinetic modeling approach used in the *Proposed Approaches* documents. These recommendations did not correspond to a specific charge question.

The Panel recommends that EPA should better characterize the uncertainty that results from different parameters/ assumptions by considering sensitivity analyses or Monte Carlo simulations with a range or distribution of values. For instance, the Goeden et al. (2019) transgenerational toxicokinetic (TK) model includes at least central and upper bound estimates for different parameters, which could serve as the basis for a sensitivity analysis.

Because the Reference Doses developed in the draft MCLG document are intended to support the development of drinking water MCLGs, the Panel recommends that EPA's analysis ensure that the predicted serum levels are protective for all life-stages. Specifically, such model results would be used along with life-stage-specific changes in ingestion rates at a fixed water concentration to be the basis of an MCLG.

I.E4.1 EPA Response:

EPA revised the assessments to provide additional explanations for selecting and customizing the Wambaugh et al. (2013) model as the basis of the pharmacokinetic (PK) modeling for animal internal dosimetry, which is included in Section 4.1.3.1 of the *Proposed MCLG* documents. In response to SAB recommendations, EPA validated the animal PK model to ensure the model predicted serum concentrations for all relevant life stage and conducted sensitivity analyses (see Appendix F). For the sensitivity analyses, EPA evaluated the potential bias and precision of the customized Wambaugh et al. (2013) model by expanding on the comparison of fits to training datasets used, including a scatter plot of model-predicted serum concentrations and literature-reported concentrations. A similar scatter plot was also provided for recently published studies that were not part of the original Wambaugh et al. (2013) parameterization. In addition to these visual checks, EPA also provided the mean squared log error (MSLE) for each adult and developmental dataset. Furthermore, EPA conducted a local, one-at-a-time sensitivity analysis to examine how parameter sensitivity varied across the adult and developmental models.

Likewise, the rationale for the selection of the Verner et al. (2016) model for modeling human dosimetry is provided in Section 4.1.3.2 of the *Proposed MCLG* documents. In response to SAB recommendations, EPA reconsidered alternate human modeling approaches, including the Loccisano family of models (Loccisano et al., 2011, 2012a, 2012b, 2013) and the Goeden et al. (2019) (Minnesota Department of Health (MDH) model). A detailed description of the strengths and weaknesses of these models, as well as the rationale for ultimately selecting a nonphysiologically based model, is provided in sections 6.6.2 and 6.7 of the *Proposed MCLG* document.

Additionally, EPA validated the human PK model against the available data. Specifically, EPA compared serum levels in children using the original input parameters and updated parameters for the Verner et al. (2016) model. Predicted child serum levels, compared to reported and observed values detailed in Appendix F, indicate that the application of updated parameters showed good agreement between model predictions and reported values. EPA also compared the Verner model assumptions to the MDH model assumptions, and the results of this analysis demonstrate that the Verner model assumptions best fit the data (see Appendix F). EPA also performed a one-at-a-time sensitivity analysis to examine how parameter sensitivity varied across age and between maternal and child serum (see PFOA/PFOS Appendix F).

Input parameters for animal modeling are found in Section 4.1.3.1 of the *Proposed MCLG* documents, and an expanded identification of human modeling parameters is found in Section 4.1.3.2. The model code was thoroughly checked for quality through the established quality assurance project plan (QAPP) for physiologically based pharmacokinetic (PBPK) models (U.S. EPA, 2018). EPA has provided the model code along with the supporting documentation, which can be accessed https://github.com/USEPA/OW-PFOS-PFOA-MCLG-support-PK-models.

Finally, a detailed description of the challenges, uncertainties, and limitations associated with the customized Wambaugh et al. (2013) and Verner et al. (2016) models are found in Section 6.6 of the Proposed MCLG documents, respectively.

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).*

   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.S4.2 SAB Recommendations:
The Panel recommends that the EPA include more details on model code, parameters, data, and performance to support model evaluation and parameter justifications.

I.E4.2 EPA Response:
EPA added a detailed table comparing original and updated modeling parameters in Section 4.1.3.2 of the *Proposed MCLG* documents. Justification for the half-life and volume distribution values selected by EPA is also provided within this section. A summary of PFOA half-life information and volume distribution data is included in Section 3.3.1.2.5, Section 3.3.1.4.5, and Appendix B. EPA notes that a discussion about uncertainties related to the selected values for these parameters was previously included in Section 6.6.2 of the *Proposed MCLG* documents. EPA has provided the model code and the supporting documentation, which can be accessed https://github.com/USEPA/OW-PFOS-PFOA-MCLG-support-PK-models.

b. *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.S4.3 SAB Recommendations:
The Panel recommends the following with respect to the use and application of the TK model for endpoints observed in human neonates or children:

- Although the draft MCLG documents develop RfDs and not MCLGs, EPA should develop a RfD based on serum PFOA levels that can be used to develop a drinking water concentration (MCLG) that is protective for all life-stages.
- EPA should 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 RfDs and MCLGs. While the Verner et al. (2016) model predicts dosimetry from a constant daily dose, the Goeden et al. (2019) model considers age-specific toxicokinetic factors (e.g., volume of distribution) and exposure factors (milk and drinking water intake). Additionally, Goeden et al. (2019) appears to have equal or better model fits as compared to the Verner et al. (2016) model. Thus, the Goeden et al. (2019) model appears more "fit for purpose" for deriving drinking water MCLGs.
- 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, which could be converted to either an equivalent external dose or an equivalent water concentration using TK modeling, as appropriate. EPA should take this approach to better account for life-stage-specific changes in ingestion rates at a fixed water concentration that form the basis of an MCLG.

I.E4.3 EPA Response:
To address this SAB recommendation, EPA evaluated the Goeden et al. (2019) model and considered other approaches to address the question of how to account for age-specific TK factors. EPA also considered the advantages and disadvantages of the different outputs of the models and approaches. The Goeden et al. (2019) and Verner et al. (2016) models are structurally very similar, with a single compartment for mother and child, first-order excretion from those compartments, and a similar methodology for describing lactational transfer from mother to child. One advantage of the Verner model for use in MCLG derivation is that it explicitly models PFOA/S serum concentrations across the mother's life—from the mother's birth through childbearing and the end of breastfeeding—as opposed to making assumptions about maternal blood levels before childbirth. Another advantage of the Verner model is that it accounts for the effect of dilution of PFOA/S levels during childhood growth, such as the changes in blood levels that occur after weaning. EPA also evaluated the use of an age-dependent V_d in children and the treatment of exposure as a drinking water intake rather than a constant exposure relative to body weight, which are two substantial differences SAB noted between the two models. It was determined that neither of those factors substantially improved the fit of the Verner et al. (2016) model, with updated parameters, to the data sets used for validation. Comparing the published fits between the two models is not appropriate because EPA revised the parameters of the Verner et al. (2016) model to be based on the most recent data. EPA has provided a more detailed rationale for selecting the Verner et al. (2016) model over the Goeden et al. (2019) model in Section 6.7 and Appendix F. It is worth noting that the drinking water intake used for MCLGs based on noncancer effects is chosen based on the target population relevant to the critical effect that serves as the basis of the RfD. Therefore, even if the RfD does not incorporate increased drinking water intake in certain life stages, the subsequent

MCLG does take this intake variation into account and is protective of the life stages with greater water consumption.

Based on EPA's re-evaluation, EPA has decided to continue using the updated Verner model rather than adopt the Goeden model because the Verner model assumptions best fit the data (see Appendix F) and because it is important for EPA to be able to generate an RfD for these compounds. EPA used the updated Verner model to develop candidate RfD values based on serum concentrations in children and adults. As described in Section 4 of the *Proposed MCLG* documents, these RfDs can be used to develop MCLGs that are protective for all life stages and across various noncancer health effects.

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.S4.4 SAB Recommendations:
None.

I.E4.4 EPA Response:
No response necessary.

<u>Animal Toxicokinetic Model</u>

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.*

I.S4.5 SAB Recommendations:
The Panel recommends that EPA consider whether it may be appropriate to use measured serum/plasma data when available. The choice of measured versus model-predicted levels will depend on model performance in comparison with judgment as to the reliability of measured values.

I.E4.5 EPA Response:
For the animal PK model, model predictions from Wambaugh et al. (2013) were evaluated by comparing each predicted final serum concentration to the serum value in the supporting animal studies (training data set) and to the animal studies published since the publication of Wambaugh

et al. (2013) (test data set). The predictions for these two data sets were generally similar to the experimental values. Specifically, there were no systematic differences between the experimental data and the model predictions across species, strain, or sex, and the median model outputs uniformly appeared to be biologically plausible despite the uncertainty reflected in some of the 95th percentile confidence intervals (CIs) (see PFOA/PFOS appendices).

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.S4.6 SAB Recommendations:
The panel recommends that EPA characterize the uncertainty associated with using median predictions.

I.E4.6 EPA Response:
EPA has added a description of the limitations and uncertainties associated with the PK modeling approach to estimating animal internal dosimetry in Section 6 of the *Proposed MCLG* documents. Sensitivity analyses for both the adult and developmental animal PK models are provided in Appendix F. In general, the internal dose metrics used in these analyses were not sensitive to the parameters with the largest credible intervals following the Bayesian inference calibration. In other words, the results of the sensitivity analyses demonstrated that changing the most uncertain parameters from the modified Wambaugh et al. (2013) model did not impact the internal dose metrics.

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.S4.7 SAB Recommendations:
The panel recommends that EPA plot the model-predicted plasma concentrations versus the observed or measured plasma concentrations to better visualize model performance.

I.E4.7 EPA Response:
EPA added plots of the model-predicted plasma concentrations versus the observed plasma concentrations for PFOA and PFOS (see PFOA/PFOS appendices). Briefly, training and test data both show good agreement with model predictions using the male-specific parameters from Wambaugh et al. (2013), with MSLE for the adult training datasets and developmental test datasets under a half-$\log_{10}$ and about one $\log_{10}$ for the adult test datasets (MSLE presented in the

PFOA/PFOS appendices). Because experimental serum concentrations spanned many orders of magnitude, EPA presented the unity line with +/- half-$\log_{10}$ to visualize the goodness of fit.

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.S4.8 SAB Recommendations:
The Panel recommends that EPA examine Hinderliter et al. (2006) as to whether it may be useful to refine estimates of PFOA clearance in infant rats.

I.E4.8 EPA Response:
The animal PK model described in the *Proposed MCLG* documents successfully predicts infant PK during gestational/lactational exposure (See PFOA Appendix F, Figure F-12). Based on SAB's recommendation, EPA examined Hinderliter et al. (2006) and compared the reported PK data at 2 hours post-dosing and at 24 hours post-dosing for the 3-, 4-, and 5-week-old animals to determine how the model predicts single-dose PKs at this young age (See PFOA Appendix F, Figure F-13). During the post-weaning phase, the modeling framework in the analysis of the Hindlerliter et al. (2006) study uses the Wambaugh et al. (2013) model with reported juvenile body weights for post-weaning animals. Across all three age groups, this approach works reasonably well for juvenile male rats (blue and orange symbols in the PFOA Appendix F, Figure F-13). As a result of investigating Hinderliter et al. (2006), EPA found an age-dependent change in model predictions for the female juvenile rat (red symbols), where the Wambaugh et al. (2013) model dramatically underpredicts the 3-week-old female rats at 24 hours post-dosing while slightly underpredicting the 5-week-old female rats at 24 hours post-dosing. This is due to rapid female-rat-specific PFOA clearance in the Wambaugh et al. (2013) model, which was parameterized on adult female rat PK data. One possibility is that this model's underprediction for young animals could be due to a not-yet-modeled age-dependent change in PFOA urinary excretion as female pups mature to adult rats and could be attributed to changes in OAT1/OAT3 expression as the pup ages. As outlined in the PFOA Appendix F, Figure F-12, the one-compartment model approach for breastfed pups successfully predicts the reported pup prenatal and lactation life stages. Additionally, figures F-10, F-11, and F-12 (PFOA Appendix F) demonstrate that the switch to the Wambaugh et al. (2013) for post-weaning and pup maturation successfully predicts steady-state PFOA concentrations in the post-weaning male and female rats at postnatal week 19 when the endpoint of interest from NTP (2020) is measured. While it would be possible to use Hinderliter et al. (2006) to estimate an age-dependent clearance for these young rats, EPA's assessment of the study indicates that, due to the single-dose study design and age at which the measurements were reported (i.e., 3–5 weeks of age), incorporation of the results would not impact the current risk estimation of the endpoints used in the NTP study because those measurements were taken at 19 weeks of age with continuous dosing between 15 and 19 weeks.

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.S4.9 SAB Recommendations:
The Panel recommends the EPA perform two analyses to better justify its parameter choices.

1. Sensitivity or uncertainty analyses to better characterize the impact of uncertainty of these parameters.

2. Lactational transfer involves movement into the milk and from milk back into the mother's blood supply. Instead of assuming unidirectional movement into milk, EPA should evaluate the impact of not accounting for movement from milk back into the blood supply.

I.E4.9 EPA Response:
EPA conducted sensitivity analyses to examine how parameter sensitivity varied across the adult and developmental models (see Figure F-5 in PFOA/PFOS appendices). For each parameter/dose metric pair, sensitivity coefficients were calculated to describe the relative change in a dose metric relative to the proportional change in a parameter value. Overall, the dose metrics used in this analysis were least sensitive to the parameters with large, credible intervals from Wambaugh et al. (2013), indicating that uncertainty in these parameters does not impact median concentration predictions.

The animal PK model accounts for gestation, lactation, and post-weaning phases (see Section 4.1.3 in the *Proposed MCLG* documents). A lactational phase involved PFOA/PFOS transfer from the breastmilk to the suckling pup where the pup was modeled with a simple one-compartment PK model. The methodology was adapted from Kapraun et al. (2022), where rapid equilibrium between maternal serum PFOA/PFOS and milk PFOA/PFOS is assumed and modeled using a serum:milk partition coefficient. Hence, the comment from the SAB does not apply because the model does not assume unidirectional flow between maternal serum and breast milk; the reverse movement back into serum is rapid enough for the two fluids to reach equilibrium.

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.S4.10 SAB Recommendations:
The Panel recommends that EPA examine Hinderliter et al. (2006) to potentially develop sex-specific parameters for neonatal clearance.

I.E4.10 EPA Response:
EPA agrees that sex-specific differences in transporters most likely begin to appear during this post-weaning window, where an age-dependent shift in female rat PFOA clearance is observed in Hinderliter et al. (2006). However, these differences between juvenile and adult rats are resolved by 19 weeks and do not impact dose metric predictions (PFOA Appendix F, figures F-

10 and F-11). The one-compartment modeling approach in Hinderliter et al. (2006) is only applied during lactational transfer to the pup, making it difficult to apply these post-weaning data to the current model structure. For a more in-depth discussion of Hinderliter et al. (2006), see response I.E4.8 EPA Response:, above.

Charge Question #5A - 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 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.S5A.1 SAB Recommendations:

The Panel recommends the following improvements on confounding:

- Consider multiple studies of a variety of endpoints in different populations to provide convergent evidence that is more reliable than any single study or health endpoint in isolation.

I.E5A.1 EPA Response:

In response to SAB recommendations, EPA describes at least two epidemiological studies conducted in populations with differing characteristics for POD derivation for each endpoint of interest for the four priority noncancer health outcomes. As the SAB noted, multiple studies in different populations support the selected endpoints and reduce potential concerns about confounding or other biases that would operate similarly across different studies and outcomes. EPA is limited by the human populations that have been studied in the published literature on PFOA and PFOS. See Section 4 of the *Proposed MCLG* documents for additional information on the multiple studies considered for POD derivation.

I.S5A.2 SAB Recommendations:

- Closely review sensitivity analyses and limitations noted by study authors, such as in Starling et al. (2017) and Wikstrom et al. (2020) as noted above, in determining if a study is appropriate for dose-response assessment.

I.E5A.2 EPA Response:

Wikström et al. (2020) assessed maternal PFAS serum concentrations primarily in the first trimester (at a median of 10 weeks of gestation) and also adjusted for week of serum sampling in sensitivity analyses resulting in no differences on the effect, which minimizes concerns surrounding bias due to pregnancy-related hemodynamic effects.

All the selected PODs were derived based on single PFAS models, including the association between PFAS exposure and birthweight. As noted by the SAB, Starling et al. (2017) performed sensitivity analyses to examine the potential for confounding by other correlated PFAS exposures by multiple regressions. These results suggest that associations, while diminished, were still evident for PFOA although PFOS was not ultimately selected in their penalized elastic net model. Another penalized elastic net analysis by Lenters et al. (2016) showed that only PFOA was included in the multiple exposure model as the PFAS-representative variable. In

contrast, two other studies examining the impact of multiple-PFAS modeling showed attenuation of BWT results but showed no change for PFOS (Bell et al., 2018; Darrow et al., 2013) and attenuation of PFOA results. Thus, the results across different studies do not appear consistent, which raises the possibility that co-amplification bias (Weisskopf et al., 2018) from the inclusion of highly correlated co-exposures in the multi-PFAS studies is possible in some combinations of modeling multiple PFAS exposures and some endpoints—but not in all combinations.

To further evaluate this, EPA compared the PODs derived for decreased tetanus and diphtheria antibodies from Budtz-Jorgensen and Grandjean (2018). This comparison showed that for PFOA, there was very little change in the POD estimate with and without the inclusion of PFOS in the regression models (see appendices), indicating that there was little potential confounding by PFOS in the PFOA-only results. The results for PFOS were somewhat more sensitive to the inclusion of PFOA in the models for some combinations of the age of antibody measurement and antibody type, and the multi-PFAS model generally fit less well than the single-PFAS models.

Ultimately, EPA examined all the available information on single-PFAS results versus multi-PFAS results and ultimately judged that the single-PFAS results were not substantially confounded by other measured covariates. As the SAB noted, health effects observed after PFOA/S exposure in different studies in separate populations provide additional support for the selected endpoints and reduce the potential concerns about confounding.

I.S5A.3 SAB Recommendations:

- Do not use incomplete adjustment for socioeconomic status as the primary basis for excluding a study without establishing that socioeconomic status is likely to be a confounder within the context of the study.
- Acknowledge potential confounding by race/ethnicity for health endpoints that may exhibit racial/ethnic disparities.

I.E5A.3 EPA Response:

Epidemiological studies were subject to extensive study-quality evaluation as part of a systematic review, which included evaluating study results for risk of biases (ROBs), such as the potential for confounding from known confounders and other covariates that may be of interest. The systematic review protocol (Appendix A) in the MCLG drafts for PFOA and PFOS includes additional details on the study-quality evaluation domains, such as prompting questions and suggested considerations. Evaluation results for specific studies of interest can be found on the HAWC project page.

EPA acknowledges that in observational epidemiologic studies, potential residual confounding may result from socioeconomic status and racial/ethnic disparities; however, epidemiological studies were not excluded from consideration (i.e., rated as *low* confidence or *uninformative*) based primarily on a lack of or incomplete adjustments for socioeconomic status or race/ethnicity. Appendix D of the MCLG drafts for PFOA and PFOS provides detailed information on identified epidemiological studies and includes the study-specific confounding variables considered, such as socioeconomic status.

EPA's mission is to protect human health and the environment. Protection of health pertains to all Americans, including those who are overburdened by exposure or more sensitive to exposure.

In the systematic review of the health effects after PFOA or PFOS exposure, it is important to understand susceptibility factors, such as SES and race/ethnicity. However, data gaps exist in this area, including exposure study data gaps for disadvantaged communities.

Charge Question #5B - 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?

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?*

Critical Study

I.S5B.1 SAB Recommendations:

The Panel recommends that additional clarification and detail, including summary tables, be included to support the selection of the critical study and the specific dataset within the critical study, and why other studies demonstrating suppressed vaccine responses were not selected as the critical study.

The Panel also recommends that results of the systematic review evaluation of the critical study also be included; if it was not systematically reviewed, the review needs to be performed.

The Panel recommends that the conclusions from other agencies about Grandjean et al. (2012) be reviewed and potentially included.

I.E5B.1 EPA Response:

EPA updated Section 4 of the *Proposed MCLG* documents to include more detail and rationale, including summary tables, to support the selection of the multiple studies and endpoints considered for POD derivation, including effects on the immune, hepatic, developmental, and cardiovascular systems. Multiple candidate RfDs from epidemiological and animal toxicological studies were derived and are discussed in Section 4.

For example, for PFOA and decreased vaccine response, multiple medium-confidence studies were considered for POD derivation: Budtz-Jørgensen and Grandjean (2018), Timmerman et al. (2021), Granum et al. (2013) and Looker et al. (2014). As described in Section 4, while all these studies demonstrate decreased vaccine response and contribute to the hazard judgment in Section 3, Budtz-Jørgensen and Grandjean (2018) and Timmerman et al. (2021) were selected for POD derivation because they offered data characterizing antibody responses to vaccinations in children using a variety of PFOA-exposure measures across various populations and

vaccinations. Additionally, the analytic models in these publications were amenable to BMD modeling, while Granum et al. (2013) and Looker et al. (2014) were not (see Table 4-1 and Appendix E for additional details).

In the previous *Proposed Approaches* documents that SAB reviewed, EPA considered Grandjean et al. (2012) to be systematically reviewed under the HAWC entry for Grandjean et al. (2017a), as this was the most recent follow-up study conducted for the Grandjean et al. (2012) cohort. However, based on SAB recommendations, EPA incorporated epidemiological studies analyzed in the 2016 HESDs into the *Proposed MCLG* documents, which included systematic review evaluations of Grandjean et al. (2012). Please see Figure 3-15 for the results of the study-quality evaluations for the immune studies. EPA considered the conclusions from other health agencies about both the immune endpoints in general and the Grandjean et al. (2012) study in particular. EPA's consideration included reviewing publicly available documents from other health agencies. EPA developed conclusions about the epidemiology studies that evaluated PFOA/S exposure and immune endpoints by following the systematic review process described in the IRIS Staff Handbook (U.S. EPA, 2022b) when developing the *Proposed MCLG* documents. EPA's systematic review was performed by a number of experts in epidemiology and systematic review.

Other Critical Effects

I.S5B.2 SAB Recommendations:

The Panel recommends that additional clarification and detail be included to support the selection of the critical effect and why this effect, beyond having the lowest PODHED, is the most scientifically appropriate choice as well as being the most protective of public health.

The Panel recommends that candidate RfDs be developed for other health endpoints that have been consistently reported in epidemiology studies, as well as from the PODs for effects in experimental animal studies (in both cases including serum ALT, discussed above).

The Panel recommends that the final choice of the health-effect specific RfDs and the overall RfD consider the strength and limitations of the data upon which each is based. A meta-analysis approach also should be considered.

I.E5B.2 EPA Response:

EPA updated Section 4 of the *Proposed MCLG* documents for PFOA/PFOS to include more transparent detailed information, including summary tables, to support the selection of the critical studies and effects for all endpoints from the priority health outcomes (cancer, hepatic, immune, cardiovascular and developmental). EPA derived several candidate RfDs for each health outcome based on both epidemiological and animal toxicological studies (see Table 4-11 in PFOA/PFOS). This also includes candidate RfDs for increased serum ALT from epidemiological studies. The health effects observed in the available animal toxicological studies are consistent with the effects observed in the epidemiological studies. EPA selected amongst the candidate RfDs to identify an RfD representative for each of the four prioritized noncancer health outcomes (i.e., health outcome-specific RfDs), as well as an overall RfD that is protective of the effects of PFOA/PFOS on all health outcomes and endpoints (see Figure 4-5 in PFOA and Figure 4-4 in PFOS). The candidate RfDs considered for the health outcome-specific and the overall RfDs were selected based on the weight of evidence for each endpoint and the strengths

and limitations of the databases for each health outcome, as further described in Section 4 of the *Proposed MCLG* documents.

As part of the evaluation of benefits from cardiovascular disease (CVD) reduction (*Analysis of Cardiovascular Disease (CVD) Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water* (U.S. EPA, 2021b)), EPA performed a meta-analysis to evaluate associations between PFOA/PFOS exposure and effects on serum lipids, specifically total cholesterol (TC) and high-density lipoprotein cholesterol (HDLC). EPA also considered available meta-analyses while evaluating evidence of the associations between PFOA/PFOS and other health outcomes considered. For PFOA, 17 meta-analysis studies were identified and summarized in tables A-42 and A-43 in the PFOA Appendix A; for PFOS, 13 meta-analysis studies were identified and summarized in Table A-42 in the PFOS Appendix A. EPA incorporated the results of recent meta-analyses as another line of evidence considered in the evidence integration.

Additional analyses or rationales:

Clinical Relevance of the Critical Effect

I.S5B.3 SAB Recommendations:

The Panel recommends that information on infectious disease outcomes be added to the evidence integration section of both documents including strength of evidence conclusions.

The Panel recommends that EPA add clarification and detail to better support the finding of decreased antigen-specific antibody responses as a well-established adverse outcome, in and of itself, even in the absence of definitive evidence of increases in infectious disease/infectious disease risk.

I.E5B.3 EPA Response:

To respond to this comment, EPA added a detailed description of the available evidence for infectious disease outcomes in Section 3.4.2.1.2.2 and the appendices of the *Proposed MCLG* documents. Studies reporting on this effect were used to inform the evidence integration conclusions for the immune health outcome (see 3.4.2.4). As mentioned in EPA's response to SAB recommendations under Charge Question #1, EPA incorporated a systematic evidence integration approach and determined that the endpoint of decreased antibody response to vaccination in children had sufficient weight of evidence to consider quantitatively. Additional rationale supporting the adversity of this endpoint that indicates impacts on the development of the immune system, even in the absence of definitive data collection regarding infectious disease/risk levels, has been added to Section 4.1 of the *Proposed MCLG* documents.

Use of Epidemiological Data Rather than Experimental Animal Data as Basis for RfD

I.S5B.4 SAB Recommendation:

The Panel recommends that EPA provide a clear and thorough discussion of the strengths and limitations of PODs based on studies from both human and experimental animal models in selecting RfDs.

I.E5B.4 EPA Response:
In Section 4 of the *Proposed MCLG* documents for PFOA and PFOS, EPA describes the strengths and limitations for each POD considered for candidate RfD derivation. EPA also discusses the uncertainties associated with the use of both PFOA and PFOS animal toxicological studies and human epidemiological studies as the basis of RfDs, noting that there is greater total uncertainty associated with animal toxicological studies based on the interspecies uncertainty factors (UFs). In Section 6, EPA describes the uncertainties with the use of the available PFOA and PFOS epidemiological studies for quantitative dose-response analyses and compares toxicity values derived from animal toxicological and epidemiological studies, providing potential explanations for the differences in RfD values derived from the two data types.

Application of Toxicokinetic Model

I.S5B.5 SAB Recommendation:
The Panel recommends that candidate RfDs be expressed in internal dose units; these could then be converted either to traditional continuous oral dose units or to drinking water concentration units (e.g., for supporting a MCLG). The latter of which would consider varying exposure factors depending on lifestages. Additional clarification and justification are needed to explain why the approach to convert to a continuous oral dose is adequately protective across lifestages.

I.E5B.5 EPA Response:
To respond to SAB's recommendation, EPA considered expressing RfDs in internal dose units. EPA does provide points of departure in units of internal dose (Table 4-8), from which RfDs are derived using dosimetric extrapolation and application of UFs. EPA applies UFs based on established guidance, which is distinct from the scientific practice of dosimetric extrapolation. UFs have been designed for application to an external exposure, which is consistent with human health risk assessment best practices.

As stated previously in this Response to Comments document under Charge Question #4 - Toxicokinetic Modeling, the MCLG derivation incorporates an estimate of body weight-adjusted drinking water exposure that is based on life-stage-specific drinking water intake per body weight per EPA's *Exposure Factor Handbook* (U.S. EPA, 2019). Since the goal is to protect all ages and subpopulations, the drinking water intake rate is selected for the most sensitive or susceptible life stage based on the available data. Although data gaps can prevent the identification of the most sensitive population (e.g., not all windows of exposure or health outcomes have been assessed for PFOA), the critical effect and point-of-departure (e.g., human equivalent BMD) can inform the identification of sensitive life stages because the critical effect is typically observed at the lowest tested dose among the available data. When multiple potentially sensitive populations or life stages are identified based on the critical effect or other health effects data, EPA selects the life stage with the greatest body weight-adjusted drinking water intake (DWI-BW) to be protective of the most sensitive population that may be exposed. This results in an MCLG value that is protective across life stages, even if the RfD itself is not based on response after exposure to this specific life stage. For endpoints in pregnant women and children, the external RfD reflects the changes in the maternal accumulation of PFOA/S over time and the transfer of accumulated chemical to the offspring through gestational and lactational transfer—this was accounted for in the PK model used to develop POD_{HEDs}.

Ultimately, EPA decided to maintain the units of the RfD as an oral exposure that is constant relative to body weight, a decision that is consistent with current EPA risk assessment practices and used in other risk assessment applications (e.g., human health criteria, regional screening levels (RSLs)). Further consideration of developing the PK model to incorporate life stage information and to derive an MCLG directly is discussed in sections 6.6 and 6.7 of the *Proposed MCLG* documents.

Duration(s) of Exposure to Which RfDs Apply

I.S5B.6 SAB Recommendation:

The Panel recommends that the durations of exposure to which the RfDs apply be clearly stated, with explanatory text. This is critical in addressing situations of drinking water contamination with PFOA and/or PFOS in regard to the timeframe in which intervention is needed. These durations (and corresponding lifestages) should also be considered when implementing the above recommendation to convert RfDs from serum levels to drinking water concentrations.

I.E5B.6 EPA Response:

EPA updated Section 4.1.6 of the *Proposed MCLG* documents for PFOA and PFOS to include the durations of exposure to which the RfDs apply.

Benchmark Dose (BMD) Model and BMD Level

I.S5B.7 SAB Recommendations:

The Panel recommends that EPA provide supplemental data from the Budtz-Jorgensen and Grandjean (2018) publication used for BMD modeling as well the conclusions of EPA's review of the modeling in the publication, and additional rationale for the selection of specific BMDLs from this publication.

Overall, it is essential that details of the BMD modeling that forms the basis of the PODs is transparently available for evaluation of the methods, approaches, and results.

I.E5B.7 EPA Response:

In the *Proposed Approaches* document reviewed by the SAB, EPA used the BMD modeling results from Budtz-Jørgensen and Grandjean (2018) to derive candidate RfDs. Based on recommendations from the SAB, EPA reviewed and reevaluated the modeling from Budtz-Jørgensen and Grandjean (2018) and elected to conduct additional modeling of data from this study, the results of which are provided in Appendix E. EPA conducted modeling of these data using a BMR of 1/2 SD, the rationales for which are also described in the PFOA/PFOS appendices, along with justification for benchmark dose lower bound limit (BMDL) selection. Additional supplemental information obtained from the study authors has been included in Appendix E and was added to HERO (https://hero.epa.gov/hero/index.cfm/search/index; HEROID 10328962, 10328963, 9959716).

Detailed modeling results are provided in Appendix E for all studies considered for POD derivation.

Charge Question #5C - 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.

I.S5C.1 SAB Recommendations:

The Panel recommends that EPA provide a stronger and more transparent justification of BMRs for not only decreased antibody response, but also other endpoints for which BMDs were developed. Ideally, BMR levels correspond to a similar level of adversity or risk across endpoints.

I.E5C.1 EPA Response:

EPA appreciates this comment to increase transparency and consistency of BMR selections. Based on rationales described in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), the IRIS Handbook (U.S. EPA, 2022b), and published EPA assessments, BMRs were selected in a consistent manner for dose-response modeling of PFOA/PFOS-induced health effects for individual study endpoints across studies. EPA describes the selected BMRs for each endpoint along with the rationales for their selection in Section 4 and Table 4-2 of the *Proposed MCLG* documents. EPA conducted sensitivity analyses for some endpoints to compare BMDLs derived using differing BMRs. These comparisons are described in the PFOA/PFOS appendices.

Charge Question #5D - Uncertainty Factors

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

I.S5D.1 SAB Recommendations:
The Panel recommends that EPA acknowledge the approach of considering an extra 10-fold uncertainty factor when developing the MCLG for compounds with "suggestive" evidence of carcinogenicity, for which a slope factor is not available, and explain the rationale for its inclusion or exclusion.

The Panel recommends that EPA consider adoption of a probabilistic framework to calculate risk-specific doses, in particular as to whether applying this approach would be useful for MCLG derivation and/or regulatory impact assessments needed to set MCLs.

The Panel did not reach consensus on methods for accounting for effects of mixtures due to PFOA and PFOS usually occurring with other PFAS but recommends that EPA evaluate the potential applicability of different approaches and their implications for setting MCLGs.

I.E5D.1 EPA Response:
EPA appreciates the SAB panel's comments. Since the draft *Proposed Approaches* documents, EPA reviewed the weight of the evidence for PFOA and PFOS using the MOA framework in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005). EPA's review led to a change; both PFOA and PFOS were determined to be *Likely to Be Carcinogenic to Humans*. Therefore, applying an additional 10-fold safety factor for a suggestive carcinogen is no longer applicable to PFOA and PFOS.

The current EPA human health risk assessment practice is to develop deterministic toxicity values for noncancer health effects (e.g., RfDs; U.S. EPA, 2002). EPA's IRIS Handbook (U.S. EPA, 2022b) summarizes this standard EPA approach. The IRIS Handbook describes how one can estimate the distribution of a probabilistic "risk-specific dose," defined as the dose at which a pre-specified risk occurs and cites a case study application of a World Health Organization/ International Programme on Chemical Safety (WHO/IPCS) probabilistic approach in an IRIS assessment context (Blessinger et al., 2020). However, while this probabilistic approach and others are being explored through case studies and EPA research projects, these methods are not routinely applied in EPA human health assessments of either individual chemicals or mixtures of chemicals. This recommendation is outside the scope of these *Proposed MCLG* documents. EPA proposed several approaches to consider how to best address mixtures, described in the draft *Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS*) that was reviewed by this SAB panel along with the *Proposed Approaches* documents. See additional responses to mixtures approaches in SECTION II - Mixtures approaches of this document.

Charge Question #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.

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.

I.S6.1 SAB Recommendations:
While the Panel supports the selection of an RSC of 20%, the Panel recommends that EPA revise certain aspects of the RSC sections in the draft MCLG documents to better describe and explain the rationale for arriving at an RSC of 20%. This will also help ensure that the selection of the 20% RSC is consistent with the approach provided in the U.S. EPA (2000). Specifically,

- Statements in the draft MCLG documents that suggest that the percent of exposure from drinking water is relevant to RSC selection should be removed.
- The relationship between the selection of the RSC and the numerical value of the RfD should be made clear.
- EPA should consider using available data on non-drinking water exposures to PFOA and PFOS to clearly support the choice of an RSC of 20% for MCLGs based on the RfDs presented in the draft document.
- Similarly, EPA should also explain how an RSC of 20% is supported by data on serum PFOA and PFOS levels from the U.S. general population.
- EPA should clarify the relevance of the RSC selection, or more generally to the MCLG development, to factors such as the protection of disproportionately affected subpopulations and sensitive subpopulations, including infants and children.

I.E6.1 EPA Response:
These comments generally support an RSC of 0.2 for PFOA and for PFOS. In response to SAB recommendations, EPA has revised several portions of the RSC section to be consistent with EPA's Exposure Decision Tree in the 2000 *Methodology for the Derivation of Ambient Water Quality Criteria* (U.S. EPA, 2000b). The revisions include updates to sections describing drinking water exposure and clarification of the relationship between the RSC and RfD. Specifically, EPA clarified that the RSC is intended to ensure that the level of a contaminant in drinking water, when combined with exposure via other identified sources that are common to the population of concern, will not result in exposures that exceed the RfD (U.S. EPA, 2000b).

EPA considered the available data on nondrinking water exposures to PFOA and PFOS for RSC determination, per SAB recommendations. Following a systematic literature search approach, EPA identified potential sources and pathways of PFOA and PFOS exposure, including food and food-contact materials, consumer products, ambient air, and indoor dust. When deriving the RSCs, EPA considered sensitive populations such as children because of their differing sources and routes of exposure (e.g., increased hand-to-mouth contact). Although exposure data is available for pathways other than drinking water, there is not enough information available for

each potential pathway, particularly dust, air, consumer products, and food contact materials, to characterize PFOA or PFOS exposure in the general population or in potentially sensitive populations.

However, two of the recommended bullets above require further discussion. First, EPA does not agree that the percent exposure from drinking water is irrelevant to RSC selection and should be removed. As described in the 2000 *Methodology for the Derivation of Ambient Water Quality Criteria* (U.S. EPA, 2000b), "the RSC describes the portion of the RfD available for [MCLG]-related sources," in this case, drinking water. The goal of the RfD is to "maintain **total exposure** below the RfD (or POD/UF)," and, therefore, the RSC necessarily takes into account exposure from drinking water (U.S. EPA, 2000b). Application of a 20% RSC to the RfD assumes "that the major portion (80%) of the **total exposure** comes from other sources" (U.S. EPA, 2000b). Second, biomonitoring data is not typically used to determine the RSC. While biomonitoring data provides valuable aggregate exposure information, the 2000 *Methodology for the Derivation of Ambient Water Quality Criteria* (U.S. EPA, 2000b) does not describe an approach for deriving RSCs that use serum concentrations from the U.S. general population. To clarify that this information was not used in the derivation of the RSC, the information on biomonitoring data has been moved to Section 1 of the *Proposed Approaches* documents, separate from the discussion of the RSC now located in the PFOA/PFOS appendices.

Importantly, based on further evaluation, both PFOA and PFOS are now designated as "likely to be carcinogenic to humans." As a result, an RSC was not used to derive the MCLGs. An MCLG for linear carcinogens is not determined "with regard to an individual's total risk from all sources of exposure" (U.S. EPA, 2000b). The proposed MCLG is zero—a value that is by definition as protective as possible for all populations, including those disproportionately exposed and sensitive populations such as infants and children. However, as information used to support the RSC is still relevant to noncancer health risks, EPA has retained the discussion on RSC derivation in the PFOA/PFOS appendices. This discussion includes updated text and figures to clarify the selection of an RSC for noncancer effects as recommended by the SAB.

SECTION II - Mixtures approaches

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

Overarching Comments from Final SAB Report (Cover Letter; August 22, 2022)

II.S0.1 SAB Recommendations:
The SAB supports dose additivity based on a common outcome, instead of a common mode of action as a health protective default assumption and does not propose another default approach. However, EPA should more thoroughly and clearly present the uncertainties associated with this approach along with information supporting this approach.

II.E0.1 EPA Response:
These comments generally support basing dose additivity (DA) on a common health outcome. EPA has added more text in the "Dose Additivity for PFAS" section of the *Framework* document to address the SAB's comments related to uncertainties associated with assuming dose addition as the default assumption for assessing PFAS mixtures. EPA has added further discussion on deviations from DA, such as synergy or antagonism, but available evidence suggests that dose addition should be considered the default model.

II.S0.2 SAB Recommendations:
The SAB expressed concern regarding the requirement for "external peer review" of toxicity values developed by states and recommends that this phrase in the draft framework be broadened to recommend the need for scientific input and review in general.

II.E0.2 EPA Response:
In response to this point of clarification, EPA has removed the text related to external peer review. The text now reads, "If *de novo* derivation of toxicity values is necessary, it is recommended that experts in hazard identification and dose response assessment be consulted for scientific input and review, and the associated uncertainties (e.g., data gaps) be transparently characterized."

II.S0.3 SAB Recommendations:
EPA should consider using a menu-based framework to support selection of fit-for-purpose approaches, rather than a tiered approach as described in the draft Mixtures document. Tiered approaches that require increasingly complex information before reaching a final decision point can be extremely challenging for data-poor chemicals such as PFAS.

II.E0.3 EPA Response:
Based on this and other SAB comments, EPA has eliminated the tiered approach and restructured the framework as a data-driven, flexible approach to facilitate PFAS mixture assessment in various decision contexts (e.g., contaminated site, water system) (see Section 4.2 and Figure 4-1 of the *Framework* document). With "fit-for-purpose assessment" in mind, EPA has included a discussion of key steps in the framework, including problem formulation and scoping, assembling information, evaluating data objectives, considering the data landscape to select a component-based approach(es), and performing component-based mixture assessment approach(es) (see Section 4.2.1 of the *Framework* document).

II.S0.4 SAB Recommendations:
EPA should provide clarification regarding the conceptual similarities and differences between the target-organ-specific hazard index (TOSHI) approach, the relative potency factor (RPF) approach, and the mixture benchmark dose (BMD) approach, since all are based on health effect-specific values (i.e., Reference Values (RfVs) or RPFs) for the individual PFAS in the PFAS mixture. More discussion and comparison of approaches, as well as when they converge, is needed. For instance, given the mathematical correspondence between the RPF and mixture BMD approaches, EPA should consider revising the discussion of these two approaches to present them as essentially the same (or highlighting any essential differences), and perhaps also merging them into a single section.

II.E0.4 EPA Response:
EPA has added a new section (Section 8.0 of the *Framework* document) that describes the similarities and differences among the different component-based mixtures assessment approaches. In addition, EPA has made the revision to use the same hypothetical example mixture of five PFAS (ranging from data-poor to well-studied) for all the illustrative examples so that the user can better understand the similarities/differences among the approaches.

II.S0.5 SAB Recommendations:
For both the RPF and mixture BMD approach, EPA's approach would be strengthened by using PODs from animal studies that are based on human equivalent doses (HEDs) rather than administered doses. The SAB found it difficult to envision situations in which the mixture BMD was advantageous; therefore, EPA should provide additional information on how the proposed Mixtures BMD approach will be applied in practice.

II.E0.5 EPA Response:
To respond to this recommendation, text has been added in several places to indicate that it is optimal to calculate and use HEDs rather than the oral-administered dose in test animals where and when possible. This includes additional text that walks the reader through EPA's logic flow for cross-species scaling (see the new Section 5.2.1 of the *Framework* document). Regarding the mixture BMD (M-BMD), text has been added to better articulate when this specific approach is more appropriate (e.g., component chemical data that indicate a common health outcome but with noncongruent dose-response functions). Further, Section 7.3 of the *Framework* document has been revised to reiterate the conditions that warrant consideration of this specific component-based mixtures approach (as opposed to the RPF method).

Charge Question #1- Dose Additivity Assumption

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 a t the level of toxicity endpoint/health effect rather than MOA.

A. *Please comment on the appropriateness of this approach (dose additivity based on common endpoint of toxicity or health effect) for a component-based mixture valuation of PFAS under an assumption of dose additivity.*
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).*

Assumption of Dose Additivity

II.S1.1 SAB Recommendations:

The draft mixtures document presents information supporting the assumption of dose additivity for chemical mixtures in general, including mixtures of PFAS. Several examples are discussed in the draft mixtures document (e.g., dioxin-like chemicals, organophosphate chemicals) that alter shared pathways which typically produce at least dose additive responses. The information included in the draft framework supports the conclusion that toxicological interactions of chemical mixtures are frequently additive or close to additive. It also supports the conclusion that dose additivity is a public health protective assumption that typically does not underestimate the toxicity of a mixture.

II.E1.1 EPA Response:

These comments are generally supportive, and no response is necessary.

II.S1.2 SAB Recommendations:

The SAB Panel agrees with use of the default assumption of dose additivity when evaluating PFAS mixtures that have similar effects and concludes that this assumption is health protective. It is noted that the assumption of dose additivity can provide an estimate of composite effects when individual PFAS are below their NOAELs. However, it is recommended that, when data clearly indicate interactions other than dose additivity, the approach indicated by the data should be used.

It is further noted that the physical-chemical, toxicological, and toxicokinetic properties of PFAS are different than for other classes of chemicals that have been studied as mixtures, and that there are still many unanswered questions about their interactions in mixtures. While the assumption of dose additivity may be reasonable at low concentrations, factors such as competition for transport may result in non-additive interactions at higher concentrations. Information on the doses at which such transitions may occur is needed. The Panel recommends that EPA reevaluate the default assumption of dose additivity as additional data become available.

II.E1.2 EPA Response:

EPA has added the following text in Section 3.4, PFAS Dose Additivity of the *Framework* document: “EPA will continue to review how mixtures of PFAS and other chemicals interact. Dose additivity is proposed as the ‘default’ model and other models will be evaluated when data empirically support or demonstrate significant deviations from dose additivity.”

II.S1.3 SAB Recommendations:
As discussed in the draft EPA mixtures document, a recent EPA Office of Research and Development (ORD) study of PFOA and PFOS (Conley et al. – Appendix A of draft EPA mixtures document) indicates dose additivity for developmental toxicity of these two PFAS in rats. Other studies that indicating a common MOA and dose additivity for PFAS are also reviewed in the draft framework. For example, the draft EPA mixtures document discusses that Wolf et al. (2014) reported additivity for PPAR-α activation in binary mixtures of PFOA and four other PFAS in cultured cells transfected with the mouse or human PPAR-α receptor. While the dose additivity assumption is recommended for the reasons discussed above, the Panel suggests that the discussion of studies of toxicological interactions in PFAS mixtures in the EPA mixtures document be expanded to also include studies that do not indicate dose additivity and/or a common MOA 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.

For example, a recent paper not cited in the draft EPA mixtures document, Marques et al. (2021), 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. Surprisingly, this appears to be the first mammalian study of defined mixtures of PFAS to be published in a peer reviewed journal. 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." The Panel suggests that discussion of this paper be added to the EPA mixtures document. Another recent study, Nielsen et al. (2021), that was not included in the draft EPA mixtures document did not find dose additivity for activation of PPAR-α by PFAS mixtures in cultured cells transfected with a full length human PPAR-α construct. The Panel suggests that discussion of Nielsen et al. (2021) be included in the final EPA mixtures document. Nielsen et al. (2021) found that the potency (EC50) for PPAR-α activation varied among the seven PFAS tested. They also reported that the efficacy (maximal PPAR-α activation compared to positive control) was lower for perfluorosulfonic acids (PFSAs) than for perfluorocarboxylic acids (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 include, Kjeldsen and Bonefeld-Jorgensen (2013) who studied PFAS activation of the estrogen and androgen receptor in a cultured cell line transfected with these

receptors; Ojo et al. (2020) 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 in zebrafish; and Menger et al. (2020) who studied behavioral effects in zebrafish of nine PFAS individually and a mixtures of equal concentrations of all nine PFAS.

II.E1.3 EPA Response:
The Marques et al. (2021) paper was included in the draft document (page 25 of the November 2021 draft *Framework*), and the Nielsen et al. (2022a) paper (cited as Nielsen et al. (2021) in the comment above) had not been published at the time of completion of the initial draft document. Those studies and the others identified in the SAB report have been included in the revised *Framework* document. Importantly, the mammalian PFAS mixture studies that have been published to date (including Marques et al. (2021) and Roth et al. (2021)) did not include individual chemical dose-response data or conduct any evaluation of mixture model predictions (i.e., dose addition versus response addition) compared to observed mixture effects. Thus, conclusions of mixture interactions (e.g., synergy or antagonism) were speculative and not supported by data published in those papers. In regard to Nielsen et al. (2022a), the GCA model of additivity has been developed for use with *in vitro* data that clearly identify full versus partial receptor activity (i.e., agonism or antagonism); however, there are no available data supporting the use of this model in estimating the *in vivo* effects from mixtures exposure. Regarding PFAS mixture studies of zebrafish, it has been clearly reported that fish peroxisome proliferator-activated receptors have low sequence homology to mammalian receptors and do not respond to many ligands that are active in mammalian systems; thus, the relevance of data reported from fish systems to the estimation of human health are unclear for PFAS. Overall, as referenced in Section 3.0, Dose Additivity for PFAS of the *Framework* document, two large systematic reviews published in the literature regarding data on deviations from additivity in published studies have concluded that when interactions occur, they are rarely more than a factor of 2–3-fold difference from simple additivity. These discussions have been added to the *Framework* document (see Section 3.4).

Assumption of Similarity of Toxicity Endpoint Rather than Common MOA in Mixtures Evaluation

II.S1.4 SAB Recommendations:
The Panel agreed with use of a similar toxicity endpoint/health effect instead of a common MOA as a default approach for evaluating mixtures of PFAS. This approach makes sense because multiple physiological systems and multiple MOAs can contribute to a common health outcome. Human function is based on an integrated system of systems and not on single molecular changes as the sole drivers of any health outcome. The Panel concluded that rather than the common MOA, as presented in the EPA draft mixtures document, common physiological outcomes 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 Panel notes that the assumption of dose additivity for chemicals that cause a common toxicological effect through different MOAs is supported by results of a recent study of effects of mixtures of compounds with different MOAs on craniofacial malformations in zebrafish (Van Der Ven et al., 2022) and an accompanying commentary (Kortenkamp, 2022).

Furthermore, many PFAS, including the four used in the examples in the draft EPA mixtures document and others, 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). For clarity, the Panel recommends that the difference between a MOA and a health outcome be defined in the framework. Additionally, when data clearly indicate that an approach based on common toxicity rather than common MOA is not supportable, the approach indicated by the data should be used.

II.E1.4 EPA Response:
These comments support using a similar toxicity endpoint/health effect instead of a common MOA as a default approach for evaluating mixtures of PFAS. EPA has included the definition of MOA and has added text to explain the difference between MOA and health outcome (Section 1.6 and multiple places throughout Section 3 of the *Framework* document).

II.S1.5 SAB Recommendations:
The Panel notes that the U.S. 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 document, and this should be clarified.

Finally, although 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. That being said, dose additivity for a common toxicological effect can still apply even if the MOA for the effect differs among chemicals in a mixture. For completeness, it is the Panel suggests that a summary of information indicating that different MOAs for PFAS be included in the framework. For example, PFOA, PFNA and PFOS cause the same general types of hepatic toxicity. However, as summarized by Post et al. (2017), the hepatic effects of PFOS in rodents appear to be primarily PPAR-α independent (DWQI 2018), while hepatic effects of PFOA (DWQI, 2017) and PFNA (DWQI, 2015) 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 in DWQI, 2015) in mice are PPAR-α dependent, but the developmental effects of PFOS (reviewed in DWQI, 2018) appear to be independent of PPAR-α.

II.E1.5 EPA Response:
The important factor of exposure duration has been added in text throughout Section 4 (e.g., step 2 of the overall approach "Evaluate data objectives" (see Section 4.2.1 of the *Framework* document)). The MOAs for most, if not all, PFAS have not been clearly characterized. The literature indicates that those PFAS that are relatively data rich have multiple operative MOAs with varying potencies across PFAS structures. The revised *Framework* includes a discussion of data that identify similarities and differences in PFAS MOAs (see Section 3), including a discussion of the peroxisome proliferator-activated receptor-alpha (PPAR-α) knockout studies in rodents.

Consideration of Human Data

II.S1.6 SAB Recommendations:

The examples of mixtures assessments provided in the draft framework are based on the four PFAS (PFOA, PFOS, PFBS, GenX) that currently have final EPA Reference Doses (RfDs); all of these RfDs are based on animal data. However, the RfDs for PFOA and PFOS and the cancer slope factor for PFOA in the EPA's draft MCLG documents are based on human data, and additional toxicity factors based on human data may be developed in the future for other PFAS. The Panel suggests that EPA consider how toxicity factors based on human data could be used in evaluations of PFAS mixtures, including for mixtures where toxicity factors for some PFAS are based on animal data and for other PFAS are based on human data.

II.E1.6 EPA Response:

The use of human exposure-response data from epidemiological studies of sufficient quality in human health risk assessment has the advantage of known human relevance. Human dose-response metrics (e.g., PODs and corresponding human health assessment values) can be used directly, as no dosimetric adjustments are required other than for duration (if needed). However, as human exposure-response data are limited for most PFAS, experimental animal data are leveraged where/when available. Because the EPA has existing peer-reviewed guidance supporting the practice of cross-species dosimetric scaling to human-equivalent doses in oral exposure scenarios (U.S. EPA, 2011; see https://www.epa.gov/risk/recommended-use-body-weight-34-default-method-derivation-oral-reference-dose), conversion of animal doses to HEDs should be performed when possible. Integrating PODs or health assessment values across human epidemiological and experimental animal dose-response data can be performed because the animal data are converted to HEDs; as such, in a mixture assessment context, each component chemical's dosimetry is, in essence, a human dose. Text has been added in several places indicating that it is optimal to calculate and use HEDs rather than the oral-administered dose in test animals where and when possible.

Use of NAMs Data in Component-based Mixtures Approaches for PFAS

II.S1.7 SAB Recommendations:

The potential use of data derived from new approach methodologies (NAMs; e.g., high throughput assays, read-across) for hazard identification and dose-response evaluation for PFAS mixtures is mentioned in several places in the draft mixtures framework (p. 12, 27, 34, 37, 52). The Panel agrees with the draft framework's statement (p. 41) that the use of NAMs data would allow for evaluation of toxicity of "data-poor PFAS" detected in environmental media that would not otherwise be considered.

However, current EPA risk assessment guidance does not provide for the use of NAMs data as the basis toxicity factors such as RfDs, and state environmental agencies generally follow EPA risk assessment guidance in developing health-based standards and guidance values for environmental contaminants. Therefore, EPA and states may face difficulties in justifying and implementing standards or guidance values (either chemical-specific or mixture-based) based on NAMs data for contaminants (PFAS or others) in drinking water or other environmental media. Regarding this issue, EPA stated at the SAB Panel meeting on December 16, 2021 that the agency 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. They further 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 document, and the Panel recommends that it be added to the final framework.

II.E1.7 EPA Response:
Various EPA guidance documents (e.g., *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005)) or technical frameworks (e.g., *Human Health Risk Assessment to Inform Decision Making* (U.S. EPA, 2014)) allude to the importance of leveraging alternative toxicity data types such as structure-activity, -omics, and/or computational platforms in hazard identification. However, EPA agrees with the SAB comment that current EPA guidance(s) does not describe or define how these NAM data could be used to inform dose-response assessment (e.g., RfV derivation). It is agreed that such technical guidance is much needed in the toxicology and risk assessment community in general. Clarifying text of "fit-for-purpose" for NAM data has been added in the revised *Framework* document, particularly in sections 1 and 4 of the *Framework* document. Further, conceptual and illustrative (quantitative) application of NAM data has been added to sections 4–7 of the *Framework* document in the context of the three approaches (HI, RPFs, and M-BMD); these additions are intended to provide the reader with some context of how NAM data (like *in vitro* bioactivity) might be envisioned to be applied quantitatively in the future.

II.S1.8 SAB Recommendations:
Additionally, it is important to recognize 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 concern 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. EPA's decision to minimize animal studies in its toxicology research has added to this issue, although a few recent EPA ORD animal studies have yielded high impact, 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) and Conley et al. (2021); Nafion Byproduct 2 - Conley et al., (2021); and mixtures of PFOA and PFOS (the recent studies highlighted in the draft mixtures framework).

II.E1.8 EPA Response:
EPA agrees with the SAB comment that data availability is not unique to PFAS or mixtures. It is recognized that leveraging NAM data across a diverse environmental chemical space will likely increase in human health (and ecological) risk assessment over time. How NAM data are used to inform qualitative hazard identification, and more importantly, quantitative dose-response assessment for single chemicals and mixtures, are key questions that EPA is actively evaluating. EPA agrees that more toxicity data from all assay domains (e.g., whole animal; NAMs) for PFAS, in general, are greatly needed.

Development of Toxicity Factors for PFAS for Which Final EPA Toxicity Factors Are Not Available

II.S1.9 SAB Recommendations:

The draft EPA mixtures document (p. 33, last paragraph) states that toxicity values are needed to address PFAS (and other contaminants) for which final EPA toxicity factors have not been developed. The draft mixtures framework also notes that several states have developed toxicity factors for several PFAS for which there are no EPA toxicity factors (see Post, 2021). As noted in the draft EPA mixtures document, EPA has developed guidance for the derivation of subchronic and chronic oral RfDs, and most or all states follow this EPA guidance.

The SAB Panel agrees with EPA's 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 document and replacing it with, for example, "development of toxicity values should include opportunities for scientific input and review" may be more applicable. 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 independent peer review. In fact, the Minnesota Department of Health oral toxicity values mentioned in the draft mixtures document for potential use in HI calculations (p. 33, first paragraph) did 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 mixtures document 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 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."

II.E1.9 EPA Response:

EPA has removed the text related to "external peer review." The text now reads: "If *de novo* derivation of toxicity values is necessary, it is recommended that experts in hazard identification and dose response assessment be consulted for scientific input and review, and the associated uncertainties (e.g., data gaps) be transparently characterized."

Overall Recommendations

II.S1.10 SAB Recommendations:

The SAB PFAS Review Panel supports dose additivity based on a common outcome, instead of a common mode of action as a health protective default assumption and does not propose another

default approach. However, it is recommended that the uncertainties associated with this approach be more thoroughly and clearly presented along with information supporting this approach. Additionally, for clarity, the difference between a MOA and a health outcome should be defined.

II.E1.10 EPA Response:
See responses to comments under the "Assumption of Dose-Additivity" (II.E1.2 EPA Response:) and "Assumption of Similarity of Toxicity Endpoint Rather than a Common MOA in Mixtures Evaluation" (II.E1.4 EPA Response:) subheadings.

II.S1.11 SAB Recommendations:
The Panel recommends that when data clearly indicate interactions other than dose additivity, the approach indicated by the data should be used and that EPA reevaluate the default assumption of dose additivity as additional data become available.

II.E1.11 EPA Response:
See responses to comments under the "Assumption of Dose-Additivity" (II.E1.2 EPA Response:) subheading.

II.S1.12 SAB Recommendations:
Currently, studies that indicate a common mode of action and dose additivity for PFAS are reviewed in the draft framework. The Panel recommends that the discussion be expanded to include studies that do not indicate dose additivity and/or a common mode of action for PFAS.

II.E1.12 EPA Response:
See responses to comments under the "Assumption of Dose-Additivity" (II.E1.2 EPA Response: subheading.

II.S1.13 SAB Recommendations:
The Panel recommends 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. The Panel recommends that EPA clarify potential uses of NAMs data in PFAS mixtures assessments.

II.E1.13 EPA Response:
See responses to comments under the "Consideration of Human Data" (II.E1.6 EPA Response:) and "Use of NAMs Data in Component-based Mixtures Approaches for PFAS" (II.E1.7 EPA Response:; II.E1.8 EPA Response:) subheading.

II.S1.14 SAB Recommendations:
The Panel expressed concern regarding the EPA's stated requirement for "external peer review" of toxicity values developed by states and recommends that this phrase in the draft framework be broadened to encompass processes that include the need for scientific input and review in general.

II.E1.14 EPA Response:
See responses to comments under the "Overarching Comments" (II.E0.2 EPA Response:) and "Development of toxicity factors for PFAS for which final EPA toxicity factors are not available" (II.E1.9 EPA Response:) subheadings.

Charge Question #2 - Hazard Index Approach

Section 4.3 (Hazard Index, HI) of the framework 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.*

II.S2.1 SAB Recommendations:
The Panel agrees with the use of Hazard Index (HI) as a screening method and decision-making tool (see "limitations" discussion below).

In general, the screening level Hazard Index (HI) approach, in which Reference Values (RfVs) 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. Toxicological studies to inform human health risk assessment are lacking for most members of the large class of PFAS, and mixtures of PFAS that commonly occur in environmental media, overall. For these reasons, 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 dose additivity (DA) that has been validated and used by EPA. The HI does not provide quantitative risk estimates (i.e., probabilities) 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 draft framework, this approach has advantages and limitations that were adequately described.

II.E2.1 EPA Response:
These comments are generally supportive of EPA's approach. No response is necessary.

II.S2.2 SAB Recommendations:
The Panel also found that the approaches described in the draft framework would be better described and used as a menu-based approach rather than a tiered one. Given the agency's desire to support fit-for-purpose approaches, not every PFAS mixture scenario will be one that warrants a tiered or hierarchical approach. In some instances, an HI or target-organ-specific hazard indices (TOSHI) might provide enough information for decision-making about PFAS (or other chemicals) contamination in drinking water (or other media). Tiered approaches that require

increasingly complex information before reaching a final decision point can be extremely challenging for data poor chemicals such as PFAS. Data gaps identified in a such tiered methodologies could result in a bottleneck through which these chemicals may never emerge.

II.E2.2 EPA Response:
Based on this and other comments, EPA has eliminated the tiered approach from the revised document and restructured the framework as a data-driven, flexible approach to facilitate PFAS mixture assessment in various decision contexts (e.g., contaminated site, water system) (see Section 4.2 and Figure 4-1 of the *Framework* document). With "fit-for-purpose assessment" in mind, EPA has included a discussion of key steps in the framework, including problem formulation and scoping, assembling information, evaluating data objectives, considering the data landscape to select a component-based approach(es), and performing a component-based mixture assessment approach(es) (see Section 4.2.1 of the *Framework* document).

II.S2.3 SAB Recommendations:
However, it is important to recognize that the information provided for decision-making by HI and TOSHI (e.g., that a mixture poses a potential risk) differ from the quantitative toxicity information provided by more refined approaches such as relative potency factor (RPF).

II.E2.3 EPA Response:
EPA added language throughout the *Framework* document to draw distinction between the HI and RPF approaches; in particular, see steps 4a and 4b in Section 4.2.1 as well as the new Section 8.0.

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

Lack of Toxicology Data

II.S2.4 SAB Recommendations:
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 draft framework. Additional guidance will likely be necessary for most users.

II.E2.4 EPA Response:
Based on this and other SAB recommendations, a new data-driven framework has been added in the revised *Framework* document that replaces the tiered approach and provides the user some context for decision-making in situations when formal human health assessments are not available for mixture PFAS. While the HI necessitates availability, or *de novo* derivation, of toxicity values, the RPF (Section 6.0) and M-BMD (Section 7.0) approaches do not. Rather, these two approaches only require that dose-response data are available for mixture components. As such, the new data-driven framework provides opportunities for users to leverage data without the formal derivation of toxicity values.

Use of TOSHI Approach

II.S2.5 SAB Recommendations:

The TOSHI approach necessitates endpoint/health effect-specific reference values, not just overall reference values. Therefore, the draft framework should be clearer in explaining that endpoint/health effect-specific reference values must be developed for individual PFAS. 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 approach 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.

II.E2.5 EPA Response:

The issue raised in this comment has been addressed through the removal of the prior version's tiered approach and the addition of the newly developed data-driven framework. The General HI and TOSHI are simply variations of the HI and can each be applied depending on data availability. As such, they are no longer presented as equivalent (tiered) approaches but rather are options in a HI domain based on data availability across one or more health outcome domains. Further, the revised TOSHI subsection better articulates and illustrates how the approach works; it uses the same hypothetical five-component PFAS mixture as used in the general HI approach (as well as the RPF and M-BMD methods).

Consideration of Probabilistic Methods for HI/TOSHI Calculations to Estimate Risk

II.S2.6 SAB Recommendations:

The National Academies of Sciences, Engineering, and Medicine (NASEM, 2021) review of the IRIS Handbook recently endorsed the IRIS program's development of probabilistic risk-specific doses to replace traditional deterministic reference values. In the future, 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."

II.E2.6 EPA Response:

EPA's current human health risk assessment practice is to develop deterministic toxicity values for noncancer health effects (e.g., RfDs; U.S. EPA, 2002). EPA's IRIS Handbook (U.S. EPA, 2022b) summarizes this standard EPA approach. The IRIS Handbook does describe how one could estimate the distribution of a probabilistic "risk-specific dose"—defined as the dose at which a pre-specified risk occurs—and cites a case study application of a WHO/IPCS probabilistic approach in an IRIS assessment context (Blessinger et al., 2020). However, while this probabilistic approach and other approaches are being explored through case studies and EPA research projects, these methods are not routinely applied in EPA human health assessments of either individual chemicals or mixtures of chemicals.

Challenges with Implementation

II.S2.7 SAB Recommendations:

An HI and TOSHI do not provide quantitative estimates of risks associated with PFAS mixtures in a given exposure (see discussion of probabilistic methods above that could provide risk estimates). Nonetheless, these approaches could be useful for categorizing a specific mixture as to its potential hazard. Additionally, HI/TOSHI estimates need to be interpreted with caution in that different mixture exposure scenarios that contain the same chemicals may result in the derivation of identical HIs/TOSHIs. However, due to factors specific to each exposure scenario, they may not necessarily exhibit the same potential for causing adverse health effects.

II.E2.7 EPA Response:

The challenges in interpreting HI/TOSHI estimates provided in the comment are clear. The nuances and issues raised in the comment have been summarized in the new Section 8.0 and in sections 5.4 and 5.5 of the revised *Framework* document.

II.S2.8 SAB Recommendations:

Another disadvantage of the HI/TOSHI approach for 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 chemical-specific toxicity values (e.g., Reference Doses). As shown in Table 4-3 (p. 39) of the draft EPA mixtures document, development of health-based water concentrations (HBWCs) requires chemical-specific toxicity values) and chemical-specific exposure assumptions (e.g., ingestion rates, Relative Source Contribution factors). Additionally, HBWCs may apply to different exposure durations (i.e., short-term, subchronic, chronic). The draft framework 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 document) are the U.S. EPA (2016) Health Advisories (Has) for PFOA and PFOS (USEPA, 2016a; USEPA, 2016b). 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 PFBS or GenX are present in breastmilk.

II.E2.8 EPA Response:

When developing HBWCs, the goal is to protect all ages of the general population, including potentially sensitive populations or life stages (e.g., children). The approach to select the DWI-BW rate for the HBWC includes a step to identify the sensitive population(s) or life stage(s) (i.e., populations or life stages that may be more susceptible or sensitive to a chemical exposure) by considering the available data for the contaminant. Although data gaps can make it difficult to identify the most sensitive population (e.g., not all windows or life stages of exposure or health outcomes may have been assessed in available studies), the critical effect and POD that form the

basis for the RfD can provide some information about sensitive populations because the critical effect is typically observed at the lowest tested dose among the available data. Evaluation of the critical study, including the exposure interval, may identify a particularly sensitive population or life stage (e.g., pregnant women, formula-fed infants, lactating women). In such cases, the user can select the corresponding DWI-BW for that sensitive population or life stage from the Exposure Factors Handbook (U.S. EPA, 2019) to derive the HBWC. In practice, when multiple populations or life stages are identified based on the principal study design and critical effect or other health effects data (from animal or human studies), EPA selects the population or life stage with the greatest DWI-BW because it is health-protective of the most sensitive population. This approach improves the protection of all populations and life stages at the HBWC. In the case of the HI approach, each component hazard quotient (HQ) and the overall HI are protective of all populations and life stages. In the absence of information indicating a sensitive population or life stage, the DWI-BW corresponding to all ages of the general population may be selected (see Table 5-4 in the revised *Framework* document).

II.S2.9 SAB Recommendations:
Additionally, the U.S. EPA (2016a; 2016b) 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 use HBWCs.

II.E2.9 EPA Response:
EPA has clarified that HIs should be derived using HBWCs that are based on the same exposure duration. The example HBWCs provided in Section 5.2.2 of the *Framework* document are derived using chronic oral RfDs; thus, they are considered health-protective values for *a lifetime* of exposure.

II.S2.10 SAB Recommendations:
In the example in Table 4-4 of the draft framework, the individual concentrations for PFOA and PFOS are of 20 ng/L for each which is 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 of the draft framework, the individual concentrations of PFOA and PFOS of 400 ng/L exceed the HBWC 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 health advisory (HA)), yet the combined HI that considers both PFAS exceeds 1. For example, 40 ng/L for PFOA and 50 ng/L for PFOS.

II.E2.10 EPA Response:
Thank you for the suggestion. EPA has developed an illustrative example of five PFAS with varying levels of available data that are now included in the revised *Framework* document. In the HI example (Table 5-6 in Section 5.2 of the *Framework* document), PFAS 2 and 5 have HQs of 0.9 and 0.3, respectively. In isolation, these component PFAS would be below levels of concern (i.e., HQ < 1.0), but when combined, the HI would be 1.2, which exceeds 1.0, indicating risk.

Limitations

II.S2.11 SAB Recommendations:

There are some limitations and potential complications in terms of the intended users such as states and public water systems applying this framework in the 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 National Primary Drinking Water Regulations (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.

II.E2.11 EPA Response:

Based on this and other SAB comments in this report (e.g., see "Overarching Comments from Final SAB Report (Cover Letter; August 22, 2022)"), the tiered approach has been replaced with a data-driven/menu-based framework for the selection of component-based approaches for PFAS mixture assessment. As such, the interpretation of any approach as being "screening" or preliminary has been minimized by the newly proposed framework in the revised document. The framework and example calculations that are presented in the revised document are intended to demonstrate the data-driven application of EPA's component-based mixture methods based on gradations of data availability and completeness, which are anticipated to occur in real-world scenarios for PFAS. While the examples provided are focused on drinking water, the approaches described in the framework could also be applied to other environmental media with oral exposure routes (e.g., soil, fish/shellfish, food); therefore, they are not intended to be limited to drinking water-related actions. For information related to EPA's proposed NPDWR for PFAS, please see Docket # EPA-HQ-OW-2022-0114.

II.S2.12 SAB Recommendations:

The equivalency of HI calculations using the different categories of toxicity assessment information available as presented in Table 4-2 should be clarified. 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.

II.E2.12 EPA Response:

To respond to this comment, EPA revised the *Framework* to include an illustrative hypothetical PFAS example in which data availability across the component chemicals were purposefully designed to span data-poor to data-rich. The objective of the example is to clearly communicate how to select a component-based approach(es) for a given mixture assessment, based on data availability for individual PFAS.

Overall Recommendations

II.S2.13 SAB Recommendations:

The Panel recommends that EPA consider using a menu-based approach rather than a tiered approach as described in the draft Mixtures document. Tiered approaches that require increasingly complex information before reaching a final decision point can be extremely challenging for data-poor chemicals such as PFAS.

II.E2.13 EPA Response:

In response to this and other SAB recommendations, EPA has eliminated the tiered approach and restructured the framework as a data-driven, flexible approach to facilitate on-the-ground PFAS mixture assessment in various contexts (e.g., contaminated site, water system) (see Section 4.2 and Figure 4-1 of the *Framework* document). With "fit-for-purpose assessment" in mind, EPA has included a discussion of key steps in the framework, including problem formulation and scoping, assembling information, evaluating data objectives, considering the data landscape to select a component-based approach(es), and performing a component-based mixture assessment approach(es) (see Section 4.2.1 of the *Framework* document).

II.S2.14 SAB Recommendations:

In the future, 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.

II.E2.14 EPA Response:

See the responses to the comment under the "Consideration of probabilistic methods for HI/TOSHI calculations to estimate risk" (II.E2.6 EPA Response:) subheading.

II.S2.15 SAB Recommendations:

The Panel recommends that EPA provide additional clarity and guidance for implementing the framework to mitigate any inadvertent uncertainties, such as use different ingestion rates when basing mixtures assessments on HBWCs.

II.E2.15 EPA Response:

In response to this recommendation, EPA has added a lengthy discussion about the selection of exposure factors and relative source contribution for HBWC development (Section 5.2.2 of the *Framework* document).

Charge Question #3 - Relative Potency Factor

*Section 4.4 (**Relative Potency Factor, RPF**) of the framework 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.*

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

II.S3.1 SAB Recommendations:
The draft EPA mixtures document describes three different approaches for assessing mixtures. The Hazard Index (HI), Target-Organ Specific HI (TOSHI), and Benchmark Modeling (BMD) that lead to either the derivation of Relative Potency Factors (RPFs) and Internal Chemical Equivalency Concentrations (ICECs) or a mixture BMD. RPFs quantify relative potencies of substances with respect to an effect and can be used to express combined exposures of multiple substances in terms of the exposure value of the chosen index substance (i.e., as index substance equivalents) (MCRA 9).

To summarize, the Panel agreed that the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS, and did not suggest an alternative methodology. The Panel noted that the RPF approach is a more data intensive approach, as compared to the Hazard Index methods, which is likely to see a greater application for PFAS. They expressed concern that there are many PFAS with little or no data and an approach is needed to address mixtures where comprehensive datasets do not exist. The Panel agreed that the EPA should reconsider the tiered approach that is presented in Figure 4-1 of the draft mixtures document. They also noted that New Approach Methods (NAMs) may be useful in filling data gaps for some PFAS given the large number of these substances that lack data.

II.E3.1 EPA Response:
Based on multiple SAB recommendations in this report (e.g., see "Overarching Comments from Final SAB Report (Cover Letter; August 22, 2022)"), the tiered approach has been replaced with a data-driven/menu-based framework for the selection of component-based approaches for PFAS mixture assessment. The revised *Framework* document also includes a detailed presentation of the component-based analysis of a hypothetical PFAS mixture, including the application of NAM data (e.g., *in vitro* cell bioactivity) for data-poor PFAS.

II.S3.2 SAB Recommendations:
The Panel concluded that the framework needs further elaboration and clarification before it can be implemented. Firstly, the draft framework delves into high level details about the various methodologies proposed without substantial discussion of the methodologies (e.g., HI, TOSHI, RPF, BMD) until later in the framework. Secondly, the Panel noted that it would be helpful to have guidance about the types of data sets are most applicable for each approach. Alternatively, an additional figure/figures could be included with a flow diagram as provided in Figure 4-1, beginning with a particular type of data set and then providing guidance on which methodology to use. As discussed in the response to Charge Question 1, the Panel also agreed that the draft framework should re-evaluate the tiered approach as they questioned whether HI or TOSHI is needed before moving on to RPF and suggested using a menu of options based on the available data instead. Overall, there was agreement that removal of tiers would enhance the framework.

II.E3.2 EPA Response:
Based on this and other comments in this SAB review report (e.g., see "Overarching Comments from Final SAB Report (Cover Letter; August 22, 2022)"), the tiered approach has been replaced with a data-driven/menu-based framework for the selection of component-based approaches for

PFAS mixture assessment. EPA agrees with the SAB recommendation and recognizes the need to provide better context for the application of each component-based method (e.g., HI/TOSHI, RPF, and M-BMD). To address this recommendation, significant additional text has been added that more transparently details the basic tenets of each method, including the data objectives and strengths and limitations of each, but more importantly, an illustrative example application of each method using the same five-component hypothetical PFAS mixture. As a result of these revisions, the reader can now follow how the baseline dataset for each PFAS is used or considered in each approach in the revised *Framework* document.

II.S3.3 SAB Recommendations:
The Panel suggested that it would be helpful if the draft framework provided clarification regarding the conceptual differences between the TOSHI approach and the RPF approach, since both are based on health effect-specific values (i.e., RfVs or RPFs) for the individual PFAS in the PFAS mixture. Moreover, the Panel agreed that 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 unnecessary (e.g., if one is supposed to be more "conservative" for screening, rather than more "predictive.").

II.E3.3 EPA Response:
Based on this comment and others, EPA has added a section describing similarities and differences among all the approaches (see Section 8.0 in the *Framework* document). Regarding the HI/TOSHI and RPF approaches, one key difference is the application of UFs. As the HI or TOSHI methods necessitate the use of RfVs, the use of UFs will always be a consideration. That is, while the PODs used in the HI and RPF methods for any two mixture components could be identical, the application of UFs may not be the same. Thus, the ratio of PODs might be the same across methods, but it is not accurate to say that the HI/TOSHI and RPF methods would give the same result (unless the composite UF applied to any two components (with the same POD) is identical). Further, the HI/TOSHI method necessitates the use of RfVs based on PODs; in contrast, the RPF method is flexible because any point along the dose-response continuum (e.g., dose response effective dose 50% (ED_{50}), dose response inhibitory concentration 50% (IC_{50}), lowest observed adverse effect levels (LOAELs), BMDs) may be used.

II.S3.4 SAB Recommendations:
Overall, the Panel concluded that more discussion and comparison of approaches, as well as when they converge, is needed. As noted below in Charge Question #4, an example was provided demonstrating that the RPF and mixture BMD approaches can be very similar or even equivalent, indicating that differences between them should not be exaggerated. The draft framework should provide further discussion and explanation focusing on this concern. Lastly, the framework should give guidance on the approaches, and which approach is preferable- a more conservative versus a predictive one.

II.E3.4 EPA Response:
EPA responded to this recommendation by adding a discussion that compares the HI/TOSHI vs. RPF. vs. M-BMD approaches throughout the revised *Framework* document (e.g., new Section 8.0). Further, text has been added in several places that delineates when the RPF method is optimal (e.g., same shape and general slope of the dose-response functions across mixture

components) versus when the M-BMD method is optimal (e.g., when dose-responses across components are not congruent). Therefore, there should not be an opportunity for convergence across methodology using the same mixture component datasets. In the revised framework, language was added to advise the user to apply each method where possible, contingent upon the characteristics of the available mixture component dose-response data.

II.S3.5 SAB Recommendations:
The Panel also agreed that the framework should increase the discussion, rationale, and justification with regard to Index Chemical (IC) selections. The Panel suggested that the framework would be strengthened by providing a flowchart or process in which the IC can be determined. An example appreciated by the Panel was the comparison of PFOA to PFOS as the IC versus PFOS to PFOA.

II.E3.5 EPA Response:
To respond to this recommendation, EPA augmented text in Section 6.1 of the *Framework* document with additional details regarding the selection of an IC. It is beyond the scope of the *Framework* document to provide a formalized process for IC selection; indeed, it would be beyond what is even provided in EPA Mixtures Guidance (i.e., Section 4.4.2.4.1 of the EPA's 2000 *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000a)). The *Framework* description presented in Section 6.1 provides clear data objectives associated with selecting an IC.

II.S3.6 SAB Recommendations:
Lastly, the RPF approach is based on the assumption 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 mixtures framework 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. (2021), 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.

II.E3.6 EPA Response:
EPA agrees with the SAB that the application of the component-based methods contained in the framework should employ HEDs in all instances where/when possible.

<u>Overall Recommendations</u>
II.S3.7 SAB Recommendations:
The Panel recommends that the framework provide further elaboration and clarification before it can be implemented, including providing guidance about the types of data sets that are most applicable for each approach.

II.E3.7 EPA Response:
See response II.E3.2 EPA Response:.

II.S3.8 SAB Recommendations:
The Panel recommends that the draft framework provide clarification regarding the conceptual similarities and differences between the TOSHI approach and the RPF approach, since both are based on health effect-specific values (i.e., RfVs or RPFs) for the individual PFAS in the PFAS mixture. Therefore, more discussion and comparison of approaches, as well as when they converge, is needed.

II.E3.8 EPA Response:
See response II.E3.3 EPA Response:.

II.S3.9 SAB Recommendations:
The Panel recommends that the framework increase the discussion, rationale, and justification with regard to Index Chemical (IC) selections

II.E3.9 EPA Response:
See response II.E3.5 EPA Response:.

Charge Question #4 - Mixture BMD

*Section 4.5 (**Mixture BMD**) of the framework 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.

II.S4.1 SAB Recommendations:
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.

In general, the Panel agreed that the Mixture BMD approach is a reasonable methodology for estimating a mixture-based POD.

II.E4.1 EPA Response:
This comment generally supports EPA's approach. No response is necessary.

<u>Relationship to Other Approaches</u>
II.S4.2 SAB Recommendations:
While the mixture BMD approach was deemed reasonable, some caveats were identified. As with the RPF approach, the framework should also discuss scenarios in which the TOSHI and Mixture BMD approach will give essentially the same answer. That is, when the ratio of the BMD values used to calculate the mixture BMD is equal to the ratio of the endpoint-specific RfD values used to calculate the HI. Also, the extent to which this consistency is appropriate or inappropriate should be clarified (e.g., if one approach is intended to be more "conservative" for screening, rather than more "predictive.").

II.E4.2 EPA Response:
In response to the SAB recommendations, a summary section (Section 8.0) has been added to clarify some of the differences between the approaches described in the *Framework* document. The HI and TOSHI approaches both provide an estimate of risk based on the specific POD for each component PFAS in the mixture, whereas the RPF and BMD approaches provide for a mixture dose-response function for estimating a continuum of effect levels for a given exposure level of the mixture.

II.S4.3 SAB Recommendations:
Further, the RPF and mixture BMD approaches appear to be very similar or even equivalent; differences between them should not be exaggerated. Both approaches appear to be a summary measure of the toxicity of a mixture, ICECMIX for the RPF approach and tadd for the mixture BMD approach. Both approaches are weighted sums of the component concentrations, with weights proportional to some measure of toxicity (e.g., inverse of BMD or of ED10).

II.E4.3 EPA Response:
The document has been revised to address this recommendation by including the important distinction that the RPF and M-BMD approaches provide equivalent results, but only when the dose-response slope factors are congruent across component PFAS in the mixture. The RPF approach requires congruent slopes, whereas the M-BMD does not; when the component chemicals in the mixture display disparate slope factors for the common effect, the assumptions of RPF are violated, and the risk estimates differ from the M-BMD approach. An example of this discrepancy in model assumptions has been added to Section 7.1, and the assumptions of the two approaches regarding slope have been clarified throughout the *Framework* document. Further, the conditions under which RPF and M-BMD provide similar or different risk estimates are summarized in Section 8.0 of the *Framework* document.

II.S4.4 SAB Recommendations:
Comparing these results shows that ICECMIX and (tadd)-1 differ only in inessential details and are essentially proportional to one another, as follows: It is first noted that the RPF approach can use any common toxicity value, e.g., one can replace ED10 with BMD in equation (1). Second, di (the "component chemical's concentration") and ai ("the fixed proportions of the component PFAS in the mixture") are either identical or strictly proportional to each other. Third, ICECMIX includes a constant proportionality factor (ED10IC).

Having constructed ICECMIX and tadd, one can presumably use them to evaluate a risk in analogous ways. The summary tadd 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 ICECMIX by ED10IC to calculate a hazard index or use it in a dose-response function for the index chemical as in equation (4.4).

Given these mathematical correspondences, EPA should consider revising the discussion of these two approaches to present them as essentially the same (or highlighting any essential differences), and perhaps also merging them into a single section.

II.E4.4 EPA Response:
See response II.E4.3 EPA Response:.

II.S4.5 SAB Recommendations:
Utility of the mixture BMD. The draft framework did not clearly present the practical utility of this approach as compared to other mixture approaches, and the Panel found it difficult to envision situations in which the mixture BMD was advantageous. 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 SAB meeting on December 16, 2021, 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. The method as described in the draft mixtures 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 the inclusion of the possibility of NAMs filling data gaps was suggested, for thyroid and developmental endpoints, current NAMs are quite limited and thus could be a limiting factor in the use of this method. The proposed approach would also benefit from additional information on how the method will be applied in practice, e.g., whether for specific mixtures, i.e., those that are found in a specific location or water system or whether 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). Development of 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.

II.E4.5 EPA Response:
The BMD approach is appropriate for use when component PFAS in the mixture have *noncongruent* dose-response slope factors for the common effect. Congruent dose-response slopes are required for RPF but not for BMD; thus, the BMD is expected to provide a more accurate estimate of risk when component chemicals display disparate slopes. This distinction has been clarified throughout the *Framework* document, and a demonstration of the different mixture response functions using the different models has been added to Section 7.1 of the *Framework* document for clarification. Other than the difference in the assumptions of component chemical dose-response functions, the two methods can be applied to water samples with quantified levels of PFAS and appropriate toxicity data for modeling equivalent BMD points. The resulting M-BMD can then be converted to an HBWC to which the original sample is compared (i.e., the site/sample data informs the ratio of PFAS in the mixture and, in turn, is the value for comparing the resulting M-BMD-based HBWC). Regarding NAMs, EPA included a hypothetical example of five PFAS in a mixture, including a data-poor chemical for which NAMs data are leveraged (“PFAS 5”).

II.S4.6 SAB Recommendations:
In general, the Panel agreed that the approach is scientifically supported for PFAS mixture risk assessment, and that both its criteria for application and its potential limitations are well described. Throughout the draft framework for PFAS, the EPA clearly explained the BMD process and approach and appear to have followed the basic recommendations in the EPA’s Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures (2000).

II.E4.6 EPA Response:
This comment generally supports EPA's approach. No response is necessary.

II.S4.7 SAB Recommendations:
The Panel noted 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. Case studies that illustrate these points, using real-world scenarios, could be useful in highlighting this change.

II.E4.7 EPA Response:
In response to the SAB's advice, this section has been edited to clarify that POD_{HED}s should be used in calculating the M-BMD, similar to the approach for RPF. Text has been added in several places to indicate that it is preferable for comparison purposes to use HEDs rather than oral mg/kg-day doses administered to test animals, if HEDs are available. Where applicable, all illustrative examples in the revised document portray calculation of mixture assessment metrics (e.g., HQs, RPFs, M-BMDs) using HEDs.

Overall Recommendations

II.S4.8 SAB Recommendations:
The Panel recommends that additional information on how the proposed Mixtures BMD approach will be applied in practice be provided.

II.E4.8 EPA Response:
Based on this comment, the M-BMD of the *Framework* document (Section 7.0) has been expanded to include more detail regarding the assumptions of this approach as compared to RPF and an example of how it can be applied in practice.

II.S4.9 SAB Recommendations:
The Panel recommends that PODs based on HEDs be used in the Mixture BMD approach and EPA should clarify this with case studies that illustrate these points, using real-world scenarios to highlight this change.

II.E4.9 EPA Response:
The illustrative example Section 7.2 has been edited to demonstrate the use of POD_{HED}s in the risk-based calculation.

II.S4.10 SAB Recommendations:
Finally, the Panel also included in Appendix B of this report specific areas needing clarification in the PFAS Mixtures document.

II.E4.10 EPA Response:
The areas needing clarification that were identified by the SAB panel in Appendix B of the *Framework* document have been addressed.

SAB Appendix B Comments

II.SB.1 SAB Recommendations:

p. 4, last paragraph. Suggest adding that PFAS salts fully dissociate within the body, as well as in the environmental media mentioned here.

p. 5, last paragraph. Suggest also citing more recent studies that include emerging PFAS such as the study of PFAS in the Cape Fear River in NC. (McCord, J., & Strynar, M. 2019. Identification of Per- and Polyfluoroalkyl Substances in the Cape Fear River by High Resolution Mass Spectrometry and Nontargeted Screening. Environmental science & technology, 53(9), 4717–4727).

p. 6, second full paragraph. Suggest including statewide NJ study of PFAS in fish. (Goodrow *et al.* 2020. Investigation of levels of perfluoroalkyl substances in surface water, sediment and fish tissue in New Jersey, USA. The Science of the total environment, 729, 138839).

p. 7, last paragraph. PFOS and PFHxS were phased out prior to the EPA PFOA Stewardship agreement. See EPA press release (May 16, 2000) at https://archive.epa.gov/epapages/newsroom_archive/newsreleases/33aa946e6cb11f35852568e1005246b4.html and Butenhoff at al. (2009): "Between the years 2000 and 2002, due to persistence and evidence of widespread exposure of the general population, 3M Company discontinued production of PFHxS along with perfluorooctanoate (PFOA) and chemistries based on perfluorooctanesulfonyl fluoride, including perfluorooctanesulfonate (PFOS)" at https://www.sciencedirect.com/science/article/abs/pii/S0890623809000173?via%3Dihub.

p. 7, last paragraph. When mentioning that the EPA PFOA Stewardship Program includes "higher homologues," suggest specifically mentioning that this includes PFNA and longer-chain PFCAs, since it is has often been incorrectly stated that PFNA is a "replacement" for PFOA.

p. 8, first paragraph and Table 4, including footnote c on p. 9-10. The information about New Hampshire using the EPA Health Advisory of 70 ng/L for PFOA and PFOS combined as of July 2021 is not correct. Although there was a court injunction that stopped NH from enforcing the lower MCLs that it had developed, the state legislature adopted the lower MCLs into law in July 2020 and the court injunction is no longer in effect. NH is currently implementing the MCLs that it developed for PFOA, PFOS, PFNA, and PFHxS individually. See https://www.jdsupra.com/legalnews/update-on-new-hampshire-pfas-standards-68854/ and https://www.seacoastonline.com/story/news/2020/09/04/judge-rules-for-nh-in-3mrsquos-bid-to-block-pfas-protections/42435483/.

p. 8, last paragraph. Although it does not apply specifically to drinking water, it is suggested that the EFSA (2020) Tolerable Weekly Intake for the total concentration of PFOA, PFOS, PFNA, and PFHxS be mentioned here. See https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2020.6223.

p. 23, last paragraph. It is unclear what "other straight chain compounds" means since PFOA and PFOS, mentioned earlier in the sentence, can exist as linear and branched isomers. Suggest rewording to "other perfluoroalkyl acids" which refers to other PFCAs and PFSAs of longer and shorter chain length.

p. 24, last paragraph. Lau *et al.* (2006) should be cited along with the other studies that show that developmental exposure to PFOA causes reduced survival/viability and reduced body weight in offspring.

p. 36, second to last line. "…judged to…" should be "…judged adequate to…"

p. 37, 2nd full paragraph and two flow diagrams (Bioactivity-based, Read-across) that follow. It is stated that the information shown is "currently, the general process" for developing RfVs from NAMs data. It is not clear if this information and the diagrams come from another source or if they were developed for the draft EPA mixtures framework document. If they come from another source, a citation should be provided. If they were developed for this draft document, this should be clearly stated.

p. 42, second paragraph. It is mentioned that EPA has applied the RPF approach to disinfection byproducts. Can a citation be provided?

II.EB.1 EPA Response:
Thank you for the corrections. For all editorial recommendations above, the *Framework* document was revised as suggested. Additionally, EPA added or removed references as recommended by the SAB.

SECTION III - Benefits from CVD reduction

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

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.

III.S0.1 SAB Recommendations:
The Panel recommends that EPA provide a clearer rationale and list the main assumptions at the outset before launching into the considerable detail that follows.

The Panel recommends more discussion as to the rationale for selecting this particular endpoint for risk reduction analysis (e.g., strength of the 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), as well as considering risk reduction analyses for other endpoints. The SAB also recommended that EPA ensure that recommendations for the draft MCLG documents relating to evidence identification and synthesis are applied to the CVD endpoint.

While recognizing the need to manage the complexity and volume of results, the Panel recommends performing a sensitivity analysis for each step from beginning to end.

III.E0.1 EPA Response:
EPA has provided the key analytical assumptions within the Introduction of the Economic Analysis for the proposed rule (see Section 2.2 of the Economic Analysis). Additionally, EPA included a comprehensive summary of all limitations and uncertainties associated with benefits analysis for the proposed rule (see Section 6.8 of the Economic Analysis).

In response to the comments requesting that EPA describe the rationale for selecting CVD and other health endpoints in its benefits analysis, EPA has summarized its decision-making process for identifying health endpoints that can be considered in quantitative benefits analysis (see Chapter 6 of the Economic Analysis for the proposed rule). Moreover, EPA has provided a robust discussion of adverse health effects associated with PFAS exposure in the Economic Analysis for the proposed rule and has provided a clear rationale for the health effects selected for valuation (see Section 6.2 of the Economic Analysis for the proposed rule for the complete assessment of adverse effects considered in health-risk-reduction analyses). The adverse health effects summary in the Economic Analysis aligns with the MCLG documents' summaries of the adverse effects and weight/strength-of-evidence conclusions. Based on SAB feedback on the draft MCLG documents' assessment of CVD-related risks, EPA has developed an RfD for TC within the proposed MCLG documents. All studies in EPA's CVD benefits analysis were evaluated for ROB, selective reporting, and sensitivity as applied in EPA's MCLG documents. The derivation of an RfD for this endpoint and the alignment of evidence identification and

synthesis for all investigated health effects addresses the SAB's concerns about the inconsistency between the two documents.

Based on SAB feedback, EPA expanded its documentation and conducted additional sensitivity analyses to evaluate the impact of the inclusion or exclusion of certain studies in the meta-analyses of exposure-response estimates. Based on recommendations from the SAB, EPA also assessed the inclusion of HDLC effects in a sensitivity analysis. Further, EPA expanded its documentation and conducted additional sensitivity analyses to assess the effects of using a key single study approach versus the meta-analysis approach to inform the exposure-response estimates (see Appendix K of the Economic Analysis for the proposed rule for additional detail on the methodology and results of EPA's sensitivity analyses).

Charge Question #1 - EPA's Meta-Analysis

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.*

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.*

III.S1.1 SAB Recommendations:

The recommendations from Section I (MCLG documents) with respect to evidence identification and evaluation should also be applied here where applicable.

The Panel recommends that EPA list the studies that were excluded from the meta-analysis and provide a brief description of these studies and explain why each was excluded.

The Panel recommends that the evidence synthesis protocol 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.

The Panel recommends that EPA provide additional discussion as to the rationale for excluding HDLC from the detailed consideration given to total Cholesterol.

III.E1.1 EPA Response:

Based on SAB feedback on the draft MCLG documents' assessment of CVD-related risks, EPA has developed an RfD for TC (for more information, see U.S. EPA, 2021b; *Analysis of Cardiovascular Disease (CVD) Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water*). The derivation of an RfD for this endpoint addresses the SAB's concerns about the perceived inconsistency between the CVD document and the MCLG documents. The SAB also recommended that EPA ensure that recommendations for the draft

MCLG documents relating to evidence identification and synthesis are applied to the CVD endpoint. All studies in EPA's CVD benefits analysis were evaluated for ROB, selective reporting, and sensitivity as applied in EPA's derivation of MCLGs for PFOA and PFOS in drinking water.

In response to the SAB's recommendations, EPA expanded its documentation of the meta-analysis and conducted additional sensitivity analyses to evaluate the impact of the inclusion or exclusion of certain studies in the meta-analyses of exposure-response estimates. Further, EPA expanded its documentation and conducted additional sensitivity analyses to assess the effects of using a key single-study approach versus the meta-analysis approach to inform the exposure-response estimates. EPA identified two suitable key studies for use in the single-study approach. EPA found that the single-study approach resulted in increased benefits. This trend was driven by the larger estimates of PFAS-TC slope factors and inverse associations in the HLDC effect for one or both contaminants in the key single studies. Chapter 6, Table 6-16 in the Economic Analysis summarizes the 14 studies that EPA identified from literature reviews and used to derive slope estimates for PFOA and PFOS associations with serum TC levels. The Economic Analysis also details which studies were included/excluded from the cholesterol meta-analysis (see Appendix F of the Economic Analysis). Appendix F of the Economic Analysis also provides details on the studies' selection criteria, meta-data development, meta-analysis results, and discussion of the uncertainty and limitations inherent in EPA's exposure-response analysis.

EPA responded to the SAB's recommendation to assess the impacts of study quality on the overall effect estimate by conducting sensitivity analyses that excluded the studies considered to have higher ROB. There were 10 studies considered medium confidence and four studies considered low confidence based on the ROB study quality evaluations. To assess the potential impact of study quality on the overall effect estimate, EPA conducted sensitivity analyses that excluded the four low-confidence studies. For PFOA, when all the studies were combined, EPA observed nonsignificant positive increases in TC of 0.003 (95% CI: −0.001, 0.006) mg/dL per ng/mL serum PFOA (p-value = 0.177, I2 = 89%) and for HDLC of 0.001 (95% CI: −0.001, 0.004) mg/dL per ng/mL serum PFOA (p-value = 0.291, I2 = 71%). When the low-confidence studies were excluded, the results for both TC and HDLC were similar to the associations observed when all the studies were included in the meta-analysis, with 0.003 (95% CI: −0.003, 0.008) for TC (p-value = 0.321, I2 = 89%), and 0.002 (95% CI: −0.002, 0.005) for HDLC (p-value = 0.290, I2 = 58%). This information is summarized in Table F-2 within Appendix F of the Economic Analysis. For PFOS, when all studies were combined, EPA observed a borderline statistically significant positive increase in TC of 0.066 (95% CI: −0.001, 0.132) mg/dL per ng/mL serum PFOS (p-value = 0.055, I2 = 100%) and a nonsignificant increase for HDLC of 0.0003 (95% CI: −0.001, 0.001) mg/dL per ng/mL serum PFOS (p-value = 0.631, I2 = 89%). When the analysis excluded the four higher ROB studies, the association was significantly positive for TC (0.086, (5% CI: 0.001, 0.17, p-value = 0.047, I2 = 100%) and remained the same for HDLC (0.001, 95% CI: −0.001, 0.002, p-value = 0.606, I2 = 83%). This information is summarized in Table F-3 within Appendix F of the Economic Analysis.

Based on the comments and recommendations from the SAB on EPA's analysis of CVD risk reductions resulting from changes in PFOA/PFOS exposures, EPA assessed HDLC in a sensitivity analysis (see Appendix K of the Economic Analysis for the proposed rule). Although

the evidence of associations between HDLC and PFOA and PFOS was mixed, certain individual studies reported robust associations in general adult populations.

Charge Question #2 - EPA's Life Table Approach

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.

III.S2.1 SAB Recommendations:
The Panel recommends that EPA describe how the current application of the life table methodology differs in the use of prevalence statistics and other key input data and assumptions from prior applications.

The Panel recommends that EPA clearly list the assumptions and modeling decisions in the proposed methodology that may affect the estimates of the mortality/morbidity impacts, such as excluding individuals with pre-existing conditions and tracking post-acute CVD mortality for up to five-years after a CVD incident (e.g., in Table 7).

III.E2.1 EPA Response:
In response to the SAB's recommendations, EPA added more discussion on the life table approach and its application for the CVD benefits analysis in Appendix K of the Economic Analysis for the proposed rule. Additional CVD model assumptions and decisions have been added to Appendix K to provide further clarity on the methodology. Additionally, consistent with the SAB recommendations and to maximize transparency, EPA describes the limitations associated with EPA's life table application and how they may affect the estimates of the mortality/morbidity in Section 6.8 of the Economic Analysis for the proposed PFAS rule.

For estimating CVD events, EPA's model inputs required information on the baseline prevalence of the past hard CVD event history in the U.S. population because the population evaluated for the first hard CVD event estimation excludes those with a history of hard CVD events. The CVD model integrates the atherosclerotic cardiovascular disease (ASCVD) model predictions and post-acute CVD mortality estimates in the series of recurrent calculations that produce a life table estimate for the population cohort of interest. In addition to the standard life table components, such as the annual number of all-cause survivors and deaths for all ages, for ages 40+, the CVD model estimates the number of surviving persons with and without a history of hard CVD events, the number of persons experiencing hard CVD events at a given age, and deaths from CVD and non-CVD causes at a given age (see Appendix G of the Economic Analysis for the proposed rule for additional detail).

Consistent with the SAB recommendations, Section 6.1.4 of the Economic Analysis provides an example of a prior application of the life table approach used in another rulemaking. The 2015 Benefit and Cost Analysis for the Effluent Limitations Guidelines (ELGs), Standards for the Steam Electric Power Generating Point Source Category rulemaking used the life-table approach for bladder cancer benefits modeling (U.S. EPA, 2015). Similarly, the CVD benefits analysis used the life-table approach; detailed methods can be found in appendices G and H of the

Economic Analysis. Key differences between the prior application of the life-table model in the Steam Electric ELGs and the CVD benefits analysis include which endpoints were considered (i.e., bladder cancer versus CVD), underlying occurrence statistics and outcomes (i.e., prevalence, incidence, and survival rates), and the ages included in the model (i.e., lifetime modeled for bladder cancer versus adults aged 40+ modeled for CVD).

For SAB's recommendation regarding assumptions and modeling decisions, EPA updated Appendix K of the Economic Analysis to provide additional details on how EPA adjusted the modeled population cohort to exclude individuals with pre-existing conditions, as the ASCVD risk model does not apply to these individuals. EPA notes that elevated mortality for hard CVD event survivors may persist beyond five years of the initial event. However, EPA did not identify U.S.-based studies with sufficiently long follow-up to quantify mortality impacts beyond five years of the initial event.

Charge Question #3 - ASCVD Risk Model

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.

3i. 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.

3ii. 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.

3iii. 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.

III.S3.1 SAB Recommendations:

The Panel recommends that EPA provide further discussion of the accuracy of the model predictions in sub-groups with varying levels of social deprivation.

The Panel recommends that EPA evaluate whether inclusion of HDLC would influence the results of the modeling.

III.E3.1 EPA Response:

Regarding the comment on model predictions in subgroups, the SAB noted that although the ASCVD model is a reasonable choice for estimating the probability of first-time CVD events, it has limitations. The panel recommended that EPA include more discussion of the accuracy of its predictions, particularly for subpopulations. As such, EPA expanded its evaluation of the ASCVD model's limitations, including a comparison of the ASCVD model predictions with race/ethnicity and sex-specific CVD incidence from CDC's public health surveys (see Section 6.5.3.2 and Appendix G of the Economic Analysis for the proposed rule for additional detail).

Results of EPA's validation exercise indicate that the ASCVD model coefficients for the non-Hispanic Black model are more consistent with data on CVD prevalence and mortality for Hispanic and non-Hispanic other race subpopulations than the ASCVD model coefficients for the non-Hispanic White model.

Regarding the SAB's comment on the inclusion of HDLC, EPA found that, as expected, the inclusion of HDLC effects decreases annualized CVD benefits and the inclusion of blood pressure effects slightly increases annualized CVD benefits. Because HDLC was shown to have a stronger effect than blood pressure on annualized CVD benefits, the inclusion of blood pressure and HDLC effects together decreases annualized CVD benefits. For more information, see the sensitivity analyses evaluating these effects in Appendix K of the Economic Analysis for the proposed rule.

The inclusion of HDLC effects in EPA's national analysis would marginally reduce national benefits estimates but would not change the EPA's bottom-line conclusion that the quantifiable and nonquantifiable benefits of the rule justify the quantifiable and nonquantifiable costs. After further examination of the evidence for HDLC and blood pressure effects, EPA elected to include blood pressure effects because the findings from a single high-confidence study and several medium-confidence studies conducted among the general population provided consistent evidence of an association between PFOS exposure and blood pressure. EPA did not include HDLC effects in its national benefits analysis because the available evidence of associations between PFOS exposures and HDLC levels is inconsistent, and there is no evidence of an association between PFOA exposures and HDLC levels.

Charge Question #4 - Limitations and Uncertainties

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?

III.S4.1 SAB Recommendations:

See Appendix A of this report for specific areas needing clarification. Some notable highlights include:

- Quantified uncertainty about the slope of the relationships between TC and either PFOA or PFOS should be clarified and should account for the sensitivity of the meta-analysis results to restrictions on the functional form of the included estimates.
- The exclusion of non-TC CVD-related outcomes may not result in an "underestimate" due to the exclusion of HDLC in the analysis. The Panel recommends changing the "effect on estimate" to "uncertain" and explaining this in the "details" column (Row 7 of Table 7).

III.E4.1 EPA Response:

Based on SAB's feedback on Appendix A, EPA expanded the discussion on the relationship of TC with PFOA and PFOS. Specifically, EPA quantified the uncertainty associated with cholesterol slope factors. The slope factors that express the effects of serum PFOA and serum PFOS on cholesterol are based on EPA meta-analyses that provide a central estimate and a CI. EPA assumed that the slope factors would have a uniform distribution within their range. EPA also conducted additional sensitivity analyses to evaluate the impact of the inclusion or exclusion of certain studies in the meta-analyses of exposure-response estimates as outlined in Appendix K

of the Economic Analysis for the proposed rule. Further, EPA expanded the documentation and conducted additional sensitivity analyses to assess the effects of using a key single-study approach versus the meta-analysis approach to inform the exposure-response estimates.

EPA also summarized limitations and sources of uncertainty associated with the estimated serum cholesterol dose-response functions in Appendix F of the Economic Analysis for the proposed rule. The effects of these limitations and the sources of uncertainty on estimates of risk reduction and benefits evaluated in the PFAS NPDWR are uncertain.

Based on SAB's recommendation, EPA updated Table 6-51 to "uncertain" with the appropriate discussion details (see Section 6.8 of the Economic Analysis).

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