SCIENCE ADVISORY BOARD
A Federal Advisory Committee to the U.S. Environmental Protection Agency

August 22, 2022

EPA-SAB-22-008

The Honorable Michael S. Regan
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Subject: Transmittal of the Science Advisory Board Report titled, "Review of EPA's Analyses to Support EPA's National Primary Drinking Water Rulemaking for PFAS"

Dear Administrator Regan,

Please find enclosed the final report from the Science Advisory Board (SAB) titled, "*Review of EPA's Analysis to Support EPA's National Primary Drinking Water Rulemaking for PFAS*." As part of the proposed rulemaking process, EPA prepared four documents to support the development of 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). The EPA's Office of Water requested that the SAB review four EPA documents titled, *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; 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; EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS); and EPA's Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water.*

In response to the EPA's request that the SAB review EPA's four draft documents listed above, the SAB Staff Office identified subject matter experts to augment the SAB Chemical Assessment Advisory Committee (CAAC) and assembled the SAB PFAS Review Panel to conduct the review. The SAB PFAS Review Panel met virtually on December 16, 2021, and then at three (3) subsequent meetings on January 4, 6 and 7, 2022 to deliberate on the agency's charge questions. Another virtual meeting was held on May 3, 2022, to discuss the Panel's draft report. Oral and written public comments were considered throughout the advisory process. This report conveys the consensus advice of the SAB.

The SAB recognizes the time constraints for completing the rule-making process and is supportive of the EPA's efforts to the utilize the latest scientific findings to inform their decisions. The SAB applauds the agency's efforts to develop new approaches for assessing the risk of PFAS mixtures and the benefits arising from reducing exposure to these chemicals. However, the SAB considers the supporting documents related to approaches for deriving the PFOA and PFOS MCLGs to have a number of methodological flaws. For instance, it is unclear whether the EPA used the processes from the 2020 Office of Research and Development (ORD) draft handbook for developing IRIS assessments for their evaluation steps, used a modified version of these processes, or used a completely different process. The SAB also found that the inclusion and exclusion of epidemiologic and animal studies was inconsistent across endpoints, leading to confusion about the criteria being used. In addition, several concerns about the study evaluation and evidence synthesis process used by the EPA and the lack of availability of mechanistic data in the draft MCLG documents were identified. The SAB urges that these problems be addressed with revisions that represent the state-of-practice for gathering, evaluating, synthesizing, and using evidence for decision-making.

In general, the SAB agreed with many of the conclusions presented in the assessments, framework and analysis. The SAB also identified many areas that would benefit from further clarification to enhance transparency and utility. While the SAB provided numerous recommendations, we would like to highlight the following ones, with additional details described within the enclosed full report. The SAB recommends that EPA consider the following points as they revise their documents:

Draft MCLG documents for PFOA and PFOS

- EPA should provide additional transparency and completeness in its evidence identification methodology, including development of a protocol with clear inclusion/exclusion criteria and study evaluation approaches.
- Studies, particularly human studies, that were included in the 2016 health effects summary documents (HESDs) should be considered in the same manner as the more recent studies.
- A consistent framework and descriptors should be used for evidence synthesis and integration for each health outcome.
- In the short-term and in consideration of the agency's time constraints, EPA should initially focus on those health outcomes that have been concluded to have the strongest evidence, including the liver disease, immune system dysfunction, serum lipid aberration, impaired fetal growth, and cancer.
- EPA should include an evaluation of mechanistic/mode of action data for those effects considered as the potential basis for the reference doses (RfDs) and cancer slope factors (CSFs).
- The process of hazard identification should be separated from the process of dose-response assessment.
- The rationale and criteria for selection of endpoints and specific studies for point of departure (POD) development should be more clearly presented.
- EPA should use Alanine Aminotransferase (ALT) as an endpoint in light of the numerous studies in the literature that support an association between slight elevations in ALT and increased risk of morbidity and/or mortality.

- While the SAB agrees with the "likely" designation for PFOA carcinogenicity based on new evidence and prior evidence included in the 2016 HESD, a more structured and transparent "weight of evidence" discussion to support the rationale behind this designation is needed.
- EPA should develop multiple candidate CSFs for PFOA, 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 and a judgment made as to whether to select one or more studies to represent the overall slope factor.
- EPA needs to provide additional details and transparency for all quantitative modeling, including that used for CSF development, toxicokinetic modeling, and benchmark dose modeling for POD derivation. It is essential that details of the Benchmark Dose (BMD) modeling that forms the basis of the PODs are transparently available for evaluation of the methods, approaches, and results.
- EPA should reconsider its choice of the Verner et al. (2016) human toxicokinetic model and consider whether the Goeden et al. (2019) model, which incorporates age-specific toxicokinetic and exposure factors in predicting internal dose, is more appropriate for use in the development of the PFOA and PFOS RfDs and MCLGs.
- For the development of a non-cancer RfD, EPA should consider multiple human and animal studies for a variety of endpoints in different populations so as to provide convergent evidence that is more reliable than any single study or health endpoint in isolation.
- EPA should provide a stronger and more transparent justification for the choice of benchmark responses (BMRs) - not only for decreased antibody response, but also other endpoints for which BMDs were developed.
- In deriving RfDs, EPA should consider using an "internal dose" POD, which is then further adjusted for inter-species and intra-species uncertainty/variability, so that the RfD is expressed in a dose metric equivalent value. This internal dose RfD can then be converted using toxicokinetic (TK) modeling to either an equivalent external dose or an equivalent water concentration, as appropriate.
- EPA should clearly state that the RfDs apply to both short-term and chronic exposure durations. This is critical in addressing situations of drinking water contamination with PFOA and/or PFOS and the timeframe in which intervention is needed.
- While the SAB agrees with the uncertainty factors (UFs) used in deriving RfDs, EPA should consider adoption of a probabilistic framework to calculate risk-specific doses and evaluate potential methods for accounting for mixtures in establishing MCLGs.
- The SAB supports the selection of a relative source contribution (RSC) of 20%, but EPA should 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%.

Draft mixtures document

- 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.

- The SAB 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 additional processes for scientific input and review in general.
- 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.
- 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, including when they converge, are 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.
- 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.

CVD document

- The SAB supports the overall approach to estimating reductions in cardiovascular disease (CVD) risk associated with reductions in exposure to PFOA and PFOS in drinking water. However, the SAB expressed a concern about the apparent discrepancy between the draft CVD document's focus on CVD risk, and the draft MCLG documents' conclusions that the evidence of CVD was not sufficient to form the basis of a RfD. EPA should provide more discussion as to the rationale for selecting this particular endpoint for risk reduction analysis, as well as consider risk reduction analyses for other endpoints.
- EPA should ensure that recommendations for the draft MCLG documents relating to evidence identification and synthesis are applied to the CVD endpoint.
- Although the SAB generally agrees with the meta-analysis, life-table and risk estimation methods, EPA should provide additional clarity as to the application of these approaches, including conduct of sensitivity analyses.
- While the Atherosclerotic Cardiovascular Disease (ASCVD) model is a reasonable choice for estimating the probability of first time CVD events, it is not without limitations. EPA should include more discussion of the accuracy of its predictions, particularly for sub-populations.
- EPA should evaluate whether inclusion of High-Density Lipoprotein Cholesterol (HDLC) data would influence the results.

As the EPA finalizes the draft MCLG, Mixtures and CVD documents, the SAB encourages the

EPA to address the SAB's concerns raised in the enclosed report and consider their advice and recommendations. The SAB appreciates this opportunity to review EPA's Analyses to support EPA's National Primary Drinking Water Rulemaking for PFAS and looks forward to the EPA's response to these recommendations.

Sincerely,

/s/

Alison C. Cullen, Sc.D.
Chair
EPA Science Advisory Board

/s/

Weihsueh Chiu, Ph.D.
Chair
EPA SAB PFAS Review Panel

Enclosure

NOTICE

This report has been written as part of the activities of the EPA Science Advisory Board, a public advisory committee providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The Board is structured to provide balanced, expert assessment of scientific matters related to problems facing the Agency. This report has not been reviewed for approval by the Agency and, hence, the contents of this report do not represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use. Reports of the EPA Science Advisory Board are posted on the EPA website at http://www.epa.gov/sab.

U.S. Environmental Protection Agency
PFAS Review Panel

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Dr. Deborah Cory-Slechta, Professor, Department of Environmental Medicine, School of Medicine and Dentistry, University of Rochester, Rochester, NY

Dr Jamie C. DeWitt, Associate Professor of Pharmacology and Toxicology, Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC

Dr. Jeffrey Fisher, Senior Research Fellow, Computational Biology Division, ScitoVation, Durham, NC

Dr. James K. Hammitt, Professor, Center for Risk Analysis, Harvard University, Boston, MA

Dr Lisa Kamendulis, Assoc Prof, Department of Environmental Health, School of Public Health, Indiana University, Bloomington, IN

Dr. David Keiser, Professor, Department of Resource Economics, University of Massachusetts Amherst, Amherst, MA

Dr. Loren Lipworth, Professor and Associate Director, Division of Epidemiology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

Dr. Lala Ma, Assistant Professor, Department of Economics, Gatton College of Business and Economics, University of Kentucky, Lexington, KY

Dr. Sheila Olmstead, Professor of Public Affairs, Lyndon B. Johnson School of Public Affairs, The University of Texas at Austin, Austin, TX

Dr. Gloria Post, Research Scientist, Division of Science and Research, New Jersey Department of Environmental Protection, Trenton, NJ

Dr. Kristi Pullen-Fedinick, Executive Director, Center for Earth, Energy, and Democracy, Minneapolis, MN

Dr. David A. Savitz, Professor of Epidemiology, Brown University School of Public Health, Providence, RI.

Dr Angela L Slitt, Professor, Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

SCIENCE ADVISORY BOARD STAFF

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Dr. Aimin Chen, Professor of Epidemiology, Department of Biostatistics, Epidemiology and Informatics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Dr. Amy Childress, Professor and Director of Environmental Engineering, Sonny Astani Department of Civil & Environmental Engineering, University of Southern California, Los Angeles, CA

Dr. Weihsueh Chiu, Professor, Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX

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Dr. Selene Hernandez-Ruiz, Director, Laboratory and Analytical Services Division, Water Resources Mission Area, U.S. Geological Survey, Lakewood, CO

Dr. Elena G. Irwin, Distinguished Professor of Food, Agricultural and Environmental Sciences in Economics and Sustainability and Faculty Director for the Sustainability Institute, Department of Agricultural, Environmental, and Development Economics, The Ohio State University, Columbus, OH

Dr. David Keiser, Professor, Department of Resource Economics, University of Massachusetts Amherst, Amherst, MA

Dr. Mark W. LeChevallier, Principal, Dr. Water Consulting, LLC, Morrison, CO

Dr. Angela M. Leung, Clinical Associate Professor of Medicine, Department of Medicine, Division of Endocrinology, Diabetes, and Metabolism, David Geffen School of Medicine; VA Greater Los Angeles Healthcare System, University of California Los Angeles, Los Angeles, CA

Ms. Lisa Lone Fight, Director, Science, Technology, and Research Department, MHA Nation, Three Affiliated Tribes, New Town, ND

Dr. Lala Ma, Assistant Professor, Department of Economics, Gatton College of Business and Economics, University of Kentucky, Lexington, KY

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Dr. Enid Neptune, Associate Professor of Medicine, Department of Medicine, Division of Pulmonary and Critical Care Medicine, Johns Hopkins University, Baltimore, MD

Dr. Sheila Olmstead, Professor of Public Affairs, Lyndon B. Johnson School of Public Affairs, The University of Texas at Austin, Austin, TX

Dr. Austin Omer, Sustainable Systems Agronomist, Crop Science Commercial, Bayer U.S., Morton, IL

Dr. Gloria Post, Research Scientist, Division of Science and Research, New Jersey Department of Environmental Protection, Trenton, NJ

Dr. Kristi Pullen-Fedinick, Executive Director, Center for Earth, Energy, and Democracy, Minneapolis, MN

Dr. Amanda D. Rodewald, Garvin Professor and Senior Director of Center for Avian Population Studies, Department of Natural Resources and the Environment, Cornell Lab of Ornithology, Cornell University, Ithaca, NY

Dr. Emma J. Rosi, Senior Scientist, Cary Institute of Ecosystem Studies, Millbrook, NY

Dr. Jonathan M. Samet, Dean and Professor, Departments of Epidemiology and Environmental and Occupational Health, Office of the Dean, Colorado School of Public Health, Aurora, CO

Dr. Elizabeth A. (Lianne) Sheppard, Rohm and Haas Professor in Public Health Sciences, Department of Environmental & Occupational Health Sciences and Department of Biostatistics, Hans Rosling Center for Population Health, University of Washington, Seattle, WA

Dr. Drew Shindell, Nicholas Distinguished Professor of Earth Science, Duke Global Health Initiative, Nicholas School of the Environment, Duke University, Durham, NC

Dr. Genee Smith, Assistant Professor, Department of Environmental Health and Engineering, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

Dr. Richard Smith, Professor, Department of Statistics and Operations Research, University of North Carolina, Chapel Hill, Chapel Hill, NC

Dr. Daniel O. Stram, Professor, Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA

Dr. Peter S. Thorne, University of Iowa Distinguished Chair and Professor and Director of Human Toxicology Program, Department of Occupational & Environmental Health, College of Public Health, University of Iowa, Iowa City, IA

Dr. Godfrey Arinze Uzochukwu, Senior Professor, Waste Management Institute, North Carolina Agricultural and Technical State University, Greensboro, NC

Dr. Wei-Hsung Wang, Professor, Center for Energy Studies and Director of the Radiation Safety Office, Louisiana State University, Baton Rouge, LA

Dr. June Weintraub, Senior Epidemiologist and Manager of Water and Noise Regulatory Programs, San Francisco Department of Public Health, San Francisco, CA

Dr. Sacoby Wilson, Associate Professor and Director of the Center for Community Engagement, Environmental Justice, and Health (CEEJH), Maryland Institute for Applied Environmental Health, School of Public Health, University of Maryland-College Park, College Park, MD

Dr. Dominique van der Mensbrugghe, Research Professor and Director of the Center for Global Trade Analysis, Department of Agricultural Economics, Purdue University, West Lafayette, IN

SCIENCE ADVISORY BOARD STAFF

Dr. Thomas Armitage, Designated Federal Officer, U.S. Environmental Protection Agency, Washington, DC

Review of EPA's Analyses to Support EPA's National Primary Drinking Water Rulemaking for PFAS
FINAL REPORT, dated August 22, 2022

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ACRONYMS AND ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, Excretion
ALT	Alanine Aminotransferase
APFO	Ammonium Perfluorooctanoate
ASCVD	Atherosclerotic Cardiovascular Disease
BMD	Benchmark Dose
BMDL	Benchmark Dose lower bound limit
BMR	Benchmark Response
CSF	Cancer Slope Factor
DA	Dose Additivity
EFSA	European Food Safety Authority
GCA	General Concentration Addition
GenX	HFPO dimer acid and HFPO dimer acid ammonium salt
HAWC	Health Assessment Workspace Collaborative
HBWC	Health-Based Water Concentrations
HDL	High-Density Lipoprotein
HDLC	High-Density Lipoprotein Cholesterol
HESD	Health Effects Support Document
HED	Human Equivalent Dose
HFPO	hexafluoropropylene oxide
HI	Hazard Index
IC	Index Chemical
IRIS	Integrated Risk Information System
LOAEL	Lowest Observed Adverse Effect Level
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MOA	Mode of Action
NAM	New Approach Method
NASEM	National Academies of Sciences, Engineering, and Medicine
NPDWR	National Primary Drinking Water Regulations
OR	Odds Ratio
PBPK	Physiologically-Based Pharmacokinetic
PECO	Population (including animal species), Exposure, Comparator, and Outcomes
PFAS	Per- and Polyfluoroalkyl Substances
PFBA	Perfluorobutanoic Acid
PFBS	Perfluorobutane Sulfonic Acid
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonic Acid
PFCA	Perfluorocarboxylic Acid
PFSA	Perfluorosulfonic Acid
POD	Point of Departure
PPAR-α	Peroxisome Proliferator-Activated Receptor-alpha
PWS	Public Water System
QSAR	Quantitative Structure-Activity Relationship
RCC	Renal Cell Carcinoma
RfD	Reference Dose

RfV	Reference Values
RPF	Relative Potency Factor
RSC	Relative Source Contribution
SDWA	Safe Drinking Water Act
TC	Total Cholesterol
TOSHI	Target-Organ-Specific Hazard Index
UF	Uncertainty Factor

INTRODUCTION

The U.S. Environmental Protection Agency (EPA), having 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), is seeking comment from the EPA Science Advisory Board (SAB) on key scientific issues related to the development of the NPDWR. As part of this proposed rulemaking, EPA has prepared the following four 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)*

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

The draft *EPA's Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for PFOA and PFOS* documents (henceforth referred to as "the draft MCLG documents") provide a number of values, including toxicity values, that could be use in a human health risk assessment. The EPA stated that these documents do not constitute risk assessments; however the values presented were derived using human health risk assessment guidance, guidelines, and current methods.

The *EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)* document (henceforth referred to as "the draft mixtures document") provides a draft approach for a tiered, data-driven framework for estimating the likelihood of noncancer human health risks associated with oral exposures to mixtures of PFAS, based on EPA guidelines and guidance (U.S. EPA, 1986, 2000). The EPA maintains that although the framework and illustrative examples provided in this document include examples for PFAS in water, the framework itself is not limited to specific media and may be useful for understanding the potential non-cancer health effects of PFAS mixtures under various authorities or decision contexts. The EPA contends that the approach presented is not intended to be used to assign groups or subclasses or otherwise classify PFAS. Rather, the framework is designed for practical application of EPA chemical mixtures approaches and methods for a particular exposure to gain insight on the potential health risk(s) associated with exposure to mixtures of PFAS. The EPA notes that the draft mixtures document with the associated illustrative examples is intended to inform PFAS evaluation(s) by federal, state, and tribal partners, as well as public health experts, drinking water utility personnel, and other stakeholders interested in assessing the potential human health hazards and risks associated with PFAS mixtures. The EPA further emphasized that the draft mixtures document is not a regulation, does not impose legally binding requirements on EPA, states, tribes, or the regulated

community, and might not apply to a particular situation based on the circumstances.

EPA stated that it is considering several adverse health effects for quantified health risk reduction assessment. Among the adverse health effects that EPA found to have sufficient weight of evidence and available data to inform estimates of avoided adverse health outcomes were the effects of PFOA and PFOS on serum lipids, specifically total cholesterol (TC), a well-established risk factor for cardiovascular disease. EPA's *Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water* document (henceforth referred to as "the CVD document") presents EPA's methodology to determine the avoided cases of CVD events (e.g., first heart attack, first stroke, death from coronary heart disease) for a hypothetical public water system (PWS).

The EPA's Office of Water requested that the SAB review EPA's four draft documents listed above. In response to the EPA's request, the SAB identified subject matter experts to augment the Science Advisory Board (SAB) Chemical Assessment Advisory Committee (CAAC) and assembled the SAB PFAS Review Panel to conduct the review. The SAB PFAS Review Panel met virtually using a video meeting platform on December 16, 2021, and then at three (3) subsequent meetings on January 4, 6 and 7, 2022 to deliberate on the agency's charge questions. A virtual meeting was also held on May 3, 2022 to discuss the Panel's draft report. Consideration of oral and written public comments was encouraged throughout the advisory process.

The Panel identified numerous instances in which the analyses and approaches in EPA's documents could be revised to be more thorough and transparent. This report is organized into three sections, one for each topic area (i.e., MCLG derivation, Mixtures approaches, and Benefits from CVD reduction). In each section, the charge questions raised by the agency are presented and then followed by the consensus response and recommendations. The Panel provided key recommendations that are necessary to improve the critical scientific concepts, issues, and/or narrative within the EPA's documents. The Panel deemed these recommendations as important for improving the transparency of the agency's conclusions and to bolster the supporting evidence for them.

A list of acronyms and abbreviations can be found at the front of this report to assist in orienting the reader to the terminology used in the EPA's documents and throughout the Panel's responses to the Charge Questions. Editorial comments are presented within Appendices A, B and C corresponding to each of the three topic areas mentioned above. All materials and comments related to this report are available at:
https://sab.epa.gov/ords/sab/f?p=114:18:31389248712411:::RP,18:P18_ID:2601

SECTION I - MCLG derivation

Proposed Approaches to the Derivation of a 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.

Overall Comments

While the Panel agreed that some aspects of the process were clearly described (e.g., the literature search and screening processes), they also identified multiple inconsistencies and deficiencies in both the description and execution of the systematic review process utilized in the evaluation of both PFOA and PFOS. The Panel notes that the document's transparency would be enhanced through a diagram or flowchart depicting the overall process of study identification and inclusion.

The following sections, organized by steps in the systematic review process, offer a short background on the step, provide feedback from the Panel on the step, and close with recommendations for moving forward.

Problem Formulation and Criteria

Background

Before initiating a systematic review process, it is essential to clearly define the study question to be addressed and to develop a protocol. Often a preliminary literature review is conducted, which *a priori* describes the approach and criteria that will be used to guide the review. A systematic review protocol makes both the process and the methods of the review transparent and offers an opportunity for the peer review of methods. A clearly defined and published protocol creates a public record of the steps that will be used to conduct each step of the review – including evidence identification, evaluation, and synthesis.

Review of EPA process

The Panel and the public commenters noted significant concerns that the reviews for PFOA and PFOS do not appear to have established a predefined protocol. The lack of a protocol led to a lack of clarity across each of the major systematic review steps for both chemicals and was seen as a major deficiency of the reviews.

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.

Literature Search Strategy and Screening Process

Background

During the literature search strategy and screening step of a systematic review, evidence related to the study section is sought and selected based upon eligibility criteria from the Population (including animal species), Exposure, Comparator, and Outcomes (PECO) statement. Searching for and selecting evidence should be conducted using a detailed search plan that includes databases to be searched, gray literature sources, and other search methods including whether existing systematic reviews will be utilized. Literature searches can yield large numbers of records and are usually carried out in a tiered approach – beginning with a title and abstract search followed by a full text review. A range of software tools can be used in the management of evidence review and selection.

Review of EPA process

In general, the Panel found the literature search and screening processes clearly described. The Panel noted that the use of SWIFT and DistillerSR to sort the literature and process for quality evaluation and confidence determination were reasonable steps. However, several areas of concern were noted.

The Panel found the PECO statement was deficient in the list of "relevant forms" of PFOA and PFOS due to a lack of inclusion of relevant salts (e.g., salts of PFOA such as ammonium perfluorooctanoate (APFO), sodium perfluorooctanoate, and potassium perfluorooctanoate, or salts of PFOS such as lithium perfluorooctanesulfonate, potassium perfluorooctanesulfonate (K+PFOS), ammonium perfluorooctanesulfonate, or sodium perfluorooctanesulfonate). The omission of salts of PFOA and PFOS from PECO criteria for human and animal studies is problematic because a salt of PFOA (e.g., APFO) or PFOS (e.g., K+PFOS) was used as the test compound in all of the toxicity studies of PFOA and PFOS in laboratory animals reviewed in the draft MCLG documents, and some studies of occupationally exposed workers also use these terms. These salts completely dissociate to the anionic form (PFOA, PFOS) in the body, in water, and in other environmental media. While it is likely that the publications reporting the animal toxicity studies include the term "PFOA" or "PFOS" as well the salt of PFOA or PFOS that was used, the salts should be included as "relevant forms" in the PECO criteria.

In addition to the lack of inclusion of salts in the PECO statement, the Panel found that the exact process used to inform inclusion and exclusion of evidence was unclear. The lack of documentation about excluded literature, particularly for studies that underwent full text review, made it challenging to gauge if the inclusion/exclusion criteria were followed. For example, the Panel found that Vriens et al. (2019) was not listed in the PFOA or PFOS documents despite it

being a study that could inform mechanisms. In addition to missing mechanistic studies, the Panel also found that the inclusion and exclusion of epidemiologic and animal studies were inconsistent across endpoints, leading to confusion about the criteria being used. For example, Table 2 (p. 11) of the PFOA document states: *"Studies with less than 28 days of dosing, with the exception of reproductive or developmental studies, should be tagged as supplemental,"* but Section 3.1.3 (p.24) on literature search results for animal toxicology studies states: *"of the 32 animal studies that met the inclusion criteria, most studies had either short-term (n = 15) or developmental (n = 12) study designs."* The inclusion of short-term studies other than developmental studies in Section 3.1.3 does not seem to be consistent with the information in Table 2.

The Panel concluded that the decision to exclude literature published within the timeframe of the development of the 2016 health effects support document (HESD; U.S.EPA, 2016) in the current literature search was unjustified. The 2016 HESD did not include systematic reviews of the literature and are not fully representative of the relevant literature needed for the PFOA and PFOS reviews. For example, a 2011 study by Dong et al. which reported that PFOS caused a dose-related decrease in serum levels of sheep red blood cells (SRBC)-specific IgM in mice was not included in the 2016 HESD. Dong et al. (2011) is important because it was used as the critical study for the PFOS Reference Doses (RfDs) and drinking water guidelines established by Minnesota, New Hampshire, and Washington (Post, 2021). The lack of inclusion of this key study in the 2016 HESD document erodes confidence that all the necessary and relevant studies needed for the current PFOA and PFOS reviews are included.

Additionally, the rationale for not considering studies, particularly human studies, that were included in the 2016 HESDs is not clear or supportable. There is no reason to conclude that the earlier studies are less relevant or of lesser quality than the newer studies. Consideration of all human studies is especially important because conclusions about the level of evidence for human health effects, which are generally observational rather than experimental, are based on the overall weight of evidence from all relevant data. Furthermore, specific human epidemiology studies included in the 2016 HESDs may be preferable to the more recent studies for point of departure (POD) development for some health endpoints, as illustrated by the use of data from Grandjean et al. (2012) as the basis for the final RfDs for both PFOA and PFOS. This topic is discussed in more detail, including examples, in the response to the charge question #2 on Noncancer Hazard Identification.

Finally, the Panel noted that relevant literature has been published since the exclusion date used for the draft MCLG documents. While selecting a date for the search exclusion is a critical element of a systemic review literature search, a cut-off date of greater than a year ago, creates a significant window in which relevant literature may have been published.

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 draft EPA Office of Research and Development (ORD) handbook (U.S. EPA, 2020), 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.

Study Evaluation

Background

In the study evaluation step of a systematic review, individual studies selected for inclusion in the review are assessed using predefined criteria for internal validity (also known as "risk of bias"), study sensitivity (i.e., whether there are factors in a study's design or conduct that reduce its ability to observe an effect), and study quality (i.e., whether a study is conducted at the highest possible standard for the study type). The goal of this stage of a systematic review is to evaluate whether the results of a study represent a "reliable, sensitive, and informative presentation of a true response," as described in the draft ORD handbook (U.S. EPA, 2020).

Review of EPA process

In general, the Panel agreed that the evidence evaluation process was difficult to follow and not clearly described. The seven domains that were considered are not listed in the text, and critical details about how the seven domains were selected are missing from the draft MCLG documents, as are details about whether objective criteria were used in weighing how deficiencies in certain/multiple domains contributed to the overall rating. It is unclear whether the EPA used protocols in the 2020 draft ORD handbook for their evaluation steps, a modified version of the protocol, or a completely different process.

The Panel was also concerned about the downgrading of studies based upon "study sensitivity" if they had limited exposure contrasts and/or small sample sizes. Narrow exposure ranges should not automatically lead to downgrading of studies as these can still contribute informative data within that narrow range, despite reductions in precision of effect estimates. Additionally, the reports mention exclusion of publications with specific population subsets. More transparency and information are needed on how this decision was reached and used as a basis for exclusion.

The Panel also noted that a protocol for risk of bias assessment and, more importantly, how that approach was used in the synthesis of evidence for each particular health endpoint is not clearly presented; and therefore, the results cannot be confidently evaluated for accuracy or transparency, or for consistency across health endpoints. This is especially important when a proposed systematic review protocol has not been previously registered or published.

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.

Data Extraction

Background

According to the draft 2020 ORD handbook, data extraction is the stage of the systematic review during which relevant results from each study are extracted and reviewed to facilitate the drawing of comparisons across results. The results of this process are tables, graphs, and other visual displays that help organize and present study findings.

Review of EPA process

Several issues with the data extraction process used by the EPA were identified. For example, information on the absorption, distribution, metabolism, and excretion (ADME) and mechanistic data that were extracted does not appear to be included in the draft MCLG documents.

Another example of the lack of clarity in data extraction was observed in the Dose-Response Studies subsection of the Data Extraction section in the draft MCLG documents (p. 19), where the agency states: *"Data extraction was conducted for most studies that were included in the literature inventory, except those excluded as described below. … Extractions were limited to outcomes of interest and/or the most sensitive LOAEL."* It was not clear whether this means the most sensitive LOAEL was based on internal dose (serum PFOA or PFOS level) or administered dose. For most human studies, the doses are based on serum levels (internal dose). For animal studies, the LOAEL based on administered dose may not correspond to the LOAEL based on internal dose (serum level) because of toxicokinetic (half-life) differences between species or sexes (e.g., female rats have a much quicker excretion rate vs. male rats). The Panel recognizes that evaluating this issue for all studies where it is relevant may not be possible due to resource limitations. However, the Panel suggests that this potential uncertainty in the approach be acknowledged.

Additionally, the Dose-Response Studies subsection of the Data Extraction section (p. 19) states that: "*…low confidence studies when medium and high confidence studies (e.g., on an outcome) were available did not go through data extraction."* This appears to contradict the statement in Section 2.4 that low confidence animal studies were not considered and that "all study designs" (not specifying study confidence level) were considered for human studies. This information should be clarified and should be consistent in the two sections.

Also, the draft MCLG documents (p. 19) state that: *"For human evidence, all study designs were considered; for animal evidence, only animal studies with at least two exposure groups and with high or medium for study quality were considered."* If all human studies were considered, the rationale for considering human studies, but not animal studies, that have low confidence should be provided.

Finally, a critical deficiency is that mechanistic data are not summarized in the draft MCLG documents, except for summary tables of the number of studies with each type of mechanistic information. Discussion of the mode(s) and/or mechanism(s) of action for toxicity is normally an important component of toxicity assessments such as these PFOA and PFOS assessments. As discussed in the draft 2020 ORD staff handbook, such an evaluation can provide information about the human relevance of effects observed in animal species and the plausibility of effects observed in humans, among other areas of potential uncertainty. EPA did not provide a rationale for concluding that mechanistic data does not need to be considered in cancer and non-cancer hazard identification and that this information can be added later. While the Panel recognizes that it may not be possible to include an evaluation of mechanistic data for all health effects, a mechanistic or mode of action evaluation for the noncancer endpoint(s) selected as critical endpoints for RfD(s) and for the weight of evidence for carcinogenicity should be included to support EPA's conclusions.

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.

Evidence synthesis

Background

Evidence synthesis is the stage of a systematic review when the results from individual studies are quantitatively and/or qualitatively analyzed within a specific stream of evidence. The methods that will be used for evidence synthesis should be predetermined in the systematic review protocol to ensure transparency and consistency across all evidence streams for each health outcome evaluated.

Review of EPA process

During the Panel's public meeting on December 16, 2021, the EPA noted that the evidence synthesis processes did not utilize a systematic review protocol in the draft MCLG documents. Additionally, the Health Effects Evidence Synthesis and Integration sections for each health outcome in the Hazard Identification (Section 3.3) do not appear to consistently follow the process presented in the Systematic Review (Section 2.6). Specifically, it is stated (p. 22 of the draft MCLG documents): *"a summary discussion that addresses considerations regarding causation as adapted from Hill (1965)"* is provided for each health outcome, but this was not

consistently done in the Evidence Synthesis and Integration sections under Hazard Identification (Section 3.3). The Panel noted that this represents a significant deviation from standard systematic review protocols, including the process established in the draft 2020 ORD handbook. In general, the Panel found the process used by the agency to be lacking in clarity and transparency. For example, it was noted that it was unclear who synthesized the evidence, how disagreements were resolved, how conflicting results from different studies were accommodated, and whether syntheses were independently reviewed by scientists knowledgeable about the studies.

In addition to the lack of a systematic protocol for evidence synthesis, the lack of clarity was further exacerbated by the use of non-integrated teams across the various evidence streams. EPA stated during the Panel meeting on December 16, 2021, that different sections were written by different scientists who used professional judgement as to the terms used and the way conclusions were presented. The inconsistencies introduced due to a disjointed process instead of a structured and systematic approach make it difficult to compare the conclusions of the different health effects sections.

The Panel also found the use of inconsistent language confusing and in need of standardization. For example, the conclusions in different Evidence Integration sections use a range of terms that appear to have similar meanings: "*suggestive evidence*," "*moderate evidence*," and "*consistent evidence*"; "*inconsistent evidence*" and "*mixed evidence"*; however, no definitions of these terms were provided. Furthermore, the overall nature of the conclusions for different health effects was not presented consistently (e.g., "*suggestive evidence for an association of PFOS with [the health outcome]*", "*suggestive evidence that PFOA impacts [the health outcome]"*, or "*suggestive evidence for an effect of PFOA on [the health outcome]*"). The Panel was unclear from the wording of these conclusions whether they were intended to apply to the evidence for association of the effect with PFOA or to the overall evidence that PFOA causes the effect, which is an important distinction.

Additionally, it is stated (p. 22 of both the PFOA and PFOS documents): *"The syntheses of human and animal health effects evidence focused on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of studies."* The meaning of "exposure context" here is unclear and should be clarified.

Finally, the Panel recommends that sensitivity analyses be performed to determine if interpretations or conclusions will change based on varied decisions for inclusion or exclusion and study ratings of high, medium and low confidence across various study design domains.

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 draft ORD staff handbook for developing IRIS assessments (U.S. EPA, 2020), 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, 2021). 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.

Additional peer-reviewed studies that could inform hazard identification and toxicity value derivation.

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

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?

General Comments

The charge question had requested input on the "strong" and "suggestive" designations. EPA clarified that they are seeking input on whether the evidence for the endpoints for which PODs were developed is strong enough for dose-response, and if there is strong enough evidence to derive PODs for additional endpoints. Some of the issues addressed here overlap with the responses to the earlier systematic review charge questions.

Recognizing that the EPA PFOA and PFOS assessments will provide the basis for the PFOA and PFOS MCLGs, they will likely have large practical impacts and will receive extensive

attention and scrutiny. Therefore, the Panel stresses that a strong and transparent rationale for the conclusions about strength of evidence for health outcomes, as well as other components of the assessment, should be provided.

The draft MCLG documents include levels of confidence (i.e., strength of evidence conclusions) in the evidence synthesis sections while the 2016 HESDs did not. The inclusion of such strength of evidence conclusions can clarify the rationale for the selection of health endpoints/systems for consideration for POD development. However, a consistent process for determination of the strength of evidence designations is not clearly described or followed. The sections on Human Evidence and Animal Evidence for the specific health effects/outcomes are often difficult to follow due to the highly summarized presentation of the information and the inconsistent presentation of information and conclusions for different health outcomes (discussed in detail below).

EPA stated that consistent language and a structured approach were not used to describe the level of evidence for different health effects because the health endpoints sections were written by different scientists and the conclusions are based on professional judgment. Specifically, consistent terminology and a consistent approach were not used for the synthesis and integration of human, animal, and mechanistic evidence to make overall hazard conclusions. As discussed in more detail below, the Panel recommends that a consistent approach be used.

The Panel recognizes that due to time and resource constraints the revisions suggested below may not be feasible for all endpoints included in the drafts. The draft MCLG documents, as well as evaluations by others, indicate that the human evidence for noncancer effects is strongest for effects on the liver, immune system, serum lipids, and fetal growth. The effects with the strongest evidence from animal studies could also be identified; these would likely include hepatic, immune system, and developmental effects, and possibly others. The Panel recommends that EPA focus on revisions to the human, animal, and mechanistic evidence sections and evidence integration sections for those endpoints that have the strongest evidence, rather than for all endpoints and additional endpoints also be evaluated systematically, possibly over a longer timeframe. Conclusions about weight of evidence and PODs for additional endpoints may be important to inform assessments of PFAS mixtures, as described in the draft Mixtures Framework document.

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.

Need for a consistent approach and terminology

Approach for evidence synthesis and integration

The Panel noted that the hazard identification process in the draft MCLG documents is not fully transparent or consistent, either in the evaluation of different evidence "streams" (human, animal, mechanistic), or in their integration. Consistency in the evaluation of human and animal

studies, as well as mechanistic data, is important in increasing confidence in the validity of the hazard determinations derived from each of these evidence streams. Several public comments also included similar points. For instance, although study quality/risk of bias is discussed, the "synthesis" within an evidence stream does not appear to be structured. Examples of how to structure "synthesis" include the Hill criteria (see IRIS 2020 Handbook and NASEM review of the IRIS Handbook), GRADE, NTP/OHAT, and the Navigation Guide. Similarly, there is no framework for integration of human, animal, and mechanistic data, which is also addressed in several of these examples. These frameworks should include templates for tabular summaries of evidence both "within" and "across" evidence streams that could be adapted to provide more transparency.

The Panel suggests that a format or template be developed so that the information can be presented consistently for each endpoint. For example, an existing framework could be used to make a synthesis conclusion for each evidence stream (human, animal, mechanistic), and an overall hazard conclusion based on integration across the evidence streams. This would provide additional transparency as to the hazard conclusions, as well as clarify the reasons why a particular health endpoint is chosen for candidate RfD derivations. Relevant to this point, Section 2.6 in the Systematic Review section states that modified Hill criteria were discussed for each health outcome, but it appears that this was not actually done in the Hazard Identification section. Inclusion of such a discussion would help to address the issues mentioned above.

Summary tables that present basic information for each study, such as study population (e.g., general population, children, pregnant women, occupational, etc.) for epidemiology studies and lab animal species for toxicology studies, exposure range or dose levels, and overall results for the endpoints evaluated (e.g., significant increase, significant decrease, or no effect) would be helpful to understanding the overall human and animal evidence.

Terms used for strength of evidence designations

Terms such as "suggestive evidence," "moderate evidence," and other seemingly interchangeable terms are used in sections on different health outcomes. These terms are not defined in the draft MCLG documents, and it is unclear whether there is an intended difference among these seemingly similar terms. Additionally, no strength of evidence term is used in some cases, such as, "…*the evidence indicates an association between increased serum levels of PFOS and decreased antibody production following routine vaccinations in children*," and "*EPA concluded the impaired IgM response reported in [animal studies] supported the human results and this endpoint was considered for POD derivation [for PFOA]*."

To address these issues, the Panel recommends that consistent descriptors be defined and used for human, animal, and overall strength of evidence conclusions for each endpoint. While recognizing that approaches from the draft 2020 ORD IRIS staff handbook were not used for strength of evidence determinations in the draft MCLG documents, the table of "Evidence integration judgments for characterizing potential human health hazards in the evidence integration narrative" (Table 11-5) in the draft 2020 ORD handbook provides the following descriptors: "evidence demonstrates", "evidence indicates [likely]"; "evidence suggests but is not sufficient to infer"; "evidence inadequate"; and "strong evidence supports no effect." Criteria

and examples for applying these descriptors are also provided. Such an approach would be consistent with the use of consistent descriptors and consistent criteria for selection of descriptors for the domains and overall confidence conclusions in the individual study evaluations earlier in the overall process. It would also be consistent with the use of descriptors and criteria for selection of the descriptors for weight of evidence for carcinogenic potential in the U.S. EPA (2005) Guidelines for Carcinogen Risk Assessment.

Consistency in terminology for conclusions about strength of evidence

The Panel also noted inconsistencies in the terminology used to describe the strength of evidence in the health outcomes sections of the draft MCLG documents and how they are applied. For example, in some cases it is stated that there is a certain level of evidence (e.g., suggestive) for "associations of PFOA [or PFOS] with [the effect]", while in other cases, it is stated that there is a certain level of evidence (e.g., suggestive) that PFOA or PFOS "impacts [the effect]" or a certain level of evidence "for [the effect]" (or similar language). It is not clear whether these different terms are intended to distinguish between the level of evidence for an association versus the level of evidence supporting a plausible relationship between PFOA or PFOS and the effect. The Panel recommends that the intended meaning of the strength of evidence conclusions for each health outcome be clarified and that consistent terminology be used for describing these conclusions.

As mentioned above, Section 2.6 in the Systematic Review section states, *"a summary discussion that addresses considerations regarding causation as adapted from Hill (1965)*" is provided for each health outcome. However, such a discussion does not appear to be included for many or most of the health outcomes in the Noncancer Hazard Identification section. The Panel suggests including such a summary discussion of considerations for causation adapted from Hill (1965) to address the issue mentioned above.

Focus on key health outcomes

As above, the Panel recognizes that time limitations may prevent incorporation of the suggestions discussed above for all health outcomes. A possible approach to address this issue would be to focus on those endpoints determined to have the strongest evidence, rather than for all endpoints. Moreover, the Panel recommends that additional endpoints should also be evaluated systematically, possibly over a longer timeframe. Conclusions about weight of evidence and PODs for additional endpoints may be important to inform assessments of PFAS mixtures, as described in the draft EPA Mixtures Framework document.

Considering human studies from 2016 HESD

(Note: Some of the comments on this issue are also relevant to the response to the Systematic Review charge question above.)

The Panel concluded that the rationale for not considering human studies which were included in the 2016 HESDs is not clear or supportable. There is no reason to believe that the earlier studies are less relevant or of lesser quality than the newer studies. Consideration of all human studies is especially important because conclusions about the level of evidence for human health effects,

which are generally observational rather than experimental, are based on the overall weight of evidence from all relevant data.

The 2016 HESD for PFOA concluded that there was substantial evidence for human effects of PFOA, as follows: "*Human epidemiology data report associations between PFOA exposure and high cholesterol, increased liver enzymes, decreased vaccination response, thyroid disorders, pregnancy-induced hypertension and preeclampsia, and cancer (testicular and kidney)*" and that "*…human data identified significant relationships between serum levels and specific indicators of adverse health effects…*" The reason that human studies were not considered as the basis for PODs and RfDs in the 2016 HESDs was not related to the quality of the studies themselves or to a lack of overall evidence for human effects, but was rather due to the absence of a toxicokinetic model to relate internal dose (serum levels) to external exposure (administered dose). The 2016 PFOA HESD states that human data were not used as the basis for PODs and RfDs because of "*lack [of] the exposure information for dose-response modeling*." This is no longer an issue because EPA has now accepted a model (Verner et al., 2016) that can relate serum PFOA and PFOS levels to external dose during developmental lifestages and throughout life, and it has concluded that the human data for PFOA and PFOS can be used in dose-response modeling.

The evaluation of the association of PFOA and high cholesterol in occupationally exposed workers in the draft PFOA document illustrates the need to consider all epidemiology studies, including those considered in the 2016 HESD, in making strength of evidence conclusions. As stated in Section 3.3.5.1.2.1, the 2016 HESD concluded that there was "*relatively consistent and robust*" evidence of an association of PFOA and increased serum cholesterol in the occupational worker studies. However, the draft PFOA document reviewed only the three newer occupational worker studies not included in the 2016 HESD, all of which were rated as "low confidence." The draft PFOA document states that these newer studies "*suggest no association between PFOA and TC in workers*" and that "*differences in findings from occupational studies between the 2016 [HESD] and this review may be attributable to the limitations of occupational studies in this review*." There does not appear to be a supportable rationale for making a conclusion based on only three low confidence studies when other potentially stronger studies are also available. As such, the Panel recommends that EPA also consider both the older and newer worker studies to strengthen the overall of weight of evidence conclusion.

Another important aspect of this issue is that specific human epidemiology studies included in the 2016 HESDs may be preferable to the more recent studies for POD development for some health endpoints. As an illustration of this point, the PFOA and PFOS RfD are based on a dataset from Grandjean et al. (2012), a study that was included in the 2016 HESDs. Specifically, the RfDs are based on serum PFOA or PFOS levels at age 5 and tetanus or diphtheria vaccine antibody concentrations at age 7 in Cohort 3 (born 1997-2000) from Grandjean et al. (2012). Subsequent studies from the same research group (Mogensen et al., 2015; Grandjean et al., 2017) re-published this dataset and/or used this dataset in some of their analyses, but the BMDL (from Budtz-Jorgensen and Grandjean, 2018) that was used as the POD for the final RfD comes only from the dataset originally presented in Grandjean et al. (2012). If the subsequent papers that included the dataset from Grandjean et al. (2012) in additional analyses had not been published, the Grandjean et al. (2012) study and dataset used as the basis for the RfDs would not have been considered for POD development. This example clearly demonstrates why the older human studies included in the 2016 HESD should be considered.

Additional details relevant to this point are: Grandjean et al. (2012) is not included in the table summarizing epidemiology studies (Table C-7) in the draft MCLG documents, and there is no systematic review evaluation of Grandjean et al. (2012) in HAWC. If the dataset from Grandjean et al. (2012) remains the basis for the PFOA and PFOS RfDs, the Panel suggests that a HAWC systematic review of this study be performed and included in the final document.

Consideration of mechanistic information

Evaluation of the mode(s) and/or mechanism(s) of action for toxicity is normally an important part of toxicity assessments such as these draft MCLG documents. However, there is frequently little or no discussion of mechanistic information in the draft MCLG documents, with only summary tables of the number of studies with each type of mechanistic information that were identified, a reference to the section in the 2016 HESD on this topic, and a statement that an updated evaluation will be completed after the SAB review. Mechanistic/mode of action data can help to inform conclusions about the human relevance of effects observed in animal species and the plausibility of effects observed in humans, among other areas of potential uncertainty. 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.

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).

Selection of endpoints and studies for POD development

Selection of endpoints for POD derivation

In general, the rationale and criteria for the selection of endpoints to derive a POD are not always clearly presented. PODs can potentially be used as the basis for RfDs; therefore, it is important to clearly demonstrate that the endpoints selected for POD derivation are well established, sensitive, adverse or precursor to adverse effects, and that endpoints from animal studies are relevant to humans.

Importantly, the Panel noted that the draft MCLG documents do not clearly distinguish the process of hazard identification from the process of dose-response assessment. For instance, the Evidence integration sections discuss POD derivations as part of Evidence synthesis. Separating the steps of hazard identification and identifying PODs would provide greater transparency. In particular, hazard identification should not depend on whether the data can provide PODs, but rather only health effects with a certain level of confidence in the "hazard" are considered for dose-response. This separation of hazard and dose-response is important because studies that provide strong evidence for hazard do not necessarily need to be amenable to POD derivations (e.g., epidemiologic studies with semi-quantitative exposure metrics), and conversely, the availability of studies amenable to POD derivations is not sufficient to provide strong evidence of hazard. The first step is determining that there is sufficient strength of evidence for "hazard" (independent of dose) to support POD derivation. Then, when selecting studies for POD derivation, determine both whether studies are amenable to POD derivation (especially BMD analysis), as well as study confidence/quality indicators.

Furthermore, the Panel noted that the information about POD derivation for effects described as having "suggestive" evidence in different sections of the draft MCLG documents is internally inconsistent. The documents state (PFOA - p. 317; PFOS – p. 290) that human studies showing an "association" for an effect were used for PODs, but it then goes on to indicate that human health effects with "suggestive" evidence were not used for PODs, as follows (emphasis added): "*Well-conducted … human studies were prioritized for POD derivation and compared to PODs derived from animal data when possible when the human data provided an association between PFOA and an adverse effect. Such human studies were available for immunotoxicity, developmental, serum lipid, and hepatic effects. For other* ***health effects where the epidemiological data were suggestive of adverse effects*** *…., dose response data from the animal studies were prioritized*." Although the text quoted above indicates that human health effects with suggestive evidence were not used for PODs, the human health effects that were used for PODs (immune, developmental, serum lipids [for PFOA], hepatic) were described as having "suggestive" or "moderate" evidence; a conclusion of "strong" evidence was provided only for PFOS exposure and increased serum lipids. While recognizing that EPA did not use approaches from the draft 2020 ORD staff handbook for strength of evidence determinations in the draft MCLG documents, it should be noted that the draft 2020 handbook does not recommend that PODs for health endpoints with "suggestive" evidence be used as the basis of toxicity values (i.e., Reference Doses). Because of the uncertainty associated with such PODs, the draft 2020 handbook recommends that they be used only for range finding and prioritization.

The Panel recommends that EPA address the internal inconsistencies mentioned above. Additionally, the Panel suggests that EPA consider reevaluating its strength of evidence conclusions for some human endpoints, including (but not necessarily limited to) decreased immune response, increased liver enzymes, increased serum lipids (for PFOA), and decreased fetal growth to determine if they are better described as having "likely" or "strong" evidence rather than "suggestive" or "moderate" evidence. A conclusion of "likely" or "strong" evidence would provide additional support for development of a POD to be used as the basis for the RfD.

Based on the draft 2020 handbook criteria for "likely" evidence,[1] which can be selected in certain circumstances when animal and/or human evidence are only "moderate," and the assumption that "strong evidence" mentioned in the charge question is equivalent to "evidence demonstrates" or "likely evidence" in the draft 2020 handbook, it appears that there may be sufficient evidence to classify additional endpoints as "strong" or "likely." This is particularly true if the studies included in the 2016 HESD and more recent studies published after the ending date of the literature search for the draft MCLG documents are considered. As mentioned above, consideration of the earlier studies from the 2016 HESD is necessary for determining the overall weight of evidence for each health outcome.

Using immunosuppression as one example, the Evidence Integration section (3.3.4.4) for immune effects of PFOA states: "*The evidence of an association between PFOA exposure and immunosuppressive effects in human studies is moderate based on largely consistent decreases in antibody response following vaccination … in two medium-confidence, overlapping birth cohorts*." Consideration of studies that evaluated vaccine response in populations in other locations, including older studies from the 2016 HESD and recent studies not included in the current draft MCLG documents (additional studies have been suggested by SAB Panel members), could potentially support the conclusion that the evidence for decreased antibody response to vaccines is stronger than "moderate." Associations of PFOA and PFOS exposure with infectious disease are also relevant to strength of evidence for immunosuppression; this topic is discussed in detail below.

Selection of specific studies for POD development

The Panel recommends that a clearer explanation be provided throughout the MCLG documents as to why specific studies were selected for POD development when there are a number of possible choices. As one example, the BMDL presented in Dong et al. (2019) was used by EPA as the POD for increased serum cholesterol. However, no information is provided in the PFOA document as to why Dong et al. (2019) was selected for dose-response for serum lipids from the many studies that are available. Furthermore, the BMD modeling section (Appendix B) states that few details are provided by the authors about several aspects of the BMD modeling presented in the Dong et al. (2019) publication; this lack of information does not appear to support use of this BMDL as a POD.

It is possible, although not stated in the draft MCLG documents, that Dong et al. (2019) was selected because studies included in the 2016 HESD were not considered. Again, the Panel strongly recommends that older studies that were included in the 2016 HESD be considered for

[1] For "evidence indicates (likely)", there should be "an evidence base that indicates that [PFOA] exposure likely causes [the health effect] in humans, although there may be outstanding questions or limitations that remain…," and "this conclusion level is used if there is robust animal evidence supporting an effect and slight-to-indeterminate human evidence, or with moderate human evidence when strong mechanistic evidence is lacking. This conclusion level could also be used with moderate human evidence supporting an effect and slight or indeterminate animal evidence, or with moderate animal evidence supporting an effect and slight or indeterminate human evidence. In these scenarios, any uncertainties in the moderate evidence are not sufficient to substantially reduce confidence in the reliability of the evidence, or mechanistic evidence in the slight or indeterminate evidence base (e.g., precursors) exists to increase confidence in the reliability of the moderate evidence."

POD development, and the Panel notes that some of these older studies were considered in the EPA's draft "Analysis of CVD Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water." As discussed in the response to the Systematic Review charge question above, there is no reason to assume that the more recent studies are preferable to the older studies, as illustrated by the choice of a dataset from Grandjean et al. (2012) as the basis for the final RfDs for both PFOA and PFOS in the draft MCLG documents.

Additional comments about the studies and endpoints used for PODs

The list of studies and endpoints considered for POD development in Table 15 is not totally consistent with the information in the text and the dose-response results in Appendix B. For example, some of the studies/endpoints (corpora lutea, body weight) stated in the text of the PFOA document to have been selected for dose-response/POD development were not modeled, while other endpoints (kidney weight) stated not to be selected for dose-response/POD development were modeled. Also, the Panel notes that the draft PFOA document states that prenatal loss was modeled from Lau et al. (2006), but the data from Wolf et al. (2007), not Lau et al. (2006), were actually modeled.

There is also inconsistency in the evidence integration sections for different health outcomes regarding whether any human and/or animal studies were selected for POD development and, if so, which ones. For example, the human evidence integration discussions for developmental effects do not state whether any of the human studies were selected for POD development, although several birthweight studies were later selected (Table 15) for both PFOA and PFOS. As another example, the evidence integration sections for immune effects in both MCLG documents do not state that a human study of vaccine response (which eventually was used as the basis for the final RfD) was selected for POD development. In contrast, the evidence integration sections for some other endpoints specifically mention whether or not any studies were selected for POD development, and, if so, which studies were selected.

Finally, while it is stated (in the text quoted on p. 20 above - PFOA - p. 317, first paragraph; PFOS – p. 290, final paragraph) that PODs were developed from human data for hepatic effects, human data for hepatic effects were not used for POD development (as stated for PFOA on p.148, for PFOS on p. 133, and in the charge question below).

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.

Strength of evidence designations for specific health outcomes

This section of the response provides comments on discussions and conclusions on specific noncancer health outcomes in the Hazard Identification sections of the draft MCLG documents. The comments above on general issues with the determination of strength of evidence are also relevant to this part of the response.

Evidence for effects on the immune system, liver, fetal growth, and serum lipids

The Panel agrees with EPA that the most consistent epidemiological associations with PFOA and PFOS exposure are for decreased immune system response, decreased fetal growth (e.g., decreased birthweight), increased serum lipids, and increased liver enzymes, particularly alanine aminotransferase (ALT). Multiple studies for each of these four effects are generally consistent in different populations and settings, and the total body of evidence indicates that these effects are present. While there is no single definitive study for any of these endpoints (which is not a realistic goal), multiple studies of adequate quality pointing in the same direction justifies the conclusion that PFOA and PFOS are associated with these health endpoints. Additional evidence from animal studies further supports the conclusion that PFOA and PFOS cause these effects. The available data are more limited and/or the evidence is less consistent for associations of PFOA and PFOS with other health endpoints including effects on the thyroid, ulcerative colitis, neurodevelopmental effects, and others.

For the four most consistent endpoints, most studies report relatively small changes in clinical biomarkers. While most of these studies did not evaluate the number of subjects with a clinically abnormal value for biomarkers, one or more studies, for each of the four effects, reported an association of PFOA and/or PFOS with increased risk of a clinically abnormal value. Examples of studies that reported an increased risk of clinically abnormal values are as follows: Grandjean et al. (2012) reported tetanus or diphtheria antibodies levels below a clinically protective level; Looker et al. (2014) found an increased risk of not attaining the antibody threshold considered to offer long-term protection to A/H3N2 influenza virus; multiple studies reviewed in the draft MCLG documents found clinically defined low birth weight or small for gestational age; Steenland et al. (2009) observed clinically defined high cholesterol; Gallo et al. (2012) and Darrow et al. (2016) reported clinically defined elevated ALT. In studies where the number of subjects with clinically abnormal values was not specifically evaluated, an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to PFOA or PFAS cannot be predicted on an individual basis, the increased number of individuals within a population with clinically defined abnormal values is of public health concern.

An increase in the clinical diseases related to the biomarkers mentioned above has not been consistently demonstrated for several of these endpoints. As discussed in the response to the next charge question, the limited available information does not demonstrate an increase in liver disease although data on hepatic effects of PFAS in animals and humans indicate that additional research on PFAS and liver disease is needed. Similarly, as discussed in the draft PFOA document, the limited available evidence does not demonstrate an association with cardiovascular disease and more research is needed. As mentioned above, multiple studies

support an association of PFOA and PFOS with clinically defined low birth weight/small for gestational age. However, the Panel is not aware of evidence for associations of PFOA and PFOS with adverse consequences such as developmental delays in low birth weight/small for gestational age infants.

Regarding associations of PFOA and PFOS and infectious disease, a recent review that focused on PFOS (Pachkowski et al., 2019) concluded that studies available through 2018 "provide evidence for an association between general population levels of PFOS exposure and infectious disease, a clinical meaningful measure of health risk," and another review that focused on PFOA (Steenland et al., 2020) concluded, "evidence that PFOA increases risk of human infectious disease is inconsistent." The Human Evidence subsections of the Immune sections outline the findings from studies of PFOA and PFOS and infectious disease. While results of these studies are not consistent, many of them reported associations with infectious disease. However, the Evidence Integration subsections of the Immune sections neither provide conclusions on associations with infectious disease nor mention infectious disease; the reason for this omission is unclear, especially since immunosuppression is the critical effect for the RfD. Additionally, several recent studies, not cited in the draft MCLG documents (e.g., Timmermann et al., 2020; Dalsager et al., 2021; Bulka et al., 2021), report associations for both PFOA and PFOS with increased risk of infectious disease. The Panel recommends that weight of evidence conclusions be developed for infectious disease in the revised documents.

Clarifications in Hazard Identification information about serum cholesterol

The Panel concluded that it is especially important to provide a clear and thorough discussion of the strength of evidence for the association of PFOA and PFOS and increased serum cholesterol since this effect is a major part of the basis for the separate evaluation of cardiovascular disease risk. The draft MCLG documents state that PFOA and PFOS caused decreased serum lipids in some animal studies while lipids are increased in human studies. They further state that this interspecies difference may be due to the "*difference in serum lipid composition between humans and commonly used rodent models*" and that "*food consumption may confound these results, as diet is a major source of lipids, yet studies do not consistently report a fasting period before serum collection*."

Other potentially important explanations for this human versus animal difference in the effect of PFOA and PFOS on serum lipids should be discussed in the draft MCLG documents. These include much lower human exposure levels compared to the doses used in animal studies (see discussion below), differences in the fat content of human diets versus rodent lab diet, and differences in the activity of PPAR-α in humans and laboratory animals. Studies that investigated these issues include New Jersey Drinking Water Quality Institute (DWQI, 2017), Tan et al. (2013), Rebholz et al. (2016), and newer studies such as Schlezinger et al. (2020).

In general, human and rodent data suggest that the effects of PFOA and other PFAS on lipid formation and storage results from the balance of different effects which may act in opposite directions (Das et al., 2017). The decrease in serum lipids at the higher doses used in animal studies is believed to be due to activation of PPAR–α (DWQI, 2017). PPAR–α is also active in humans, as demonstrated by the use of PPAR–α activating drugs to decrease high cholesterol in humans. However, PFOA, PFOS, and other PFAS do not activate PPAR–α in humans at lower

environmentally relevant doses, and the increased serum lipids associated with PFOA, PFOS, and other PFAS may result from activation of other receptors and/or biological pathways involved with lipid metabolism that act in the opposite direction.

Convertino et al. (2018) is a study of advanced cancer patients who were given extraordinarily high doses of PFOA. The draft PFOA document acknowledges concerns and limitations of this study. It states that "*participants dosed with extremely high levels of ammonium perfluorooctanoate (APFO), a PFOA precursor, in an open-label, nonrandomized, phase 1 trial, were found to have reduced levels of total cholesterol with increasing plasma PFOA concentrations*." It further states that Convertino et al. (2018) "*differed from the other studies in several ways. First, all participants were solid-tumor cancer patients who failed standard therapy. Second, participants ingested APFO rather than being exposed to PFOA. Third, participants' plasma PFOA concentrations were several orders of magnitude higher than those reported in the general population*," and that "*it is unclear if these factors explained the inverse association between PFOA and total cholesterol*." This study was rated as "low confidence" by EPA.

While Convertino et al. (2018) may arguably fulfill the PECO criteria for health effects studies of PFOA in humans shown in Table 2, the Panel concluded that this study does not appear to be appropriate for consideration in hazard identification of PFOA. Some of these concerns were described in the NJDEP (2020) document which states that Convertino et al. (2018) "*is not useful in the evaluation of potential health effects of chronic drinking water exposure to PFOA in the general population*," and that "*limitations of this study include small sample size, very short length, limited power of study, and potential altered metabolic state of study group consisting of late-stage cancer patients. Observations in these patients cannot be considered relevant to healthy individuals because their nutritional and physiological status was likely affected by their severe illness*."

The draft PFOA document acknowledges some of these problematic issues with Convertino et al. (2018). If consideration of this study remains in the PFOA document, the Panel suggests that the additional information below about potential PFOA-related toxicity in the study, the dose-response for increased cholesterol, and the lack of relevance of the possible increase in serum cholesterol to environmental exposures be included, as follows.

An earlier abstract about this study (Macpherson et al., 2010) stated that one of the patients dosed experienced drug related toxicity (DLT) consisting of "grade 5 renal failure and transaminitis" (indicative of liver damage), and that these effects were noted as "possibly drug related." This indicates the potential for PFOA to cause renal and hepatic toxicity in humans, and that it is unclear why the observation of "possibly drug related" kidney and liver toxicity reported by Macpherson et al. (2010) was not mentioned by Convertino et al. (2018).

Also, as mentioned above, the plasma PFOA levels in the subjects in this study were extraordinarily high. The plasma PFOA levels in the 10 exposure categories shown in Figure 4 of Convertino et al. (2018) ranged from ~4000 ng/ml to ~630,000 ng/ml. Cholesterol was decreased only in the three highest exposure categories (approximately 262,000 ng/ml or higher plasma PFOA), but not in the seven lower exposure categories that also had extremely high plasma PFOA levels of up to approximately 200,000 ng/ml. The plasma PFOA levels at which

cholesterol was decreased are many orders of magnitude above those found in the general population or in communities with contaminated drinking water. They are higher than the highest serum or plasma PFOA levels in occupationally exposed workers in data summarized in Table 5-27 of ATSDR (2021), and they are similar to the serum PFOA levels at which cholesterol is decreased in animal studies, presumably through activation of PPAR–α. The observation of decreased cholesterol at these extremely high plasma concentrations is consistent with the effects of PPAR–α activating drugs that reduce serum cholesterol in humans. In contrast, the increased cholesterol associated with PFOA in the general population and in individuals exposed through contaminated drinking water likely occurs through a different mechanism that is operational at much lower PFOA concentrations.

Consistency in consideration of medications that may affect health outcomes

The Panel also suggests that EPA be consistent in consideration of the use of medications that may affect health outcomes. Specifically, the use of blood pressure medications was not considered in evaluation of blood pressure studies, while studies of serum lipids were rated as deficient if they did not consider lipid lowering medications.

Additional endpoints that should be considered for POD derivation

The list of health effects/endpoints considered appears to be generally complete and appropriate. The Panel noted that the number of the health effects considered for POD derivation have been expanded since the 2016 HESDs from less than 10 effects, all of which were from animal toxicology studies to more than 20 effects, that include effects from both animal toxicology and human epidemiology studies, in the current draft.

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.

Charge Question #2B - Elevation of ALT

Elevation of liver serum biomarkers in humans is frequently used an indication of liver injury, although it hasnot 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

The Panel does not 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 for the following reasons:

The draft EPA documents do not provide a compelling reason for considering the changes in ALT to be substantially less supported than other endpoints such as changes in birth weight, serum lipids, or antibody responses. None of these are clinical disease endpoints, but all of them are indicative of an increased risk for adverse health effects, and all have a similar level of evidence as that for the effects due to changes in ALT. The elimination of ALT as an endpoint for POD derivation therefore does not appear to be totally consistent with the rationale for developing PODs for some other human health effects or with EPA risk assessment guidance. As stated in the charge question, increased ALT is indicative of liver damage, and U.S. EPA (2002) guidelines for RfD development state that a RfD should be based on an adverse effect or a precursor to an adverse effect. The Panel noted that, although the magnitude of PFOA and/or PFOS's effect on ALT may not be large, the same may also be true for the magnitude of the PFOA and/or PFOS's effects on other human health endpoints such as, increased cholesterol and decreased birth weight. As such, if a POD is not developed for the ALT endpoint, an explanation should be provided as to why the magnitude of the effect was not sufficient for ALT but was sufficient for other effects of similar magnitude.

Numerous observations in human epidemiological studies that associate serum PFOA and PFOS concentrations with serum ALT, and multiple rat and mouse studies that also demonstrate PFOA and PFOS administration can raise serum ALT, consistent with the epidemiological studies. This observation has been made by numerous research groups in more than one animal species, such that ALT represents a reproducible and rigorous endpoint that is predictive of adverse health effects. A recent systematic review concluded that exposure to both PFOA and PFOS is

associated with increased ALT in humans and that both compounds cause increased ALT and steatosis in rodents (Costello et al., 2022). In fact, California EPA (2021) selected increased risk of clinically elevated serum ALT as the basis for its draft RfD for PFOA. In their evaluation, California EPA (2021) considered the issues related to use of elevated ALT in humans as a critical effect that are discussed in the charge question above. The Panel suggests that EPA consider the California EPA (2021) rationale for its decision to use elevated ALT as the critical effect for RfD development. If EPA decides to develop a POD for elevated ALT, the agency should also consider the modeling approach used by California EPA for this effect.

Regarding a clinical definition for elevated ALT, the draft EPA PFOA document discusses Darrow et al. (2016), which reported an association between modeled PFOA exposure and increased risk of clinically elevated ALT in a population with elevated PFOA exposure from contaminated drinking water. Gallo et al. (2012), which was not included in the draft PFOA document but was reviewed in the 2016 HESD, also found an association between measured serum PFOA levels and increased risk of clinically elevated ALT in the same study group.

Furthermore, as noted in a recent review of epidemiological evidence for health effects of PFOA by Steenland et al. (2020): “There is also evidence that effects on ALT are more pronounced among obese subjects, who are at higher risk of non-alcoholic fatty liver disease (Lin et al. 2010; Jain and Ducatman 2019).” A more recent systematic review of human and animal data on hepatic toxicity of PFAS (Costello et al., 2022) and a commentary on this study (Ducatman and Fenton, 2022) also conclude that additional research on the PFAS and non-alcoholic fatty liver disease is needed.

Also relevant to the potential significance of elevated ALT, Steenland et al. (2020) concluded that while "the limited existing evidence does not support a link between PFOA and diagnosed liver disease," there is a lack of "adequately powered epidemiologic studies of liver disease" and PFOA. They stated for "the established liver toxicity of PFOA in experimental animal studies …, the storage of PFOA in liver tissue in humans, and extensive evidence that PFOA exposure is associated with markers of hepatocyte cell death, warrants additional research on PFAS and liver disease, particularly non-alcoholic fatty liver disease."

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.

Studies that support the ALT levels as markers of adverse liver effects

There are numerous examples of associations of elevated ALT with disease endpoints, as well as <2-fold increase in serum ALT being associated with pathology-confirmed liver disease, such as non-alcoholic fatty liver disease (NAFLD). Importantly, the American Association for the Study of Liver Diseases (AASLD) has a position paper (Kim et al., 2008) which states that serum ALT may be a predictor for overall health and mortality. Thus, the Panel views it as inappropriate to

conclude that less than a 2-fold change in serum ALT is innocuous and without risks to human health.

According to the American College of Gastroenterology (ACG; Kwo et al., 2017), "Multiple studies have demonstrated that the presence of an elevated ALT has been associated with increased liver-related mortality." Kwo et al. (2017) further state:

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

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

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

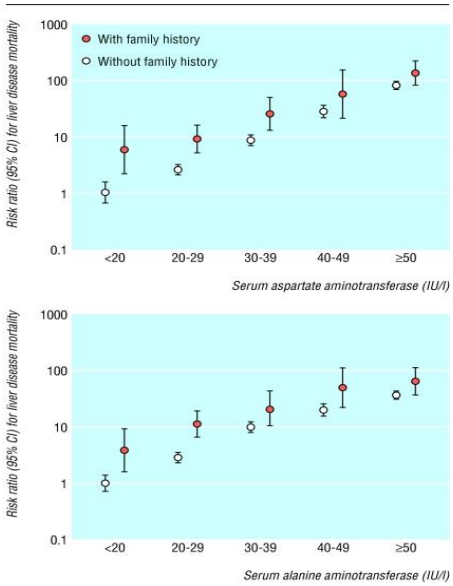
ALT, alanine aminotransferase; AST, aspartate aminotransferase; F, female; RR, relative risk; M, male; ULN, upper limit of normal.

The AASLD guidance (Kim et al., 2008) regarding serum ALT levels as an indicator of health and disease article argues that serum ALT is more than a marker for liver injury, and that ALT "may also be a good indicator of overall health, particularly in the context of obesity, the metabolic syndrome, and presence of cardiovascular disease, as many patients affected by these conditions also are at risk of having non-alcoholic fatty liver disease."

With regard to asymptomatic liver disease, a Scandinavian study (Mathiesen et al., 1999) of 151 consecutive patients with mild to moderate elevations of serum aminotransferase levels for at least 6 months in combination with liver biopsy revealed that non-alcoholic steatohepatitis and

hepatic steatosis (noted in 42%), chronic hepatitis C virus (15%), alcoholic liver disease (8%) and autoimmune hepatitis, primary biliary cirrhosis and alpha1 antitrypsin deficiency was associated with only slight to moderate elevation in serum ALT.

Kim et al., British Medical Journal (BMJ) Clinical Research, 328(7446):983, 2004. Figure 1.



Kim et al., 2004 reported a population-based study that included 142,055 individuals of ages between 35 and 59 years in whom baseline demographic and laboratory data obtained between 1990 and 1992 were available that found a positive association between the aminotransferase concentration and mortality from liver disease in serum ALT values in normal range (35-40 IU/l). The risk ratio for liver disease mortality also correspondingly increased as serum ALT increased (see Figure 1).

In Lee et al. (2008), of 47,182 county residents in Minnesota who had healthcare encounters in 1995, 6,823 (14.5%) had their ALT measured, of which 5,912 had results within normal limits and 911 (13.4%) abnormal. Abnormal AST was associated with a significantly increased standardized mortality ratio (1.32 for 1-2x ULN and 1.78 for >2x ULN).

Ruhl et al. (2013) used NHANES 1988–1994 data and compared the mortality risk of persons in ALT deciles 1, 2, 3, and 10 to that of persons in deciles 4–9 (mortality was relatively flat across these deciles) over an 18-year period (through 2006) among 14,950 viral-hepatitis-negative adults. High ALT was associated with increased mortality risk.

Moreover, there are case examples of normal ALT in the presence of histologic non-alcoholic fatty liver disease. For example, in a cohort of 458 subjects in Italy (Fracanzani et al., 2008), non-alcoholic steatohepatitis was diagnosed in 59% of the patients with normal ALT, respectively. Another cross-sectional study (Park et al., 2015) using Korean NHANES data saw an association with the upper limit of the normal range for ALT with liver disease. They defined the 95th percentile levels for ALT in healthy weight, metabolically normal, liver disease-free

Korean NHANES participants as 34 IU/L for men and 25 IU/L for women. The sensitivity for detecting chronic liver disease was significantly improved when the threshold for normal ALT was lowered.

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 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.

Consideration of other endpoints

Members of the Panel also suggested the inclusion of non-alcoholic fatty liver disease/steatosis as an adverse liver endpoint. As noted by Steenland et al. (2020), a recent study of adults from a community with elevated exposure to PFOA from contaminated drinking water showed that PFOA "was associated with cytokeratin 18 M30, a marker of hepatocyte apoptosis (Bassler et al., 2019), and a mechanism of disease progression in non-alcoholic fatty liver disease." Bassler et al. (2019) provides further evidence that PFOA causes liver cell injury, and the Panel suggests that EPA consider this study in addition to other relevant references, including:

Bassler J, Ducatman A, Elliott M, Wen S, Wahlang B, Barnett J, Cave MC. (2019). Environmental Perfluoroalkyl Acid Exposures Are Associated with Liver Disease Characterized by Apoptosis and Altered Serum Adipocytokines. *Environ Pollut*. 247:1055-1063. doi: 10.1016/j.envpol.2019.01.064. PMID: 30823334, PMCID: PMC6404528.

Costello, E., Rock, S., Stratakis, N., Eckel, S. P., Walker, D. I., Valvi, D., Cserbik, D., Jenkins, T., Xanthakos, S. A., Kohli, R., Sisley, S., Vasiliou, V., La Merrill, M. A., Rosen, H., Conti, D. V., McConnell, R., & Chatzi, L. (2022). Exposure to per- and Polyfluoroalkyl Substances and Markers of Liver Injury: A Systematic Review and Meta-Analysis. Environmental health perspectives, 130(4), 46001. https://doi.org/10.1289/EHP10092

Ducatman, A., & Fenton, S. E. (2022). Invited Perspective: PFAS and Liver Disease: Bringing All the Evidence Together. Environmental health perspectives, 130(4), 41303. https://doi.org/10.1289/EHP11149

Jin R, McConnell R, Catherine C, Xu S, Walker DI, Stratakis N, Jones DP, Miller GW, Peng C, Conti DV, Vos MB, Chatzi L. (2020). Perfluoroalkyl Substances and Severity of Non-alcoholic Fatty Liver in Children: An Untargeted Metabolomics Approach. *Environ Int*. 134:105220. doi: 10.1016/j.envint.2019.105220. PMID: 31744629, PMCID: PMC6944061.

Wahlang B, Jin J, Beier JI, Hardesty JE, Daly EF, Schnegelberger RD, Falkner KC, Prough RA, Kirpich IA, Cave MC. (2019). Mechanisms of Environmental Contributions to Fatty Liver Disease. *Curr Environ Health Rep*. 6(3):80-94. doi: 10.1007/s40572-019-00232-w. PMID: 31134516, PMCID: PMC6698418. (Table 1 of this manuscript contains additional citations exploring fatty liver disease and associations with PFOA/PFOS).

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.

Designation of PFOA as a "likely carcinogen"

EPA *Guidelines for Carcinogen Risk Assessment* (2005) provide a somewhat structured approach for assessing the weight of evidence regarding carcinogenic potential of an agent and for designation as: carcinogenic to humans, likely to be carcinogenic to humans, suggestive evidence of carcinogenic potential, inadequate information to assess carcinogenic potential, or not likely to be carcinogenic to humans.

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

- "An agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments";

- "An agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans";

- "A positive tumor study that raises additional biological concerns beyond that of a statistically significant results, for example, a high degree of malignancy, or an early age at onset";

- "A rare animal tumor response in a single experiment that is assumed to be relevant to humans"; or

- "A positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case."

In general, the Panel agrees that: a) the evidence for potential carcinogenicity of PFOA has been strengthened since the 2016 HESD; b) the results of human and animal studies of PFOA are consistent with the examples provided above and support a designation of "likely to be carcinogenic to humans"; and c) the data exceed the descriptors for the three designations lower than "likely to be carcinogenic".

The Panel notes that EPA's designation for PFOA is consistent with the California EPA (2021) conclusion that PFOA and PFOS should be evaluated as carcinogens for the setting of Public Health Goals (analogous to MCLGs) for PFOA and PFOS. It should be noted that the criteria used by California EPA for determination that a chemical is a carcinogen are not identical to the criteria in the U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*.

Supporting epidemiologic and experimental evidence for "likely carcinogenic" designation

Since the publication of the 2016 document for PFOA, at least eight epidemiological studies considering links between PFOA exposure and cancer have been published. The evidence from epidemiologic studies is primarily based on the occurrence of kidney and testicular cancer. At least one new study, the general population nested case-control study conducted in the PLCO cohort (Shearer et al., 2021), supports previous positive associations with kidney cancer observed in individuals highly exposed to PFOA (elevated exposure from contaminated drinking water near PFAS producing facility) (Barry et al., 2013; Vieira et al, 2013). EPA *Guidelines for Carcinogen Risk Assessment* state that, "When human data of high quality and adequate statistical power are available, they are generally preferable over animal data and should be given greater weight in hazard characterization and dose-response assessment, although both can be used." The EPA determined that the new epidemiologic studies were all *medium confidence* studies, including the Shearer et al. study, and that there is an absence of any *high confidence* epidemiologic studies. The Panel generally agrees that the epidemiologic evidence is consistent with the example of supporting data for the likely descriptor presented above in the Guidelines, that is, of "plausible associations between human exposure and cancer."

PFOA also caused testicular Leydig cell, pancreatic acinar cell, and/or hepatocellular tumors in male Sprague-Dawley rats in three chronic studies (Butenhoff et al., 2012 [also reported by Sibinski, 1987]; Biegel et al., 2001; NTP, 2020), including one new chronic cancer bioassay in rats (NTP, 2020) which supports previous evidence of tumorigenesis at multiple sites.

In NTP (2020), the incidence of both malignant and benign liver tumors was increased, and the incidence of pancreatic tumors was very high in all dosed groups of males. However, the occurrence of malignant tumors and the very high incidence of pancreatic tumors is not

mentioned in the draft EPA PFOA document, and this information should be added because it adds to the weight of evidence for PFOA's carcinogenic potential. There was also a marginal increase in hepatocellular carcinomas and uterine adenocarcinomas, and non-significant increases in benign and malignant pancreatic acinar cell tumors, in females in NTP (2020). The lower response in females was stated by NTP (2020) to be consistent with the lower plasma PFOA levels due to the rapid excretion of PFOA in female rats. The 2020 NTP study identified clear evidence of carcinogenic activity in the liver and pancreas in rats exposed over a lifetime, including the pre-natal, pre-weaning, and post-weaning stages. While NTP carcinogenicity studies do not include conclusions about human carcinogenicity, the 2020 NTP study concluded "clear evidence" of carcinogenic activity in male rats, which is the strongest level of evidence category used by the NTP in evaluating data from studies of this kind.

Mode of action analyses (PFOA HESD, 2016; DWQI, 2017) have concluded that the rat liver tumors caused by PFOA may not be relevant to humans, although this is not a settled issue. However, the mode of action for other types of tumors caused by PFOA in rats has not been established, and as specified in the U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*, they are considered relevant to humans.

An additional study that is relevant to the mode of action for carcinogenicity of PFOA is the initiation-promotion study in rainbow trout (Benninghoff et al., 2012). In this study, PFOA significantly increased the number of tumors and the diameter of liver tumors compared to controls treated with the same initiator. The increase in number of liver tumors was greater for PFOA than for PFOS, and PFOS did not cause a significant increase in tumor diameter. As reviewed in DWQI (2017), the overall significance of this study is that PFOA increased hepatic tumors in "rainbow trout, a species used as a model for human liver carcinogenesis because it is insensitive to peroxisome proliferation [e.g., PPAR–α activation], suggest[ing] that PFOA promotes liver tumor development through an estrogenic mechanism."

Another relevant study evaluated the ability of PFOA to promote pancreatic cancer in the LSL-$KRas^{G12D}$; Pdx-1 Cre mouse model of pancreatic cancer (Kamendulis et al., 2022). In this study, PFOA elicited temporal increases in pancreatic intraepithelial neoplasia (PanIN) lesion area and desmoplasia concomitant with the induction of oxidative stress, demonstrating that PFOA functions as a promoter of pancreatic tumor development in mice.

Finally, the draft PFOA document (p. 343-344) discusses a genotoxic versus non-genotoxic mode of action for carcinogenicity of PFOA, followed by the conclusion that PFOA is considered "likely to be carcinogenic to humans." As such, it should be made clear that the designation of "likely to be carcinogenic to humans" is independent of whether the mode of action is genotoxic or non-genotoxic.

Rationale for "likely carcinogen" designation

As discussed above, human, animal, and mode of action studies support the designation of PFOA as "likely to be carcinogenic to humans." The designation of PFOA as "likely to be carcinogenic to humans" would have large practical implications because the MCLG for "likely carcinogens" is zero. For this reason, it is particularly important for EPA to provide a strong and transparent rationale for reaching this conclusion. However, the rationale for this designation is not adequately provided in the draft MCLG document.

Additional “weight of evidence narrative” is needed, including discussion of epidemiologic data structured along the Hill criteria, assessment of evidence from animal studies, and assessment of mode of action, to justify the “likely” descriptor. It would enhance transparency and confidence to have an objective, well-described, systematic approach for evidence synthesis that incorporates the prior studies included in the 2016 HESD *and* the newer studies. In fact, this “single integrative step” of weighing all the evidence after assessing the individual lines of evidence is emphasized in the U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*.

The EPA *Guidelines for Carcinogen Risk Assessment* emphasize the importance of weighing all of the evidence in reaching conclusions about the human carcinogenic potential of agents. Specifically, the *Guidelines for Carcinogen Risk Assessment* provide at least five examples of data that support the “likely” descriptor (listed above). The draft MCLG document should explicitly demonstrate how the available data for PFOA are consistent with one or more of those examples in order to fulfill the criteria for designation as a "likely" carcinogen. These *Guidelines* are cited but the specific examples of supporting data should be added to the draft document.

Finally, the weight of evidence evaluation (i.e., determination of the appropriate descriptor for carcinogenic potential, such as "suggestive" or "likely") is part of the Hazard Identification component of the risk assessment, not the Dose-Response component. As such, the Weight of Evidence section (section 4.2) should be moved from the Dose-Response section to the Hazard Identification section (e.g., Evidence Integration) on cancer.

Consideration of other designations

The Panel agrees that neither of the categories of “not likely to be carcinogenic to humans” or “inadequate information to assess carcinogenic potential” are appropriate, as epidemiologic studies and studies of experimental animal models/chronic cancer bioassays do exist and cancers/tumors have been observed in relation to PFOA exposure. There is also agreement that the data exceed the descriptor for “suggestive evidence of carcinogenicity,” which may include a positive cancer result from only a single animal or human study with additional studies of mixed results.

The Panel also considered whether the currently available data for PFOA may exceed the descriptor for “likely” evidence and meet the criteria for the higher designation of “carcinogenic to humans”. This descriptor of “carcinogenic to humans” indicates strong evidence of human carcinogenicity. According to the *Guidelines for Carcinogen Risk Assessment*:

- This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.

- Exceptionally, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when all of the following conditions are met: (a) there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association, and (b) there is extensive evidence of carcinogenicity in animals, and (c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress

to tumors, based on available biological information.

The Panel concludes that the available epidemiologic data do not provide convincing evidence of a causal association but rather provide evidence of a plausible association, and thus do not support a higher designation of "carcinogenic to humans." While Bartell and Vieira (2021), in a critical review and meta-analysis of epidemiological literature, concluded that associations between PFOA and kidney and testicular cancer were "likely causal," they noted the limited number of studies and the need for additional larger cohort studies to support this conclusion. As discussed above, the epidemiologic data supporting the designation of "likely carcinogenic" derive largely from several *medium confidence* studies (including Shearer et al. 2021) and in the absence of any *high confidence* epidemiologic studies. Shearer et al. (2021) showed an increased risk of kidney cancer in the highest PFOA exposure quartile (odds ratio, OR=2.63) that was slightly attenuated (OR=2.19) and not statistically significant after adjusting for other PFAS. Thus, the Shearer et al. (2021) study should not be overemphasized, as "an inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality" (U.S. EPA *Guidelines for Carcinogen Risk Assessment,* 2005).

The Panel recommends that the EPA document again needs additional discussion of the "weight of the evidence" to support excluding the higher level designation of "carcinogenic." The draft document should explicitly demonstrate how the available data for PFOA do not meet one or more of the examples of the criteria for designation as a "carcinogen."

Finally, the Panel notes that the practical impact of "likely to be carcinogenic" and "carcinogenic" designations on the MCLG are the same, since the MCLG would be set at zero in either case.

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."

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.

Designation of PFOS as "suggestive evidence of carcinogenic potential"

As described above in the response to Charge Question 3a concerning the cancer classification for PFOA, EPA *Guidelines for Carcinogen Risk Assessment* (2005) provide a structured approach for assessing the weight of evidence regarding carcinogenic potential of an agent and for designation as: carcinogenic to humans, likely to be carcinogenic to humans, suggestive evidence of carcinogenic potential, inadequate information to assess carcinogenic potential, or not likely to be carcinogenic to humans.

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

- a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "Likely to Be Carcinogenic to Humans." The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system.

- a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed. (When there is a high background rate of a specific tumor in animals of a particular sex and strain, then there may be biological factors operating independently of the agent being assessed that could be responsible for the development of the observed tumors.) In this case, the reasons for determining that the tumors are not due to the agent are explained.

- evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence (such as structure-activity relationships); or

- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend.

Based on the EPA *Guidelines*, the Panel discussed whether the available data support a designation of "suggestive" which is unchanged since the 2016 categorization; or meet the higher descriptor for "likely to be carcinogenic to humans." Several new studies have been published that warrant further evaluation to determine whether the "likely" designation is appropriate. Below, the Panel provides some information that the EPA should consider in the overall weight of evidence for carcinogenicity of PFOS.

Supporting epidemiologic and experimental evidence for "suggestive" designation

The epidemiological evidence for the carcinogenicity of PFOS is mixed and/or methodological limitations of the studies preclude firm conclusions. Of the 11 new studies identified since 2016, 8 were considered medium confidence and 3 were considered low confidence. There were no new animal toxicity studies identified. A single chronic cancer bioassay in rodents (Butenhoff et al., 2012) showed increases in tumors in the liver (males and females), thyroid gland (males), and mammary gland (females); however, the liver tumors in males, thyroid gland tumors, and mammary gland tumors did not appear in a dose-responsive pattern, and liver tumors in females were significantly increased ($p \leq 0.05$) only in the high dose group.

In the draft EPA PFOS document, the Hazard Identification section on cancer (Section 3.3.1.7) discusses that Shearer et al. (2021) showed an association of PFOS with kidney cancer. Comparison of the discussion of this topic in Section 3.3.1.7 of the PFOS document with the discussion of the same topic in the PFOA document indicates that some, but not all, of the specific analyses that were statistically significant for PFOA were not significant for PFOS.

Specifically, the draft EPA PFOS document states (p. 286-287): "PFOS was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma) in a medium confidence study {Shearer, 2021, 7161466}. The study reported a statistically significant increase in risk in the highest exposure quartile and per doubling of PFOS concentration. After adjusting for other PFAS the association remained elevated in the highest quartile (i.e., adjusted OR=1.14), but it was no longer statistically significant and was lower than the second quartile; additionally, there was no association when evaluated on a per doubling of PFOS."

This can be compared to the information from the draft EPA PFOA document (p. 309): "PFOA was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma (RCC)) {Shearer, 2021, 7161466}. This large medium confidence case-control study nested within the NCI's Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO) reported a statistically significant increase in risk of kidney cancer in highest exposure quartile and per doubling of PFOA concentration. Even after adjusting for other PFAS the association remained significant in analyses on a per doubling increase in PFOA. The increase in the highest exposure quartile remained and the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS), but it was no longer statistically significant."

The Panel notes that the draft PFOS document does not mention that the Shearer et al. (2021) results were used for a CSF for PFOA. Thus, further discussion is needed on Shearer et al. (2021) epidemiologic study findings (classified as a *medium confidence* study) as they pertain to PFOS, including in the Weight of Evidence section (Section 4.2.1) because Shearer et al. (2021) is not mentioned at all in its discussion of new PFOS cancer studies identified since the 2016 HESD. In particular, the Panel concludes that the PFOS document should clearly indicate why the findings of the Shearer et al. (2021) study were judged to be less definitive for PFOS than for PFOA. The magnitude of the association between PFOS and kidney cancer was lower than that for PFOA, and after adjustment for other PFAS, the adjusted OR for the highest quartile was 1.14 and not statistically significant. However, these data should be presented clearly including a discussion of why the PFOS data from Shearer et al. (2021) were not considered sufficient for a higher designation of "likely carcinogenic."

The Panel also suggests that Li et al. (2022), a recent study that may be relevant to weight of evidence for carcinogenicity of PFOS, be included in the discussion. This study reported an increased incidence of kidney cancer in a Swedish population exposed to drinking water contaminated with a mixture of PFAS, with PFOS predominating.

An additional study that is relevant to the mode of action for carcinogenicity of PFOS is the initiation-promotion study in rainbow trout (Benninghoff et al., 2012). This study was not included in the PFOS document. In this study, PFOS significantly increased the number of tumors compared to controls treated with the same initiator; this increase was greater for PFOA than for PFOS. PFOS did not cause a significant increase in tumor diameter compared to controls

treated with the same initiator, while this effect was significant for PFOA. As reviewed in DWQI (2017), the overall significance of this study is that PFAS increased hepatic tumors in "rainbow trout, a species used as a model for human liver carcinogenesis because it is insensitive to peroxisome proliferation [e.g.., PPAR−α activation], suggest[ing] that PFOA promotes liver tumor development through an estrogenic mechanism." As discussed previously, this study is important to understanding the mode of action of hepatic tumors caused by PFOA because it has been suggested that rodent liver tumors caused by PFOA occur through PPAR−α activation that is not relevant to humans. However, this is not an issue for PFOS because the rodent liver tumors caused by PFOS do not appear to be PPAR−α dependent. For example, DWQI (2018) concluded that "available data do not support the conclusion that PFOS causes liver effects through a PPARα-dependent mode of action at the doses that resulted in tumors in [the chronic rat study conducted by] Butenhoff et al., 2012."

The Panel suggests mammary tumor development be considered as an additional endpoint associated with PFOS. Human (Bonefeld-Jorgensen et al., 2011*;* Wielsøe et al., 2017*;* Cohn et al., 2020*;* Mancini et al., 2019), animal (Butenhoff et al., 2012), and mechanistic (Pierozan et al., 2018) studies support this as an endpoint of concern, warranting a POD derivation. For mammary tumor development, there are some recent studies that have shown epidemiologic and mechanistic evidence linking PFOS with breast cancer outcomes. On the epidemiologic side, Mancini et al., 2019 found a linear dose response for PFOS and estrogen receptor positive tumors (there was also a potential association with estrogen receptor negative tumors). On the mechanistic side, Pierozan et al. (2018) showed that exposure to PFOS (at 10 microM) induced malignant transformation of MCF-10A cells.

Rationale for "suggestive" designation

Again, the findings of the Shearer et al. (2021) study for PFOS need to be presented clearly including a discussion of why they were not considered sufficient for a higher designation of "likely to be carcinogenic to humans."

The Panel also recommends that additional discussion to support the rationale for the "suggestive" designation as opposed to the "likely" designation be presented, and this recommendation stems largely from lack of inclusion of mechanistic data and inconsistency with the California EPA conclusions based on the same human, animal, and mechanistic evidence presented in the EPA PFOS document. The Panel noted that the New Jersey DWQI (2018) determined that draft PFOS should be described as having suggestive evidence of carcinogenicity, using EPA (2005) *Guidelines for Carcinogen Risk Assessment* and consistent with the designation in the draft EPA PFOS document, while observing that this conclusion was made prior to the publication of Shearer et al. (2021).

The criteria used by California EPA, for determination that a chemical is a carcinogen, are not identical to the criteria in the U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*. California has proposed (effective December 24, 2021) listing PFOS and its salts and transformation and degradation precursors on the list of chemicals known to the state to cause cancer for purposes of the Safe Water Drinking and Toxic Enforcement Act of 1986 (Proposition 65) (OEHHA, 2021a). The OEHHA summary of human evidence indicates that the results were mixed, and the summary of animal evidence highlights a chronic carcinogenicity study in rodents

supported by the tumor promotion study in rainbow trout (OEHHA, 2021b), which is mostly consistent with evidence provided in the draft EPA PFOS document.

The mechanistic data in the OEHHA summary (OEHHA, 2021b) were used to identify the shared characteristics between PFOS and other known carcinogens. Of particular importance in the OEHHA document was the finding of suggestive evidence that PFOS (and PFOA) is genotoxic – leaving the potential for a genotoxic MOA that differs from the 2016 HESD for PFOS. Given that the EPA has not completed the review of mechanistic studies for the cancer endpoint, it seems premature to have kept the 2016 determination without further evaluation of the mechanistic evidence.

In addition to the lack of inclusion of mechanistic data in the weight of evidence for PFOS carcinogenicity, the interpretation of the hepatocellular carcinoma data from the Butenhoff (2012) study in the 2016 HESD is overly conservative in dismissing the appearance of a dose-response relationship for this endpoint, particularly in females. Relevant to this point, it is noted that DWQI (2018) developed a CSF based on the incidence of hepatocellular tumors in females in Butenhoff et al. (2012). Given that multiple MOAs may be operative in this outcome, the Panel suggests that the EPA reevaluate the 2012 Butenhoff study.

Finally, as mentioned for PFOA above, the weight of evidence evaluation (i.e., determination of the appropriate descriptor for carcinogenic potential, such as "suggestive" or "likely") is part of the Hazard Identification component of the risk assessment, not the Dose-Response component. As such, the Weight of Evidence section should be moved from the Dose-Response section to the Hazard Identification section (e.g., Evidence Integration) for cancer.

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.”

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~~ 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. [wording was revised (strikeout) before Panel deliberations].

At the SAB Panel meeting on December 16, 2021, EPA clarified that the term "peak exposure" should be disregarded in the charge question, and that input is requested on the development of the CSF in general, and risk specific dose for PFOA.

In general, the Panel agrees that it is preferable to base the CSF derivation on human epidemiological data when appropriate human data are available. However, for PFOA, there is an absence of "high confidence" epidemiologic data as summarized by EPA.

EPA selected the Shearer et al. (2021) study for CSF derivation. The systematic review conducted by EPA categorized the overall confidence in this study as "medium," due to a *deficiency in controlling for confounding*, and *adequate confidence in selectivity and sensitivity* of the study (according to Figure 123, though not specifically described in the text). The Panel agreed with this classification, noting some merits and several limitations of the study design and overall significance of the results.

The Shearer et al., 2021 study is a prospective epidemiologic study that investigated the association between exposure to eight PFAS, including PFOA, and RCC risk. PFAS measurements for all subjects in serum were collected prior to RCC diagnosis. This study adjusted for diminished kidney function to control against reverse causation due to reduced kidney function in the observed associations. The study authors reported that, after adjusting for PFOS and other PFAS, the increased risk of kidney cancer in the highest PFOA exposure quartile compared to the lowest was found to be attenuated (from OR=2.63 to OR=2.19); neither that effect estimate for the highest quartile, nor the dose-response trend remained statistically significant. However, when PFOA was modeled continuously, the association remained statistically significant after adjusting for other PFAS. Although not discussed in the paper, the Panel notes that Supplementary Figure 1 shows one individual in the RCC group had much higher serum PFOA than any of the other cases or controls. The impact of this individual on the elevated RCC risk in the highest exposure group is unclear. The Panel suggests that the EPA investigate this observation, including contacting the authors of Shearer et al. (2021) if appropriate.

The study also revealed associations with RCC for PFOS and PFHxS, both of which were reported to be moderately correlated with PFOA, in models unadjusted for other PFAS. Based on this finding, it is not clear whether other PFAS (e.g., PFNA) that were also moderately correlated with PFOA were adequately controlled for. The issue of impacts of other correlated PFAS on the apparent dose-response for a specific PFAS is discussed in the response to the charge question #5A below on confounding in studies of non-cancer effects, and this issue should be included in the discussion of Shearer *et al.* (2021) in the draft MCLG documents.

Overall, the epidemiological studies have not consistently identified associations between PFOA and RCC; some epidemiological studies support RCC as a critical finding associated with PFOA exposure while others (with several limitations noted) have failed to detect an association between PFOA and RCC. Concerning the human studies that have shown an association of

PFOA with cancer (Shearer et al. (2021), Vieira et al. (2013), Barry et al. (2013), and Steenland and Woskie (2012)), the draft MCLG document did not provide the rationale for using Shearer et al. (2021) rather than of one of the other studies to derive a CSF for PFOA. However, the Panel notes that the other studies were presumably excluded from consideration because they were included in the 2016 HESD. In contrast, California EPA (2021) does provide a rationale for using Shearer et al. (2021) to derive a CSF. Furthermore, California EPA's (2021) approach for deriving a CSF for PFOA and kidney cancer was based on serum PFOA levels $(ng/ml)^{-1}$ from a study of the general population (Shearer et al., 2021) and a study of communities exposed to PFOA in drinking water (Vieira et al., 2013), with the recommended draft CSF (CalEPA, 2021) proposed as the average of the two CSFs. Given that the Panel has identified several limitations of the Shearer (2021) study, and the EPA classification of this study is of "medium" overall confidence, a clear rationale for its selection as the sole basis for the CSF should be provided.

Eight of the 13 new epidemiologic studies identified in the EPA's systematic review were considered of "medium" overall confidence and the others were considered "low" confidence, whereas the NTP 2020 chronic bioassay in rats is considered a "high" confidence study. However, a CSF was derived for only one of the new medium confidence epidemiologic studies (Shearer et al., 2021), and it is not clear whether any of the other newly identified medium confidence studies support CSF development. The Panel agrees that toxicity values should only be derived from studies with at least "medium" confidence, but the draft document needs to be more transparent as to weighing the strengths and limitations of different studies to support a CSF (including both human and animal studies).

Some human studies were excluded during the systematic review, including those of "special populations" and "occupational" exposure studies. While PFOA exposures might be higher in these populations relative to the general population, these studies could be complementary, and for consistency and transparency, should be similarly evaluated as the other studies included in the draft document. The rationale for their inclusion or exclusion in the development of candidate CSFs should be presented.

The NTP (2020) rat study reported increased incidences of hepatocellular adenomas (or carcinomas) and pancreatic acinar cell tumors. Of the three chronic bioassays that have been conducted in rodents (NTP 2020, Biegel et al., 2001, Butenhoff et al., 2012), none observed an increased incidence in kidney cancers. While concordance of tumor sites between animal and human exposures is not always observed and the EPA (2005) Guidelines for Carcinogen Risk Assessment state that "target organ concordance is not a prerequisite for evaluating the implications of animal study results for humans," the lack of "high" confidence epidemiological studies would support that "high" confidence animal studies might be at least of equal weight to "medium confidence" human studies for CSF derivation.

Overall, the Panel recommends that multiple candidate CSFs be developed, including those based on additional epidemiologic studies of sufficient quality, including any of the earlier human studies of sufficient quality that were included in the 2016 HESD but excluded from consideration in the draft document, as well as animal cancer bioassays. Each study's strengths and limitations (e.g., exposure uncertainties, confounding [including possible reverse causation], statistical power) should be discussed and then a judgment made as to whether to select one or more studies to represent the overall slope factor. The Panel does note that the CSFs derived

from the Shearer et al. (2021) study appear to be two to three orders of magnitude more potent than those derived from experimental animal studies, and thus the decision as to what slope factor to recommend needs to be carefully considered and highly transparent.

The Panel also identified other aspects of the CSF and risk specific dose for PFOA that should be addressed and/or further discussed in the document. They are listed below:

Lifetime RCC risk estimation relied on lifetime risk of kidney cancer in US males. RCC baseline risk is higher in Black individuals compared to White individuals. Discussion and/or demonstration of how the CSF might differ if different rates were applied that are representative of the population should be included.

Multiple statements in the draft MCLG documents indicated that MOA results across PFOA and PFOS are currently under review and that EPA's MOA evaluation would be deferred until after the SAB review. The Panel commented that including the final MOA evaluation for kidney cancer is important if this endpoint is used by EPA.

The draft PFOA document states that the CSF for Shearer et al. (2021) is based on the modeling approach used by California EPA (2021), and in the corrected version of Table 25 in the draft PFOA document, the California EPA (2021) CSF is based on the central tendency estimate serum level from Shearer et al. (2021). At the SAB meeting on December 16, 2021, EPA stated that they had independently replicated the modeling performed by California EPA. However, modeling details, results, and conclusions of the EPA review are not shown in the draft PFOA document. The Panel recommends that this information be included in the final EPA document with further discussion and clarification as described below.

The draft PFOA document (Table 25) shows the CSF from Shearer et al. (2021) as administered dose $(ng/kg/day)^{-1}$ as well as serum PFOA $(ng/ml)^{-1}$. However, California EPA (2021) does not provide the CSF in terms of administered dose. EPA's derivation of the CSF as administered dose $(ng/kg/day)^{-1}$ from the PFOA serum level $(ng/ml)^{-1}$ CSF is not discussed in the main document or appendices; Appendix B.1.5.1 briefly discusses the calculation of the CSF from Shearer et al. (2021). As the clearance factor was not mentioned or provided in the document, one reviewer calculated that the serum level CSF was converted to the administered dose CSF using a clearance factor of 0.12 ml/kg/day, based on a half-life of 2.7 years and a volume of distribution of 170 ml/kg (values stated to have been selected by EPA in Sections 3.2.3 and 3.2.4 of the document). Applying this clearance factor to the serum level CSFs (Table 25) would result in the administered dose CSFs that are shown. Although the numerical value of the central tendency slope factor (0.00178) is correctly shown in Appendix B.1.5.1, the units shown, $(ng/kg/day)^{-1}$ instead of $(ng/ml)^{-1}$, are incorrect. The development of the clearance factor and its use in determining that administered dose CSFs from the serum level CSFs should be clearly and completely described in the final document.

Table 25 of the draft PFOA document also shows the 95% upper confidence level of the CSF from Shearer et al. (2021). However, the 95% upper confidence level CSF is not provided in California EPA (2021), and no information on the modeling results that provided this value are shown in the draft MCLG document; these modeling results should be provided. It is also not clear from the information in the draft PFOA document whether the central tendency CSF or the

upper 95th percentile CSF would be used to develop a risk specific dose, and this should be clarified.

Finally, the Panel notes the recent publication by Steenland et al. (2022), which conducted a pooled analysis of the Shearer et al. (2021) study along with a second study by Barry et al. (2013) to derive a cancer slope factor for PFOA, as another possible modeling approach.

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.

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.

Charge Question #4 - Toxicokinetic Modeling – General SAB Comments

The transparency of the toxicokinetic (TK) modeling can be substantially strengthened by improving the documentation of the models and their applications in both humans and animals. The Panel found it challenging to locate the information necessary to piece together how the models were used as much more information is needed beyond citations and tables of model parameters. For example, the BMD calculations in the Appendix apparently use model generated blood concentrations for dose-response analyses, but the specific details are absent.

The Panel noted a need for additional transparency; a critical example of this is in the development of the PODHED for decreased vaccine response when calculating "the dose to mothers & children that results in the same serum concentration at 5 years of age." Specifically, in the draft PFOA document, it is not clear from the information provided in Section 4.1.3.2 (Toxicokinetic Model for Human Dosimetry) that prenatal and breastfeeding exposures were considered in development of the PODHED for decreased vaccine response from exposure at age 5. Information on this topic is especially important because these PODs are the basis for the final PFOA and PFOS Reference Doses in the draft MCLG documents.

This is particularly confusing, because the excerpt from Section 4.1.3.2 of the draft PFOA document copied below (emphasis added) indicates that the steady-state assumption was used to develop the PODHED for all of the human endpoints (including the PODHED based on

exposure at age 5), and that exposures during early life-stages were only considered in animal developmental studies.

"This updated [Verner et al., 2016] model was used to simulate the HED from the animal PODs that were obtained from BMD modeling of the animal studies (Section B.2). It was also used to simulate selected human studies to obtain a chronic dose that would result in the internal POD obtained from dose-response modeling. For PODs resulting from chronic exposure, such as a long-term animal study or **a human study, the steady state approximation was used** to calculate a HED that would result in the same dose metric after chronic exposure. For PODs from exposure to developmental animals, the life-stage developmental model was used to calculate a HED that results in the same dose metric during the developmental window selected by the Dose-Response team."

In contrast, in the draft PFOS document, it is indicated that prenatal and breastfeeding exposure was considered in development of the PODHED for decreased vaccine response from exposure at age 5, and EPA confirmed this at the Panel meeting on December 16, 2021. However, this topic is not discussed in the text; it is only mentioned in a footnote to the PODHED for decreased antibody response to tetanus and diphtheria vaccines in Table 21 as follows: "Calculated as the dose to mothers & children that results in the same serum concentration at 5 years of age. Note that the model predicted slightly different serum concentrations for male and female children, so the lower HED was selected to be more health protective."

The presence of such discrepancies and ambiguities necessitates clearer and more transparent documentation as to the specific models, model parameters, and other simulation inputs that are used for each HED derivation as recommended below.

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.

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.

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.

In general, the Panel agreed that for adults and chronic exposure, the PFOA and PFOS compartmental models for adults (not pregnant or lactating females) are adequate for use in HED determinations. There is also general agreement that the assumption of a constant half-life and volume of distribution for human adults is reasonable. Additionally, the assumptions of steady state are reasonable, given the long half-life of each molecule. The Panel notes that this approach is highly empirical and limited when looking to the future and using this model to ask questions about mixtures.

However, as previously noted in the overall comments, more details on model code, parameters, data, and performance are needed for model evaluation and parameter justifications. While Appendix D reviews the many different estimates, a methodology for picking a value for a particular case to use was not clearly described. For instance, Section 4.1.3.2 of the draft MCLG documents discusses parameters from the Verner et al. (2016) model that were modified by EPA, but volume of distribution and half-life were not discussed. For both PFOA and PFOS, the volume of distribution used in Verner et al. (2016) is identical to the value stated to have been selected by EPA in Section 3.2.4 of the draft MCLG documents (0.17 L/kg for PFOA; 0.23 L/kg for PFOS). However, the half-lives of 3.8 years for PFOA and 5.4 years for PFOS used by Verner et al. (2016) differ from the half-lives of 2.7 years for PFOA and 3.8 years for PFOS stated to have been selected by EPA in Sections 3.2.3 of the draft MCLG documents. It is unclear whether EPA used the half-life values that it selected or those selected by Verner et al. (2016) when applying the Verner et al. (2016) model. Also, if EPA did not use the half-life values it selected when applying the Verner et al. (2016) model, it is unclear where those values were actually used in the EPA evaluations. These points should be clarified in the final document.

Recommendations

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

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.

Overall approach for human neonates or children

The Panel agrees that compartmental models (rather than the more complex physiologically-based pharmacokinetic [PBPK] models) are reasonable for simulating dosimetry during pregnancy and lactation for endpoints in human neonates and children for PFOA and PFOS. The strengths of using this compartmental modeling approach are that it provides a data driven estimate of pharmacokinetics, avoids numerous complexities, and is less time consuming. However, a disadvantage is that this methodology may not be generally applicable because it is highly empirical and would not easily accommodate future use of mechanistic information, scaling methods, addressing mixtures, or PFAS with different pharmacokinetic (PK) behaviors and limited or no PK data. Although modeling the PK behavior of PFOS and PFOA should be straightforward given available data, the mechanisms governing the PK behavior are not simple. Protein binding and active transport of PFOS and PFOA into and out tissues by protein transporters occur to such a degree that the pharmacokinetics are altered. The impact of these factors in a growing fetus and neonate/infant is not well understood based on PFOA or PFOS longitudinal data.

While the Panel supports the general TK approach presented in the draft PFOA and PFOS documents for use in EPA's current MCLG development effort, they suggest that EPA give consideration in the future to utilizing the broader foundation of life-stage PBPK modeling (see **Box 1**) in light of the disadvantages discussed earlier. Specifically, there is a wealth of information now for pediatric drugs and PBPK models that provide a foundational approach for addressing life-stage PBPK modeling in humans, including literally hundreds of relevant publications in the literature (see example references in **Box 1**). The strategy is to construct a modeling framework that can be used for many compounds, not one or two. This includes providing PK predictions when no data are available (e.g., before human studies are conducted).

Box 1: Illustration of life-stage modeling principles

This is a brief example of some of the principles of life-stage modeling for future consideration. The Panel is not recommending this course of action now for PFOA and PFOS, but is using PFOA as an illustrative example. The PFOA pKa and the pH of plasma (and other tissue groups) would suggest that a small portion of the circulating PFPA is unionized, and the rest is ionized, bound and unbound to serum proteins. The unionized fraction is available for passive transport across membranes and the ionized fraction, by protein transporters. Urinary clearance (CLu) would be described with glomerular filtration rate (GFR, passive), secretion, and reabsorption. With PFOA, if CLu in mice, rat, or human was less than GFR, this would signal further evaluation and consideration of reabsorption by kidney protein transporters. The ontogeny of protein transporters (e.g., kidney, liver) is an active field of research and can be used for extrapolation or interpolation based on protein abundance or activity to provide simulations without the benefit of data. Ontogenies of important serum proteins are known and would be useful for describing PFOA binding. Additionally, protein expression of key renal transporters, such as Organic anion transporter 1 and 3 (Oat1 and 3), was about 50% lower in human postmortem frozen renal cortical tissues from preterm newborns and infants compared to adults. Oat1 and 3 protein expression in renal cortical tissue displayed clear maturation patterns from newborn to adult. This approach is very different from the current methods used by EPA for PFOA and PFOS to address life stages. Ultimately this methodology provides a biologically based anchoring of key parameters that can be used for understanding the pharmacokinetics of chemicals across life stages and reproductive states. Of course, there can still be gaps in data or knowledge, such as the role of tissue binding in modulating toxicity. However, if time is invested in exploring the modeling work completed over the last 11 years for pediatric drugs, and over the last 3-4 years for pregnancy and lactation, many ideas are likely to emerge.

Selected References

Cheung *et al.* (2019). A Comprehensive Analysis of Ontogeny of Renal Drug Transporters: mRNA Analyses, Quantitative Proteomics, and Localization. *Clin Pharmacol Ther*. 106(5):1083-1092.

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

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

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

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

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

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

Methodologies involve physical chemistry, QSAR, allometry, and empirical data on processes governing clearance of drugs, mainly urinary or fecal excretion and metabolism. Unbound fraction and the physiological processes that control the unbound fraction are important factors throughout the life stages. This methodology could serve as a tool, based on physiology, for extrapolating across age groups in humans. Efforts are ongoing in the development of PBPK models for pregnancy, including the fetus and for the lactating women, and nursing infants. The value in exploring this modeling approach is that human data are collected in some cases, which

is usually not the case for environmental chemicals. However, it is unclear if available laboratory animal databases are sufficient to implement this comprehensive approach for non-human species.

Justification for selection of the Verner et al. *(2016) model*

The draft MCLG documents state that the Verner et al. (2016) model was selected because "the Goeden et al. (2019) model did not account for the decrease in concentration that occurs due to growth dilution which plays a substantial role in the PK of growing infants and children." The lack of "growth dilution" in the Goeden et al. (2019) is not an adequate reason to disfavor this model, particularly since this factor is easy to add, and in any case (as noted by scientists who developed the model) represents a small correction. Additionally, the higher dose received by young children due to their higher drinking water consumption would tend to counteract the growth dilution effect. For example, Goeden et al. (2019; Figure 6) predicts that, from a certain concentration of PFOA or PFOS in drinking water, the serum PFOA or PFOS level in a 5-year-old child who was breastfed for 6 months is about 2.8-fold higher than at steady-state in adulthood. Serum levels at age 5 years would be even higher from breastfeeding for one year, as was assumed by EPA (p. 333, last paragraph of PFOA document).

The Panel questioned the choice of the Verner et al. (2016) model for several reasons. As discussed in the general comments on TK above, there was a lack of availability of adequate information (model code, parameters, data, and performance) to fully evaluate the model. The Panel notes the published model fit for PFOA appears to overpredict at 3 months and underpredict at 3 years. Additionally, if the model parameters were changed by EPA (see previous comment), then it is unclear whether the model fit would remain the same. Finally, and most importantly, the Verner et al. model assumes a constant oral dose, as opposed to a constant drinking water concentration that may be more appropriate given the context of deriving an MCLG (discussed next).

Consideration of maternal and child drinking water ingestion rates

In EPA's draft approach, it is unclear how a "dose to mothers & children that results in the same serum concentration at 5 years of age" can account for the different drinking water consumption rates in adults (mothers) and children. All the TK modeling used for deriving HEDs seems to be based on a constant dose rate in mg/kg-d. However, a constant dose rate will not equal a constant drinking water concentration due to age-dependent changes in drinking water consumption and lactational transfer. Therefore, if the goal is an equivalent "internal dose" point of departure (POD) based on area under the curve (AUC) or C_{max}, then different values will result when using dose rate first and then converting to drinking water concentrations versus calculating drinking water concentrations directly. Otherwise, the RfD will not be protective of all life-stages.

For instance, the draft MCLG documents discuss differences between the Verner et al. (2016) and Goeden et al. (2019) models for predicting serum PFAS levels in early life. However, the draft MCLG documents do not recognize that the two models have different purposes and provide different information. The Verner et al. (2016) model predicts infant and child serum PFOA or PFOS levels resulting from a constant daily PFOA or PFOS dose (ng/kg/day) to the mother and to the child after weaning. However, it is not clear how a RfD from the Verner et al. (2016) model, which predicts serum PFOA or PFOS levels at age 5 years from a constant daily

dose to the mother and the child, can be used to develop an MCLG that considers both exposure through breastfeeding, post-weaning and changing drinking water consumption rates up to age 5.

In contrast, the Goeden et al. (2019) model considers both age-specific toxicokinetic factors and the changing drinking water intakes at different age periods. Although the Goeden et al. (2016) publication presents the application of the model to PFOA, the model can also be applied to other PFAS by using chemical-specific values for half-life, volume of distribution, and other chemical-specific factors. As reviewed in Post (2021), at least four states (MN, NH, MI, WA) have used this model to develop drinking water guidelines for PFOA, PFOS, PFNA, and/or PFHxS. The Goeden et al. (2019) model predicts the serum PFOA or PFOS levels at any age (including infancy, childhood, and adulthood) that result from maternal and child consumption of drinking water with a certain concentration (ng/L) of PFOA or PFOS. Specifically, it considers the maternal drinking water intake rate which impacts PFOA or PFOS levels in breastmilk, and the varying drinking water intake rates in children of different ages after weaning. The daily water intake (L/kg/day) in young children is much higher than in older individuals, and it varies during different childhood age periods up to age 5.

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.

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.

The Panel agrees that using constant ratios for maternal to fetal serum and maternal serum to milk is reasonable given the available data. Mechanistically, the movement of PFOA or PFOS from blood across the mammary tissue into milk and back into the blood supply involves both diffusion and active transport and, thus, is not a steady state condition. Each time nursing occurs, the compartment empties and is replenished with fresh milk. For long-lived chemicals, the influence of this dynamic compartment on steady-state assumptions is assumed to be minimal when evaluated over days to months. The milk-to-plasma ratio varies to some degree with milk composition, and publications are available for estimating this ratio for drugs, including acidic drugs. Although the assumptions of a fixed ratio for the fetus and breastmilk are not correct for the timescale of each occurrence of nursing, they seem adequate for long-lived chemicals PFOA and PFOS.

Recommendations
None.

Animal Toxicokinetic Model

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

The Panel agrees with the selection of the Wambaugh et al. (2013) model for the calculation of internal dose metrics. The rationale for the choice of the model is clearly described. If a decision is made to use RfDs based on animal studies in the final document, the animal model will become an important part of the basis for the MCLGs.

Wambaugh et al.. (2013) is a comprehensive toxicokinetic model developed from 22 studies that include *in vitro* and *in vivo* dosimetry data. The *in vivo* studies include datasets for both PFOA and PFOA from mouse, rat, and monkey. The *in vivo* studies include single and repeat dose studies. Most of the rodent studies used are well powered with n= 5-25/group. Three *in vitro* assays were also included in the analysis. The Panel does not suggest an alternative model.

Regarding the development of PODs from animal studies, serum/plasma data from the study itself (e.g., at the end of the dosing period rather than the serum levels predicted by modeling)

could be used when appropriate serum or plasma PFOA/PFOS data are available. The Panel suggests that EPA consider the pros and cons of this approach, since use of the data from the study itself could reduce uncertainty. It should be noted that four of the eight states whose PFAS drinking water guidelines were reviewed by Post (2021, https://setac.onlinelibrary.wiley.com/doi/full/10.1002/etc.4863) used an approach based on serum PFAS levels measured at the end of dosing in the development of RfDs for PFOA and PFOS.

Additionally, the PFOA document (p. 330) states: "The purpose of the animal PBPK model is to make predictions of internal dose in lab animals used in toxicity studies or in humans. Therefore, to evaluate its predictive utility for risk assessment, a number of dose-metrics across life stages were selected for simulation in a mouse, rat, monkey, or human." However, toxicokinetic parameters appear to be presented only for animal species but not humans in Section 4.3.1, and application of the model to humans is not discussed. The EPA should clarify whether or how this model was used to simulate human exposures.

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.

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?

The Panel agrees with the use of median values for deriving the HED. However, the uncertainty in both the human and animal models predictions should be characterized, particularly in terms of their impact on the HED calculation. This is particularly important if a probabilistic approach is used to calculate risk-specific doses instead of the traditional deterministic RfDs. When parameter values greatly exceed biological plausibility, EPA should consider placing bounds on the parameters.

Recommendations

The panel recommends that EPA characterize the uncertainty associated with using median predictions.

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?

The Panel agrees that there are insufficient studies for proper modeling. The Panel suggests that the EPA consider re-calibration rather than selecting a sex. Specifically, the Panel suggests that EPA plot the model-predicted plasma concentrations versus the observed or measured plasma concentrations with a unity line (y = x) and lines with y=0.5x and y=2x to visualize the data and assess model performance.

Recommendations

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

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.*

The Panel agrees that a one compartment model for the developing pup or infant is adequate for deriving HEDs from experimental animal studies. Little is known regarding transfer of PFOA or PFOS from placenta or to breastmilk with active transport. While this is likely and feasible, this has yet to be demonstrated. Because renal expression of transporters is low after birth, neonatal elimination of PFOA and PFOS could likely be lower than in adults. With regard to PFOA and PFOS clearance in the neonate during lactation, the draft MCLG document assumes that clearance would be similar to that of adults at low doses. The Panel notes that Hinderliter et al. (2006) contains data on the relationship between age and serum levels of PFOA in rats between 3 and 8 weeks of age that may be useful to inform age-dependence of clearance during early-life stages.

In rats, renal Organic Anion Transporter (OAT1 and 3), and Mate1 mRNA expression are substantially lower in fetal kidney. Expression then increases during the neonatal period, with increases at weaning and adulthood (Xu et al., 2017). The Panel suggests the use of covariates such as ontogeny of transporters to greatly improve the model as information becomes available.

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.

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.

The Panel agrees with the lactation model (e.g., Loccisano et al., 2013). The parameters that dictated transfer of chemical from mother to pup were Maternal Milk:Blood Partition Coefficient (P_{milk}), Fetus:Mother Concentration Ratio (R_{fm}), species-specific *in vivo* determined half-life ($t_{1/2}$), V_d for PFOA and the species-specific milk consumption rate during lactation. The parameters and assumptions used are up to date with regard to publications and present knowledge of PFOA transfer from mother to pup.

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.

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.

The Panel notes that Hinderliter et al. (2006) mentioned above contains information on sex differences in rat PFOA toxicokinetics at different ages. Interestingly, sex differences in PFOA serum levels were not evident at 4 weeks but differences were present at 5 weeks. However, it is not clear whether it is feasible to use these data to parametrize sex differences in neonatal animals using the computational methods employed by the EPA. In adult rodents, it appears that sex differences in PFOA toxicokinetics are hormonally modulated. For example, there are a few publications that examine sex-specific OAT expression in neonatal mouse and rat kidney (Buist et al., 2004; Buist et al., 2002). While these studies have not evaluated the toxicokinetics of PFOA or PFOS in neonatal animals, Hinderliter et al. (2006) reported that sex-dependent differences in excretion of PFOA (very rapid excretion in females and decreased excretion rate in males) develops between 3 and 5 weeks of age (i.e., after weaning at about 3 weeks of age).

Potentially relevant to this point, renal excretion of PFOA in adult rats appears to be under hormonal control (Ylinen et al., 1989; Kudo et al., 2002). As reviewed in DWQI (2017), the rapid excretion of PFOA in adult female rats is due to renal tubular secretion, while renal tubular secretion of PFOA does not occur in adult males (Kudo et al., 2002). The clearance rate in male rats was increased by castration or administration of estradiol, and the increased excretion rate in the castrated rats was reversed by administration of testosterone (Ylinen et al., 1989; Kudo et al., 2002). Conversely, administration of testosterone to female rats reduced PFOA clearance to a

rate similar to that of control males (Ylinen et al., 1989; Kudo et al., 2002). Probenecid, an inhibitor of renal tubular secretion of organic anions, greatly reduced the clearance of PFOA in female and castrated male rats but had little effect on the excretion rate in control male rats (Kudo et al., 2002).

Recommendations

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

Charge Question #5A - Epidemiological Study RfD Derivation

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

The potential for confounding that results in a measured association deviating from the causal effect has been addressed to varying degrees in the epidemiologic studies. The magnitude of confounding may vary across study populations and with few exceptions has not been found to be substantial once basic demographic and social factors are considered. Known determinants of biomarkers of PFAS in blood include age, sex, body mass index, pregnancy, and breastfeeding history (for women), diet, and some health behaviors. Depending on their relationship to health outcomes of interest, these would constitute potential confounders.

Beyond these familiar concerns, there are some particular issues that need to be considered in addressing epidemiologic studies of PFAS to be used for dose-response modeling. While not all can be readily addressed, the Panel encourages EPA to carefully consider these potential sources of confounding with an explanation of how they were addressed in the studies and whether it seems likely that residual confounding is present. Because of the susceptibility of any given study or set of studies on a particular health endpoint to be affected by confounding, consideration of multiple endpoints would be beneficial. Considering multiple studies of a variety of endpoints in different populations would provide convergent evidence that is more reliable than any one study or health endpoint in isolation.

In considering the adequacy of the control for confounding, the Panel notes several different issues, including:

Correlated exposure to other forms of PFAS

Individual forms of PFAS do not occur in isolation and there tends to be a correlation among them (i.e., areas and individuals with elevated exposure to one of the chemicals often have elevated exposure to others within that class). While not necessarily acting as a conventional confounder (i.e., an independent cause of the outcome), this would distort the quantitative estimates for dose-response modeling. An effect attributed to a given change in one form of

PFAS might in fact be in part a function of other forms of PFAS that are associated with it. If this were the case, then the actual potencies of PFOA or PFOS would be lower than implied by the studies. The Panel suggests that EPA investigate and discuss this possibility. Accordingly, EPA should present information on co-exposures to other PFAS for each of the epidemiological studies selected for POD derivation, noting whether the impact of other PFAS was evaluated along with results of the evaluation. The BMDs for decreased antibody response to vaccines published by Budtz-Jorgensen and Grandjean (2018) were selected as PODs for the final PFOA and PFOS RfDs. Budtz-Jorgensen and Grandjean (2018) addressed co-exposure to PFOA and PFOS and stated that co-exposure was accounted for in the development of BMDs for PFOA and PFOS.

EPA also selected five human studies for development of PODs for decreased birthweight caused by PFOA, and four of these five studies were also used for development of PODs for this effect from PFOS. The potential impact of PFOA and other PFAS on associations of PFOS with decreased birthweight, and *vice versa* for PFOA, should be discussed in the draft MCLG documents for each of these studies. For example, Chu et al. (2020) evaluated the impact of adjustment for PFOA and PFOS on the effect of other PFAS (i.e., chlorinated polyfluorinated ether sulfonates, ClPFESAs), but it appears that the impact of PFOA on PFOS and *vice versa* were not evaluated. Additionally, it appears that Sagiv et al. (2018) did not evaluate potential confounding by co-exposure to other PFAS; the HAWC evaluation states that "there is some minor concern over potential bias due to confounding by other PFAS." The sensitivity analysis conducted by Starling et al. (2017) does not appear to support an association for PFOS with birthweight after co-exposure to other PFAS is considered. This sensitivity analysis is not mentioned in the HAWC evaluation of this study, and the Panel suggests that it should be reviewed by EPA to determine if this study is appropriate for dose-response for the effects of PFOS on birthweight. Finally, as noted in the HAWC file, Wikstrom et al. (2020) did not consider confounding by co-exposure to other PFAS, and the authors acknowledge this limitation.

Additionally, BMDs from the same human study (Dong et al., 2019) were used as PODs for increased serum cholesterol for both PFOA and PFOS. As stated in the HAWC evaluation for Dong et al. (2019), there was "no discussion of potential confounding across PFAS."

Role of shared physiology in affecting biomarkers

Most epidemiologic studies of PFAS come from the general population and are largely reflective of variations in exposure. There are relatively few studies from locations in which there are pronounced differences in water PFAS concentrations or differing occupational exposures to workers. The general consistency of results of studies from these different types of populations is a strength of the epidemiologic database for PFOA and PFOS. However, variation in physiological factors that affect toxicokinetics (e.g., kidney function, plasma volume expansion) will affect biomarkers of exposure (e.g., serum PFAS levels), and the possibility that these physiological factors are also associated with health outcomes associated with PFAS must be considered as well. For example, in studying of PFAS and kidney disease, there is the potential for those who have impaired kidney function to have elevated PFAS levels with the kidney malfunction causing the elevated PFAS levels rather than the

reverse (Watkins et al., 2013), and similarly for outcomes like early menopause (Dhingra et al., 2017).

The pitfalls of relying on exposure biomarkers, rather than exogenous exposures, for assessment of exposure to environmental contaminants in general were carefully and thoughtfully examined by Weisskopf and Webster (2017). The concern here is that measured PFAS levels in blood are determined not just by the exogenous exposure to PFAS in water, food, consumer products, air, etc., but may also be considerably impacted by physiologic variation in uptake and excretion. Studies generally do not measure exogenous exposure but rather variation in blood PFAS levels across individuals with differing biomarker levels. To the extent that these blood PFAS levels reflect physiologic differences that also impact the health effect being evaluated, there is a risk of confounding that applies to essentially all the studies considered.

The susceptibility to "physiologic confounding" is greatest when no environmental basis for differing levels among study participants has been identified. Therefore, the relatively few studies that are from populations with a wider range of exposures, such as those based on populations with differing water contamination levels (e.g., in the mid-Ohio Valley (Steenland et al., 2009) and in Ronneby, Sweden (Andersson et al., 2019), are less susceptible to this bias even when they rely on exposure biomarker levels rather than concentrations of PFAS in the water.

When the product of the study is a correlation of blood serum levels of PFAS with blood levels of antibody, cholesterol, or liver enzymes, the possibility that physiological factors may potentially jointly affect PFAS and clinical outcome measures should be considered. Birthweight studies are clearly susceptible since it is well-established that greater plasma volume expansion is associated with greater birthweight (Salas et al., 2006) and likely also associated with lower (diluted) PFAS levels, which would create a spuriously elevated estimate of the quantitative impact of PFAS on birthweight. A recent examination of the literature (Steenland et al., 2018) provided indirect support for this hypothesized bias, with studies that measured PFAS exposure later in pregnancy when this would have the greatest effect showing the strongest association with reduced birthweight. Additionally, Verner et al. (2015) concluded that some, but not all, of the decreased in birthweight associated with maternal PFAS is accounted for by differences in maternal glomerular filtration rate and that this effect may be greater in studies based on blood serum PFAS measured later in pregnancy. A new study by Chang et al.. (2022) links PFOA (and PFNA) with changes in both metabolic pathways and decreased fetal growth (small for gestational age) in pregnant African American women (i.e., a human study). Chang et al. also attempted to examine biomarkers less prone to reverse causation.

There is no direct way to overcome this uncertainty because relatively few studies have included populations with clear exogenous sources that drive differences in PFAS levels. If results of studies based on elevated environmental exposures are consistent with findings based on blood PFAS levels in the general population, then there is greater confidence overall in the weight of evidence for that effect.

Additionally, studies of birthweight measuring PFAS before or early in pregnancy are more informative than those that measure exposure later in pregnancy. If there are studies that provide some temporal separation of the measures of PFAS and clinical biomarkers, those would be preferable to those that measure them simultaneously. The draft PFOA document (p. 45) and the

PFOS document (p. 43) state that: "More confidence was placed in the epidemiologic studies [of birthweight] that adjusted for glomerular filtration rate in their regression models or if they limited this potential source of confounding by sampling PFAS levels earlier in pregnancy." However, the consideration of these factors in specific studies selected for POD development is not clearly discussed in the draft MCLG documents, and the Panel recommends EPA add this discussion.

Because susceptibility to confounding of the sort described above would likely differ across the endpoints of interest, this provides a strong justification to consider multiple endpoints rather than just one (i.e., consider antibody response to vaccines, serum lipids, liver enzymes, and birthweight, not just one endpoint or one study from among an array of broadly similar ones). Several EPA IRIS assessments, including benzo(a)pyrene (U.S. EPA, 2017) and trichloroethylene (U.S. EPA, 2011) present RfDs for several different non-cancer effects. The final RfD that was selected was supported by generally similar RfDs for other non-cancer effects.

Role of socioeconomic status (SES) and potential for residual confounding

Social factors often are associated with exposure to environmental contaminants and that possibility exists in some of the study populations. The assessment calls for thorough analysis for the presence of confounding and evaluating adjustments, specifically to determine whether residual confounding is present.

Potential residual confounding by socioeconomic status (SES) often contributes substantially to an overall low confidence rating for a study. “SES” is loosely used throughout the document and is a broad term with many contextual components. It would be helpful for EPA to describe which components of SES are suspected to confound associations of PFAS with particular health endpoints and if any studies adjusted for potential confounding from these SES components *versus* none. Assuming that any and all components of SES will always confound associations with PFAS is not supportable. For instance, exposure to PFAS from drinking water does not necessarily correlate with lower SES, depending on the characteristics of the population served by different water sources. Using incomplete adjustment for SES as the primary basis for excluding studies from further consideration irrespective of the particular circumstances and sources of PFAS in each study is not warranted.

Most studies have been conducted among predominantly white populations. Levels of individual serum PFAS levels in the general population have been shown to vary by race/ethnicity, as demonstrated in analyses of data from multiple NHANES cycles (Calafat et al., 2007). In general, PFAS levels in NHANES are lowest in Mexican Americans. Non-Hispanic White individuals have been consistently shown to have higher levels of PFOA compared to non-Hispanic Black individuals in NHANES, while for PFOS the levels are similar or somewhat lower among non-Hispanic Black compared to White individuals in NHANES. Additional analyses (Park et al., 2019; Ding et al., 2020) have also shown the importance of geography in evaluating racial disparities in PFAS exposure and serum concentrations, as well as differences in rates of decline of PFAS in blood by racial group. These studies also highlight PFAS serum level disparities between Asian populations and other racial/ethnic groups. Sensitivity analyses

restricted to studies that included or separated diverse racial/ethnic groups would be worth considering; at the very least, potential confounding by race/ethnicity should be acknowledged particularly as some of the studied health endpoints may also exhibit racial/ethnic disparities.

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.
- 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.
- 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.

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?

General Comments

Overall, the Panel agreed with the selection of the critical study, Grandjean et al. (2012), and the critical effect, suppression of a vaccine response in children exposed during development, as appropriate for the derivation of chronic RfDs for PFOA and PFOS.

The critical effect is a reduction in antibodies produced in response to a vaccine in children exposed to higher levels of PFOA or PFOS compared to children with lower levels of exposure. Reduction in antibodies to a vaccine represents the failure of the immune system to respond to a specific challenge and is considered an adverse immunological outcome. The vaccine response is a functional response of the immune system to a specific challenge, in this case, an antigen (i.e., a non-self substance that stimulates a response from the immune system) delivered in the form of a vaccine. When a vaccine is delivered, cells of the immune system coordinate a response where the antigen is recognized, and cells of the adaptive immune system are ultimately stimulated to generate antibodies to the antigen. When the vaccine response is suppressed, it indicates that some part of the immune system is not performing at the level that it should. This form of immunosuppression could indicate impacts on one or more parts of the innate and/or adaptive immune systems.

The critical effect of a suppressed antibody response to a vaccination is consistent with findings from additional epidemiological studies of different study populations across a range of vaccine types and also concurs with multiple studies in experimental animal models demonstrating that PFOA and PFOA are able to suppress the antigen-specific antibody response, the analogue to a vaccination in humans (NTP, 2016).

While the Panel agrees with the selection of the critical study and the critical effect, some expressed concerns with the process by which they were selected for the derivation of chronic RfDs for PFOA and PFOS. A more thorough rationale for selecting the critical study and critical effect should be presented with additional candidate RfDs based on both human and animal studies. These concerns and accompanying recommendations are detailed in the remaining sections.

Critical Study

Several studies have been published investigating PFAS and vaccine responses in study participants from the Faroe Islands, the population upon which the critical study is based. These studies are somewhat homogeneous in that they shared not only a study population but were conducted by the same core group of researchers who designed and interpreted the studies in similar ways. The Panel recommends that supporting and contrasting data from study populations from the Faroe Islands and other locations be included in tabular form in, for example, Sections 3.3.4.1.1 of both documents where evidence of immunosuppression in human populations is presented. In addition to the epidemiological studies discussed in the draft documents, the summary tables should also include the studies of decreased antibody response to vaccination that are not discussed in the draft MCLG documents, such as, earlier studies included in the 2016 HESD and more recent studies recommended by Panel members. Including such additional information would improve clarity and transparency for the selection of the critical study and would verify that the critical study and its study population are congruous with studies evaluating vaccine responses in other populations and not simply an outlier.

Additionally, while data from publications that studied participants from the Faroe Island (Grandjean et al., 2012; 2017a, 2017b; Mogensen et al., 2015) are summarized in the text and listed in Figures 61 (PFOA) and 62 (PFOS), it is challenging to understand the design of the study/studies from the information presented in the draft MCLG documents. These four

publications provide evaluations of one, two, or both study cohorts that include subjects born in different time periods, with measurements of serum PFAS concentrations collected from maternal or child blood at different times/ages, and responses to vaccines in children measured at different ages. For clarity, a table that presents the designs of these Faroe Islands studies is needed in the draft MCLG documents.

The Panel was also concerned that the rationale for the choice of the critical study and dataset used for RfD derivation was not provided. One initial limitation of using Grandjean et al. (2012) and other epidemiological studies examining decreased antibody responses to vaccines associated with PFAS for derivation of RfDs was that these studies lacked information about the dose-response for PFOA and PFOS individually. This issue was subsequently addressed in the development of BMDs and BMDLs from the Grandjean et al. (2012) data in the modeling done by Budtz-Jorgensen and Grandjean (2018). This modeling accounted for the impact of co-exposure to PFOA, PFOS, and other PFAS on the dose-response for each individual PFAS. While the Panel acknowledges that the availability of BMDs and BMDLs that accounted for the impact of other PFAS may have been the primary reason for the selection of Grandjean et al. (2012) as the critical study rather than one of the other studies of decreased vaccine response, no explanation for this choice is provided in the draft MCLG documents.

The Panel also notes the lack of clarity concerning why data for age five serum PFAS and age seven antibody response was selected instead of maternal serum PFAS and age five antibody response, which also are included in Grandjean et al. (2012) and Budtz-Jorgensen (2018). The fact that the PODs are based on age five serum PFAS data and age seven antibody responses does not appear to be mentioned in the text or tables in Section 4.0, although this is mentioned in Appendix B.1.1 where the BMD modeling is discussed. However, even in Appendix B.1.1, it does not appear to be mentioned that Budtz-Jorgensen and Grandjean (2018) also evaluated data for maternal serum PFOA and PFOS and age five antibody responses.

It also appears that the critical study (Grandjean et al., 2012) did not undergo the full systematic review evaluation for the domains included in the HAWC. Presumably, only those human studies that were not included in the 2016 HESD underwent a full systematic review. As data from Grandjean et al. (2012) were used as the basis for the final RfDs, it should be included in the systematic review of studies of decreased response to vaccines.

The Panel also noted that neither the California EPA nor the European Food Safety Agency (EFSA) selected Grandjean et al. (2012) as the basis for their toxicity factors (RfDs and Tolerable Weekly Intakes, respectively). The Panel recommends that EPA review the draft Public Health Goal documents produced by California EPA (2021) and the basis for the EFSA (2020) Tolerable Weekly Intakes as they may contain useful information salient to reviewing Grandjean et al. (2012).

While the research from the Faroe Islands is the basis that EPA used for BMD analysis, the Timmermann et al. (2022) study in Greenland needs to be considered. Additionally, studies of measles antibody response in Guinea-Bissau (Timmermann et al., 2020) and Hepatitis A antibody response in the Faroe Islands (Shih et al., 2021) need to be considered for transparency and consideration of other relevant health endpoints.

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.

Other Critical Effects

The Panel appreciates that effects used as the basis for an RfD must be well established, adverse or a precursor to an adverse effect, and if they are based on data from experimental animal studies, relevant to humans. Information presented in the draft MCLG documents demonstrates that decreased antibody responses to vaccinations is a well-established effect of PFOA and PFOS; this conclusion is further supported by earlier studies included in the 2016 HESDs and more recent studies as noted earlier. The Panel also agrees that decreased antibody responses to vaccinations are adverse effects, and that this effect is an appropriate critical effect for deriving RfDs for PFOA and PFOS.

The Panel notes that the volume and quality of evidence for immunosuppression is similar to other health endpoints that have been consistently reported in epidemiological studies for populations exposed to PFOA and PFOS. The Panel is therefore concerned that the preference for decreased antibody responses to vaccinations over increased serum cholesterol, changes in liver enzymes, or decreased birthweight is not clearly explained. Although the POD at a modeled human equivalent dose (POD_{HED}) for decreased antibody responses to vaccinations in children is lower than the POD_{HED} values for other endpoints for both PFOA and PFOS and likely will be protective of sensitive subpopulations, additional justification is needed to support the selection of this endpoint as the critical effect. While it is appropriate to seek to derive the RfD from the most sensitive endpoint, selecting the lowest value without consideration of other factors is not justified.

For instance, while decreased antibody response is an important developmental outcome, decreased birthweight needs to be considered as an alternate development outcome. The proposed RfD is based on studies of decreased serum antibody concentrations that are of medium quality. For PFOA, the candidate RfDs developed from high-quality studies of decreased birthweight are 3, 60, 70, 70, and 300 times larger than the candidate RfD based on serum antibody concentration (ranked by magnitude). For PFOS, the difference is smaller; the candidate RfDs for decreased birthweight are 20, 20, 20, and 100 times larger. The choice between a smaller RfD from lower-quality studies (all from the same research group and study population) and a larger RfD from higher-quality studies (from multiple groups and populations) requires a strong rationale and perhaps discussion of how much difference it makes to the result.

Because the strength of evidence and clinical relevance are similar for all four health effects mentioned above, the Panel recommends that candidate RfDs be developed for multiple human health endpoints for which appropriate dose-response data are available. Additionally, it is not clear why PODs based on experimental animal studies were not also used to derive candidate RfDs. Transparency would be increased by first deriving candidate values from all eligible human and animal studies (with adequate confidence, etc.), and then subsequently selecting health effect-specific and overall RfDs. Summary tables, as recommended for studies of vaccine responses, are also recommended for other endpoints considered for RfD derivation. Table 15 in both documents summarizes the studies across these endpoints considered for derivation of points of departure (POD) but these tables do not include important study details, such as serum PFAS concentrations.

Notably, it does not appear that health-effect-specific RfDs were developed, even for the existing candidate PODs. Such health-effect-specific RfDs are clearly needed to implement the draft mixture framework being reviewed concurrently. The strengths and limitations for each study/POD across endpoints, including risk of bias, concordance or disagreement between human and animal studies, and dose-response uncertainties, should be described to justify the selection of the dataset used to derive RfDs for each health effect. Thereafter, selection of the overall RfD from among the health-effect-specific RfDs should also discuss the strengths and limitations, including strength of evidence for hazard and the uncertainties.

Additionally, the Panel notes that in the benefits from reduction of cardiovascular disease document also being reviewed separately by this Panel, there is a meta-analysis for total cholesterol; it would seem straightforward to apply the same methodology to derive the beta-coefficients ("re-expressed," if necessary, in units of per ng/mL) for antibody responses to vaccines and other health-effect-specific endpoints. Such a coefficient could then be used for deriving PODs. Further, expressing study results in terms of a "slope" (e.g., beta coefficient) that could be used to derive a risk-specific dose should be considered. These "slope" values would be more amenable to economic benefit-cost analysis than PODs or RfDs.

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 POD_{HED}, 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.

<u>Additional analyses or rationales</u>

Clinical relevance of the critical effect

Decreased antibody responses to vaccines is relevant to clinical health outcomes and likely to be predictive of risk of disease. The conclusion that suppression of vaccine responses is an adverse finding is widely accepted in the field of immunotoxicology. Immunosuppression indicated by reductions in antibodies to an antigenic challenge such as measured by vaccine responses in humans is quite different from immunosuppression that can classified as an immunodeficiency from, for example, a genetic condition or a disease that severely depletes cells of the immune system. The form of immunosuppression that is represented by a reduction in the antibody response is a form that is similar to those who are at the extremes of age (young and old), those who have received organ transplants and are on immunosuppressive therapies, and those who are exposed to chronic stress; additionally, mild to moderate immune suppression increases the risk of infections with pathogens commonly encountered in the general population (Selgrade, 2007). When the antigen-specific antibody response is evaluated in experimental animal models, results are translatable across multiple species, including rodents and humans. Historical data associated with suppression of this response also indicates that it is highly predictive of immunotoxicity (Myers, 2018). Moreover, the immunosuppression indicated by the observed antibody decreases are not limited to those specific antigens (e.g., tetanus and diphtheria only), but rather are indicative of modulation of the general immune response.

It is not always possible to determine a level of protection from an antibody titer from a specific vaccination (Van Loveren et al., 2001). Many different factors can affect titers in individuals, and some individuals may not be appropriately protected with the vaccination. Nonetheless, even when titers in some individuals fall below a certain threshold, especially when the overall goal is protective immunity for a population, the population will likely be protected from the agent intended by the vaccination (Van Loveren et al., 2001). However, it is likely that reduced antibody responses to a specific vaccine that are associated with an environmental agent in a population may also decrease resistance to infections in that population (Van Loveren et al., 2001).

Additionally, epidemiological evidence for increased risk of infectious disease associated with PFOA and PFOS exposure is discussed in Section 3.3.4.1.1 (evidence of immunosuppression in human populations) in both documents. This effect is not, however, included in the evidence integration sections of the draft MCLG documents, and no strength of evidence conclusions are provided. While evidence for an association of PFOA and PFOS with infectious disease is mixed, a 2019 review of the immuno-toxicological literature used to support an immune-based toxicity value for PFOS in drinking water concluded that studies available through 2018 "provide evidence for an association between general population levels of PFOS exposure and infectious disease, a clinically meaningful measure of health risk" (Pachkowski et al., 2019). Several more recent publications that address PFAS exposure and infectious disease/risk of infectious disease also were not included in the draft MCLG documents (Bulka et al., 2021, Dalsager et al., 2021, and Timmermann et al., 2020).

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.

Use of epidemiological data rather than experimental animal data as basis for RfD

The Panel supports the use of human epidemiological data as the basis for RfDs for PFOA and PFOS and notes that several human health outcomes, including decreased antibody responses to vaccinations in children, increased serum cholesterol, changes in liver enzymes, and decreased birthweight, have consistently been associated with PFOA and PFOS exposure, including within the range of general population exposures. However, an expanded explanation of the rationale for the preferential use of human studies for POD derivation is needed, especially because PODs from human data are much lower than if animal data were used. It is important that the rationale for this decision be thoroughly explained and supported because it is a major shift from the approach used in the 2016 HESD, which concluded that human data were precluded from use for PODs and RfDs. This explanation should include strengths and limitations of PODs based on studies from both human and experimental animal models.

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.

Application of toxicokinetic model

Although toxicokinetic models are discussed in other Charge Questions, the Panel raises some concern that are applicable to additional analyses or rationales needed to increase the confidence in the RfDs for PFOA and PFOS. With respect to the toxicokinetic model, candidate RfDs themselves (as opposed to just the PODs) could be derived based on internal dose units (e.g., serum ng/ml). Using this approach, exposures at a constant drinking water concentration could be modeled with the toxicokinetic model and appropriate exposure factors, and the MCLG could be derived as the drinking water concentration for which the internal dose POD is not exceeded for a specified exposure duration. The distinction between drinking water concentration and oral dose is especially important because several health outcomes under consideration occur as a result of exposures during particular time periods, such as *in utero* and during childhood. During these periods, exposure factors differ substantially from those for adulthood, for example.

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.

Duration(s) of exposure to which RfDs apply

The PFOA and PFOS RfDs are based on an effect that results from shorter-than-chronic exposure (serum PFOA or PFOS levels at age five). These RfDs are considered to be protective for chronic exposures, as well as shorter-term exposures, because they are more sensitive (i.e., have lower PODs) than RfDs for chronic effects. This differs from many other contaminants (e.g., GenX) for which shorter-term (e.g., subchronic) RfDs are higher than chronic RfDs (U.S. EPA, 2021).

Although EPA stated, at the SAB meeting on December 16, 2021, that the RfDs apply to shorter-than-chronic as well as chronic exposures, the draft MCLG documents state only that PFOA and PFOS RfDs are intended for chronic exposures. Their application to shorter exposure durations needs to be clearly stated. In situations of drinking water contamination with PFOA and/or PFOS, the duration of exposure to which these RfDs apply has practical implications for the timeframe (e.g., acute, short term, longer term) in which exposure to contaminated drinking water needs to be stopped when the MCL is exceeded.

Relevant to this issue, the Panel notes that the U.S. EPA (2009) Provisional Drinking Water Health Advisories for PFOA and PFOS were stated to apply to short-term exposures. Additionally, the U.S. EPA (2016) Lifetime Health Advisories for PFOA and PFOS, which are based on developmental effects, are stated to apply to both short-term (weeks to months) and lifetime (chronic) exposure.

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.

Benchmark dose (BMD) model and BMD level

The Panel notes that EPA did not independently replicate the BMD modeling conducted by Budtz-Jorgensen and Grandjean (2018). It also appears that neither the details of the modeling nor the modeling output are included in the Budtz-Jorgensen and Grandjean (2018) publication. The Panel was informed at the December 16, 2021, meeting that EPA did not independently replicate the Budtz-Jorgensen and Grandjean (2018) modeling and the authors provided a supplemental document with details of the BMD modeling, which was reviewed by the EPA Office of Research and Development. However, the EPA's review of this BMD modeling is not discussed in the draft MCLG documents. The Panel concludes that it is critical to include the unpublished supplemental document provided to EPA by the authors of Budtz-Jorgensen and Grandjean (2018), as well as the details and conclusions of EPA's review of this BMD modeling in the final MCLG documents. While it seems unlikely that the supplemental document contains confidential information such as personally identifiable information associated with study participants, a version with the confidential information redacted could be provided if such information is included in the document.

As noted earlier and relevant to the modeling conducted Budtz-Jorgensen and Grandjean (2018), there was no rationale provided as to why EPA selected the BMDLs for age five serum PFOA or PFOS and age seven antibody responses to vaccinations, rather than maternal serum PFAS and age five antibody responses to vaccinations as the basis for the RfDs. In the absence of a rationale, the reason for this decision is unclear because the BMDLs for maternal serum PFOA and age five antibody responses to vaccinations for the piecewise model (the model that was selected by EPA) are lower, and the ratios between the BMDs and the BMDLs are smaller, than for the BMDLs for the piecewise model for age five serum PFOA at age five years and tetanus antibody responses at age seven years that was selected by EPA. Further, the rationale in the first paragraph of Section B.1.1 in the draft MCLG documents for selection of the BMDLs for the piecewise model instead of the linear model is unclear.

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.

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 andGrandjean, 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.

The Panel generally supports the use of 5% BMR for decrease in antibody response. However, stronger justification of the significance and relevance of the decreased antibody response in comparison with other adverse outcomes (e.g., decreased birth weight, elevated serum ALT, increased cholesterol) will strengthen the rationale.

The 5% BMR is supported by the fact that the decreased antibody response to vaccinations caused by PFOA and PFOS is a developmental effect and the potential severity of vaccine-preventable illnesses (i.e., tetanus and diphtheria). The developing immune system (i.e., the immune system of children) is generally regarded as more sensitive to exogenous perturbations such as those from chemical stressors than the adult immune system. Therefore, changes observed in the developing immune system reflect developmental effects. The immune response to childhood vaccines may be "an excellent indicator for developmental immunotoxicity when conducted under appropriate conditions" (Luster et al., 2005). Responses to childhood vaccines are thought to be sensitive enough to detect changes in populations with moderate degrees of immunosuppression, such as those exposed to an immunotoxic agent (Luster et al., 2005).

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

The degree to which mild to moderate immunosuppression from exposure to chemical agents produces measurable clinical outcomes can be challenging to determine in traditional epidemiologic studies (DeWitt et al., 2017). However, evidence from specific populations experiencing mild to moderate immunosuppression indicates that the risk of infections with pathogens commonly encountered in the general population is real (Selgrade, 2007). Reductions in antibody titers to a specific vaccine below a level that is considered protective does increase *the risk* of susceptibility to the disease against which the vaccine was intended (McComb, 1964; MacLennan et al., 1965; World Health Organization (WHO), 2017; WHO, 2018). Additionally, "a compromised immune system should be considered more prone to escape homeostasis, enhancing risk for disease development" (Hessel et al., 2015). The clinically significant decrease in tetanus and diphtheria antibody concentrations is generally considered to have antibody concentrations below 0.1 IU/mL. Considering the large number of people (30-40%) having antibody concentrations close to 0.1 IU/mL, a further 5% decrease in antibody concentrations could be problematic for disease protection. More susceptible individuals may be more likely to be affected by the infection. The benchmark response of 5% is conservative for people at the lower end of antibody response.

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.

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.

The Panel noted that in Section 4.1.5 of the PFOA (pp. 337 – 339) and PFOS (pp. 308 – 310) documents, EPA applied a value of 1 for interspecies (UF_A), subchronic-to-chronic (UF_S), LOAEL-to-NOAEL (UF_L), and database (UF_D) uncertainty factors. Justification for these uncertainty factors can be found in Table 22 of both documents (PFOA: pp. 337 – 8; PFOS: p. 308). Briefly summarized, a UF_A of 1 was selected because the RfDs were developed with human data. A UF_S of 1 was selected because the critical effects (decreased antibody response to tetanus or diphtheria vaccine from exposure at age 5) were the result of a shorter-than-chronic exposure that is more sensitive than the chronic effects of PFOA and PFOS. A UF_L of 1 was selected because the RfDs were based on a BMDL. Finally, a UF_D of 1 was selected because the database includes numerous medium- and high-quality studies and a more sensitive endpoint than the critical effect is not expected. For both PFOA and PFOS, EPA applied a default value of 10 for the intraspecies (UF_H) uncertainty factor to account for variability within human populations based on intrinsic and extrinsic factors that can influence response.

The Panel generally finds these values to be adequate and supported by the scientific rationale provided by the agency. The values were found to be appropriate and sufficiently protective, with rationale that was clearly described in the draft MCLG documents.

However, the Panel notes that the draft PFOS MCLG document does not discuss that the USEPA approach for developing human health goals for MCLs (i.e., MCLGs) specifies incorporation of an additional UF of 10 for potential carcinogenic effects into the RfD when there is some evidence of carcinogenicity and there are insufficient data to develop a cancer slope factor (USEPA, 1985). This approach is specified by USEPA (1985) for contaminants classified as "possible human carcinogens (Group C)" which is analogous to "suggestive evidence of carcinogenic potential" in the current terminology from the USEPA (2005) cancer risk assessment guidelines. An additional UF of 10 for potential carcinogenicity was incorporated into the RfDs for several USEPA MCLGs/MCLs including, for example, para-dichlorobenzene (USEPA, 1987).

Although PFOS is classified as a "suggestive carcinogen" in the draft MCLG document, incorporation of this additional UF was not mentioned by the EPA. The cancer slope factors for PFOS developed by NJDEP (2018) and California EPA (2021, draft) of 9 and 15.6 $(mg/kg/day)^{-1}$, respectively, can be used to estimate the daily PFOS dose associated with a 10^{-6} cancer risk as 0.11 ng/kg/day and 0.064 ng/kg/day, respectively. These daily doses are much higher than the draft USEPA RfD for PFOS of 0.0079 ng/kg/day, indicating that the RfD is protective for cancer risk and an additional UF is not needed. However, it is recommended that

EPA acknowledge that this UF is considered when developing RfDs for use in MCLGs for "suggestive carcinogens" such as PFOS and explain the rationale for its inclusion or exclusion.

While the Panel recognizes that the EPA has time and resource constraints, they recommend that the agency consider the adoption of a probabilistic framework (including UF distributions, rather than fixed values) to calculate risk-specific doses as a replacement for traditional RfDs, in line with the recommendations of the NASEM (2009) Science and Decisions report. A recent publication Chiu et al. (2018) demonstrated broad application of this approach using experimental animal studies across many chemicals and endpoints and included EPA authors from EPA's National Center for Environmental Economics. This probabilistic framework not only includes default UF distributions based on reviews of the literature, but it also enables derivation of dose-response functions (or risk-specific doses) that can be used for benefit-cost analysis. EPA should consider whether applying this approach would be useful for MCLG derivation and the regulatory impact assessments that will be needed to set MCLs.

Another suggestion is that EPA consider the appropriateness of using an additional uncertainty factor (either as a justification for an increase in an existing uncertainty factor, e.g., UF_H or UF_D, or as an independent UF) that accounts for the effects of simultaneous co-exposures to PFOA/PFOS and complex mixtures of chemical and non-chemical stressors. As the agency acknowledges, PFAS are known to occur in mixtures, with thousands of chemicals in the class. Although a UF_{TOT} of 10 could represent a conservative approach for PFOA and PFOS, it may not be fully protective of highly exposed and susceptible populations exposed to PFAS in drinking water.

One potential framework to explore is the Mixture Assessment Factor (MAF). The use of MAFs is discussed in Kortenkamp and Faust (2018) and is currently being explored by the European Commission (2020) for the assessment and management of chemical mixtures. A potential starting place for incorporating an MAF would be to create an UF_M that accounts for mixtures, with a default value equal to 10. The default value assumes that only a small number of chemicals contribute to a particular effect. The value of the UF_M could be increased or decreased based upon the number of chemicals (and to the extent possible, non-chemical stressors) expected to co-occur with the chemical being evaluated. Given that PFAS often co-occur, PFOA and PFOS are ideal chemicals for which to utilize a mixture-associated uncertainty factor with the default value of 10. However, the Panel did not reach consensus on advising this approach for the current assessments because of divergent views on the appropriate methods of accounting for the effects of mixtures on chemical toxicity. The Panel notes that reference dose is specific to the intrinsic behavior of the chemicals being evaluated, in the absence of other chemicals that cause the same effect or inhibit its effect, and there was concern about using this approach because an uncertainty factor accounting for the effects of other chemicals on the intrinsic behavior of the chemical being studied would be inappropriate. The approaches discussed in the draft EPA Mixtures Framework document—in which co-occurring PFAS that cause the same toxic effect are considered—were seen as more appropriate for accounting for toxicity of mixtures of PFAS. In these approaches, a toxicity factor or relative potency is developed for each individual PFAS, and their additive effects are considered.

Others noted that uncertainty factors should have a clear conceptual basis before being applied. There were concerns that identifying an agreed-upon framework for developing a mixture

uncertainty factor, should one exist, was beyond the scope of the charge. Furthermore, it could be argued that the concerns about mixtures are already covered by the UF_H, because human variability incorporates not just genetic variability, but variability in background and co-exposures, nutrition, etc. (Zeise et al. 2013).

In addition to considerations associated with the use of uncertainty factors, the Panel also recommends that as the agency moves forward, it should consider cumulative risk, not just from multiplicity of chemical exposures, but from other environmental factors that enhance susceptibility – including co-morbidities and/or co-exposure to social disparities (including racial, economic, and power disparities). Of particular concern are populations that may have increased susceptibility across multiple, biological, chemical and social domains (Pullen Fedinick et al., 2021). While cumulative approaches may be difficult to apply for the assessments of PFOA and PFOS for MCLG derivation, development of approaches for combined risks to health from multiple stressors is an important area of risk assessment that warrants further consideration.

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.

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 PFOAand PFOS? If so, please provide citations.*
ii. *Please provide comment on whether the recommended RSC of 20 percent (0.20) for PFOA and PFOS isadequately supported and clearly described.*

The Panel concludes that the recommended Relative Source Contribution (RSC) of 20% in the draft MCLG documents is appropriate for the PFOA and PFOS Reference Doses (RfD) development. A 20% RSC is the default value specified in the U.S. EPA (2000) guidance for deriving ambient water quality criteria for the protection of human health, which is cited as the basis for the RSC selection in the draft MCLG documents. The U.S. EPA (2000) guidance recommends an RSC range with a minimum ("floor") of 20% and a maximum ("ceiling") of 80%. A lower RSC results in a more stringent and protective water concentration, as represented by the MCLG. Hence the default value of 20%, referred to as the "floor" value in the U.S. EPA (2000) guidance, is the most stringent and public health protective choice.

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).

The most important concept that the Panel would like to reiterate is that the RSC represents the portion of the RfD that is allocated to drinking water. The intent of applying an RSC is that the total exposure from all sources at the MCLG will not exceed the RfD. The RSC is therefore dependent on the portion (percentage) of the RfD that is known or assumed to come from non-drinking water sources (e.g., food, consumer products, air, dust). Therefore, actual PFOA/PFOS exposures from drinking water, including the percentage of total PFOA or PFOS exposure that comes from drinking water and the concentrations of PFOA or PFOS in drinking water, are not relevant to RSC selection.

However, the draft MCLG documents describe the RSC both in terms of the portion of the RfD that is attributable to drinking water and in terms of PFOA/PFOS intake from water relative to total intake, where the latter is not relevant to the RfD. The RSC determination should not be based on the relative PFOA or PFOS water to non-water exposures, without the context of the RfD. In other words, the denominator in the RSC calculation, is the RfD, not the total PFOA or PFOS exposure. The Panel therefore recommends that EPA revise the RSC section in the draft MCLG documents to promote a common understanding of the concepts and methodologies involved in the RSC determination as per the 2000 EPA Guidance. The document should clearly outline why the 20% value is the most appropriate for the proposed PFOA and PFOS RfDs.

Specifically, statements in the draft document that suggest that the percent of exposure from drinking water is relevant to RSC selection should be removed. For example, Section 5.1.4 of the draft PFOA document and Section 5.4 of the draft MCLG documents discuss several studies (Hu et al., 2019; East et al., 2021; Gebbink et al., 2015; Jogsten et al., 2012), which estimate the percentage of total exposure to PFOA and/or PFOS from drinking water is less than 20%, and further state that these “estimates support a 20% RSC for drinking water.” Another example is the statement in the draft MCLG documents that “for disproportionately affected subpopulations, such as the occupationally exposed or site-impacted (e.g., by a particular source or industry)

where there may be higher average PFAS concentrations in drinking water, it may be appropriate to apply an RSC greater than 20 percent if there is sufficient information to quantitatively characterize sources other than drinking water."

Additionally, because the RSC is based on the portion of the RfD that comes from non-drinking water sources, the choice of the RSC depends on the numerical value of the RfD. The RSC will decrease as the RfD decreases since the non-drinking water exposures represent a higher proportion of a lower RfD. Because the RfDs used in the 2016 EPA Health Advisories and in state drinking water guidelines are several orders of magnitude higher than the RfDs presented in the draft MCLG documents, the RSCs used in the 2016 HESD and by states (discussed on p. 347-348 of the PFOA document) are not relevant to the selection of the RSC in the current draft MCLG documents.

These revisions and clarifications will also help address questions related to the relevance to RSC selection of factors such as protection of disproportionately affected subpopulations and sensitive subpopulations such as infants and children, PFOA and PFOS concentrations in drinking water and relative exposures from drinking water, and serum concentrations from biomonitoring data. These issues are discussed in the RSC section of the draft MCLG documents and have therefore been brought up by SAB panelists and in public comments. The Panel recommends that EPA should therefore clarify what, if any, roles these factors play in the selection of an RSC of 20% as recommended in U.S. EPA (2000) guidance, or if they are being referenced as supplementary information and are more directly accounted for elsewhere in the MCLG development.

Although the rationale for an RSC of 20% in the draft document needs clarification, available data on non-drinking water exposures to PFOA and PFOS clearly support the choice of an RSC of 20% for MCLGs based on the RfDs presented in the draft document. Estimates of daily dietary exposure to PFOA in studies from North America and Europe reviewed by DWQI (2017) range from 0.16 ng/kg/day to 6.2 ng/kg/day, and the lower bound estimate of median daily dietary exposure for adults from EFSA (2020) is 0.18 ng/kg/day, with higher values for younger age groups. The RfD of 1.5×10^{-9} mg/kg/day (0.0015 ng/kg/day) in the draft PFOA document is two orders of magnitude below the lowest of these dietary estimates. Similarly, PFOS RfD of 7.9×10^{-9} mg/kg/day (0.0079 ng/kg/day) is almost two orders of magnitude lower than the EFSA (2020) lower bound estimate of median daily dietary exposure to PFOS in adults of 0.58 ng/kg/day. Additionally, there are non-drinking water exposures from other sources such as consumer products and house dust. Therefore, exposures from non-drinking water sources far exceed the RfD, indicating the choice of the default RSC of 20% is appropriate.

An RSC of 20% is also supported by data on serum PFOA and PFOS levels from the U.S. general population. The serum PFAS levels associated with the RfDs can be determined by applying an uncertainty factor of 10 to the $POD_{\text{Internal Dose}}$ (e.g., human serum levels at the PODs) of 1.7×10^{-4} mg/L (0.17 ng/ml) from Table 21 of the draft PFOA document and 5.4×10^{-4} mg/L (0.54 ng/ml) from Table 21 of the draft PFOS document. The serum levels associated with the RfDs, 0.017 ng/ml for PFOA and 0.054 ng/ml for PFOS, are far below even the lower percentiles for serum PFOA and PFOS in the U.S. general population in the most recent (2017-18) NHANES data. In the 2017-18 NHANES, the 5^{th} percentile serum PFOA level, presumably representing those with little or no drinking water exposure, is 0.48 ng/ml, which is 28-fold

higher than the serum level associated with the RfD of 0.017 ng/ml. Similarly, the 5th percentile serum PFOS level, also presumably representing those with little or no drinking water exposure, is 1.08 ng/ml, which is 20-fold higher than the serum level associated with the RfD, 0.054 ng/ml. Therefore, serum PFOA and PFOS levels in the lowest 5th percentile of the general population (presumably without exposure to contaminated drinking water) far exceed 100% of the RfD, supporting the default RSC of 20%.

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.

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SECTION II - Mixtures approaches

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

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 biologicalorganization 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 purposesof a component-based evaluation of mixtures additivity for PFAS, EPA assumes similarity a tthe 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).*

The per- and polyfluoroalkyl substances (PFAS) chemical class includes thousands of unique chemicals that exist in environmental media often as PFAS mixtures. Toxicological studies to inform human health risk assessment are largely lacking, both for many individual PFAS and PFAS mixtures. Further, mode of action (MOA) data are limited, if not completely lacking, for the majority of PFAS. EPA's draft framework proposes a component-based approach to estimating the probability or magnitude of adverse non-cancer health outcomes for PFAS mixtures. EPA has developed this framework based on the assumption of dose additivity for the component-based approaches used to assess the risks of PFAS mixtures (i.e., HI, TOSHI, RPF, Mixture BMD) presented in the draft framework. The framework proposes that dose additivity can be applied for cases in which the given adverse outcome (i.e., toxicity endpoint/adverse outcome/effect) associated with exposure to 2 or more PFAS chemicals is similar.

Assumption of dose-additivity

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.

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.

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 studyet al. not included in the draft EPA mixtures document, Nielsen et al. (2021), 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; Dinge 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.

Assumption of similarity of toxicity endpoint rather than common MOA in mixtures evaluation

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.

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, the Panel suggests a summary of information indicating 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.

Consideration of human data

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 are based on animal data for some PFAS and based on human data for other PFAS.

Use of NAMs data in component-based mixtures approaches for PFAS

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.

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).

Development of toxicity factors for PFAS for which final EPA toxicity factors are not available

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 encompass processes that include the opportunity for scientific input and review in general, rather than specifically "external peer review."

Recommendations

Overall recommendation: 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.

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.

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.

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.

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.

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, usingavailable 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) dimeracid and HFPO dimer acid ammonium salt (referred to as "GenX chemicals").*

2A. Please provide specific feedback on whether the HI approach is a reasonable methodology for indicating potential risk associated with mixtures of PFAS. Ifnot, please provide an alternative.

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.

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. 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).

2B. Please provide specific feedback on whether the proposed HI methodologies in the framework are scientifically supported for PFAS mixture risk assessment.

There are several challenges and considerations relevant for PFAS mixture risk assessment. These challenges are articulated below.

Lack of toxicology data

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.

Use of TOSHI approach

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.

Consideration of probabilistic methods for HI/TOSHI calculations to estimate risk.

The National Academies of Sciences, Engineering, and Medicine (NASEM, 2021) review of the 2020 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."

Challenges with implementation

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.

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 for PFOA and PFOS (USEPA, 2016a; USEPA, 2016b). As shown in Table 4-3, the PFOA and PFOS health advisories 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. 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.

In the example in Table 4-4 of the draft framework, the individual concentrations for PFOA and PFOS are 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.

Limitations

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

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.

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.

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.

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.

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.

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) (Monte Carlo Risk Assessment (MCRA 9)).

Overall, the Panel agrees that the RPF approach is a reasonable methodology for estimating risk associated with mixtures of PFAS and does not suggest an alternative methodology. The Panel notes that the RPF approach is a more data intensive approach, as compared to the Hazard Index methods, the Panel expresses 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 agrees 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.

The Panel concludes 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 notes guidance would be helpful on the types of data sets that are the 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 recommends re-evaluation of the tiered approach by questioning whether HI or TOSHI is needed before moving on to RPF and suggesting a menu of options based on the available data instead. Overall, there was agreement that removal of tiers would enhance the framework.

The Panel suggests that the EPA provide clarification on conceptual differences between the TOSHI and 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 agrees that the framework should summarize when the TOSHI and RPF approach will yield 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 should indicate if consistency is appropriate or unnecessary (e.g., if one is supposed to be more conservative for screening, rather than more predictive). Overall, the Panel concludes 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.

The Panel also agrees 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.

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. (2020), 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.

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.

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.

The Panel recommends that the framework increase the discussion, rationale, and justification with regard to Index Chemical (IC) selections.

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.*

4A. Please provide specific feedback on whether the Mixture BMD approach is a reasonable methodology for estimating what is in essence a mixture-based point-of-departure. If not, please provide an alternative.

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

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

Relationship to other approaches

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.").

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, $ICEC_{MIX}$ for the RPF approach and t_{add} 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).

Combining eqns. (4.2) and (4.3) implies

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

Taking reciprocals in eqn. (4.5) yields

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

Comparing these results shows that $ICEC_{MIX}$ and $(t_{add})^{-1}$ differ only in inessential details and are essentially proportional to one another, 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, d_i (the "component chemical's concentration") and a_i ("the fixed proportions of the component PFAS in the mixture") are either identical or strictly proportional to each other. Third, $ICEC_{MIX}$ includes a constant proportionality factor ($ED10_{IC}$).

Having constructed $ICEC_{MIX}$ and t_{add}, one can presumably use them to evaluate a risk in analogous ways. The summary t_{add} can be used as a BMD, from which one can calculate a hazard index or use it as a POD from which to extrapolate a dose-response function. Similarly, one can divide $ICEC_{MIX}$ by $ED10_{IC}$ to calculate a hazard index or use it in a dose-response function for the index chemical as in equation 4-4 [i.e., $y_{MIX} = f(ICEC_{MIX})$].

Recommendations

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 merging them into a single section.

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 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 highlighting the utility of this method in a real-world sample (rather than hypothetical case) and in addressing data-poor chemicals seems essential for establishing scientific confidence in the method and evaluating whether it is fit for its intended purpose.

4B. Please provide specific feedback on whether the proposed Mixture BMD methodology in the framework is scientifically supported for PFAS mixture riskassessment.

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).

The Panel notes 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.

Recommendations

The Panel recommends that additional information on how the proposed Mixtures BMD approach will be applied in practice be provided.

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.

Finally, the Panel also included in Appendix B of this report specific areas needing clarification in the PFAS Mixtures document.

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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.

The Panel expresses concern about the apparent discrepancy between this document's focus on CVD risk and the draft MCLG documents' conclusions that the evidence of CVD was not sufficient to form the basis of a RfD. As discussed in the Panel's review of the draft MCLG document, the Panel finds the most consistent epidemiological associations with PFOA and PFOS include decreased immune system response, decreased fetal growth (e.g., decreased birthweight), increased serum lipids, and increased liver enzymes (particularly ALT), with none of these four endpoints being substantially stronger than the others. Overall, the Panel recommends more discussion on the rationale for selecting this particular endpoint for risk reduction analysis (e.g., strengthening the hazard conclusion with respect to PFOA or PFOS, availability of dose-response data to derive a dose-response function or risk-specific dose estimates, strengthening of data connecting changes in biomarker to changes in morbidity or mortality, and availability of data for monetizing benefits).

Nonetheless given that elevated serum cholesterol is one of the better-established effects of PFAS exposure in humans, the described approach is quite reasonable overall. The threshold for assessing benefits of reducing PFAS exposure levels is indicated to only require "a meaningful opportunity for health risk reduction," which seems to be the case here. Therefore, examining the sequence from regulation and reduction of PFAS in drinking water to changes in serum PFAS to changes in serum cholesterol to changes in the rate of cardiovascular events provides a reasonable basis for estimating the cardiovascular benefits of the regulation. In the draft CVD document, 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. EPA should specify additional assumptions and explanations, perhaps by expanding Section 2 of the report.

Specifically, with respect to CVD, the assumption is that a shift in cholesterol resulting from PFAS exposure will have the same impact on cardiovascular disease that cholesterol levels based on natural levels or use of cholesterol lowering medications have had. However, the epidemiologic literature that provides strong support for an effect of PFAS on cholesterol does not provide support for an effect of PFAS on the risk of cardiovascular disease. This does not negate the value of the approach, but the Panel suggests the inclusion in the draft CVD document of an acknowledgement that the approach follows the pathway that links cholesterol to cardiovascular events rather than looking at the reported effects of PFAS directly on cardiovascular disease.

The temporal sequence of events is addressed at many points in the technical details, but the Panel suggests that the draft analysis start with an overview and orientation to help the reader follow what comes next. While the population serum PFAS levels would be altered over time as PFAS levels decrease in drinking water, the serum half-life data indicate that this would not be instantaneous. The EPA draft CVD document states that with that altered serum PFAS level, an instantaneous shift in serum cholesterol would result. The altered serum cholesterol level would then result in changes in the risk of cardiovascular events (e.g., the number of such events occurring per year).

The Panel recommends that EPA perform a sensitivity analysis for each step from beginning to end, while recognizing a need to manage the complexity and volume of results. Perhaps in addition to the "most likely" approach, a chain of assumptions leading to "best case" (maximum benefit) and "worst case" (minimum benefit) would be worthwhile to present.

Additionally, while not directly relevant to the modeling of cholesterol and CVD risk, this analysis raises the question as to what methodologies will be used for estimating health benefits for other endpoints (i.e., those with candidate RfD values in the MCLG document). For instance, it seems that decreased birth weight (as a continuous endpoint or as an increase in the percentage of low-birth weight infants) would be a good candidate for estimates of risk reduction. Some studies in the economic literature that examine the economic consequences of decreased birth weight include Almond (2005), Black et al. (2007), Almond et al. (2010), Barreca et al. (2011), Fletcher (2011), Figlio et al. (2014), Bharadwaj et al. (2013), and Chyn et al. (2021). If EPA were to pursue monetizing this endpoint, the Panel recommends a more thorough literature review for this as well as other endpoints.

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.

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.

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) selectedin 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.*

The approach to identifying and managing the literature on PFAS and cholesterol is reasonable for this purpose, with little basis for more esoteric or complex approaches. What is needed, however, is some sense of the impact had other approaches been used, setting the stage for a sensitivity analysis. If, in fact, a variety of reasonable alternative options would all generate roughly the same predictions, then this would be sufficient to put aside concerns with the specific approach used. If reasonable alternatives would lead to meaningfully different estimates, it would be useful to carry through one or more alternatives to the final results in a sensitivity analysis.

The meta-analysis study selection criteria are well reasoned, and EPA identified 14 relevant studies for analysis. One interesting study (Fitz-Simon et al., 2013) was not included probably due to its different study design of modeling prospective cholesterol level changes by serum PFAS level changes in the C8 Health Study population. However, this study is very relevant to the topic of the meta-analysis. Although its design may not make it easy to be integrated into the meta-analysis, the interpretation of the meta-analysis findings should consider this study in the array of pertinent results.

Because the NHANES has been repeatedly analyzed for the association between PFAS and total cholesterol levels, using meta-analysis may not yield additional value compared with the approach Dong et al. used in the 2019 publication. If the results were from different study populations, meta-analysis results would provide a useful method to aggregate across these populations.

There are a series of related concerns regarding the basis for exclusion of studies from consideration. The criterion of "special populations" requires a clearer explanation and reconsideration. The Panel recommends that EPA list the studies that were excluded from the meta-analysis and provide a brief description of these studies and why each was excluded. EPA mentions that studies performed on specific population subsets were not considered for inclusion in the meta-analysis, but the basis for exclusion other than occupational populations is unclear. For example, what is the rationale for determining that the Steenland et al. 2009 study (C8 cohort) is not a "special population" subset? Similarly, studies of the Inuit Population of Nunavik (Château-Degat et al., 2010) and Chinese Male Adults (Yang et al., 2018) were included but might not have been.

Study quality as the basis for inclusion and exclusion is likewise a relevant consideration that needs to be carefully defined and consistently applied. In the Appendix, EPA mentions that all of the studies in the meta-analysis, except one (Lin et al. 2019) are cross-sectional with various

methodological limitations. It would be helpful to know if the quality of the cross-sectional studies is viewed any differently than Lin et al. (2019) and what the implications of the analysis are when using studies of varying quality, if any. More details are needed regarding the protocol for risk of bias assessment/confidence rankings and whether that approach was used before considering the weight of the evidence and/or carrying studies forward for meta-analysis. The evidence synthesis protocol should also include a "tiered" approach to evaluate whether results or conclusions change based on varied decisions about inclusion of high, medium and low confidence studies across various study design domains (as referenced in the overall discussion of sensitivity analyses above). As discussed in more detail in Section I (MCLG documents), excluding studies automatically for insufficient adjustment for socioeconomic status may also not be warranted.

In addition to variation due to selection of studies, the impact of modeling approach also warrants a sensitivity analysis. For example, the linear untransformed models yield by far the highest slopes, especially for PFOA, which calls for careful interpretation. For extraction of slope values for total cholesterol (TC) and high-density lipoprotein cholesterol (HDLC), Section A.2.1 (pg. 6) states that when multiple models with different confounders were reported within a single study, *either* the most adjusted results or the main model results were selected.

In the slope estimation, the associations for HDLC and PFOA and PFOS were positive, albeit not statistically significant, which may not warrant exclusion of HDLC from consideration in the CVD risk reduction analysis. Selecting endpoints solely on the basis of having statistically significant positive effects may exclude meaningful associations that are imprecise. The Panel encourages further explanation of the basis for excluding this indicator from detailed consideration. For instance, the causal connection between pharmacologically-induced changes in HDLC and changes in CVD morbidity or mortality is less clear than for TC.

Finally, the Panel agrees that the approach for estimating the dose-response function using the Atherosclerotic Cardiovascular Disease (ASCVD) risk assessment tool is likely a reasonable choice. It was developed by leading cardiovascular disease researchers, it is widely used despite some limitations, and there is no reason to invent a new tool for the purposes of this exercise. In fact, it seems the instrument was developed for exactly this purpose, to estimate the impact of interventions including modifying cholesterol levels, and the reduction of PFAS in drinking water is one of the ways this might be accomplished. For conducting sensitivity analysis, the Panel does not expect that other models would yield notably different results, or even if they would, that the others are somehow more accurate.

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.

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.

Overall, the application of the life table methodology to evaluate CVD risk reduction from treatment of PFOS/PFOA is reasonable, and the methodology is generally well-described.

As the draft EPA CVD document highlights, this approach has been used by the EPA previously (e.g., to evaluate the benefits and costs of the 2015 Steam Electric Rule, US EPA 2015 and the Clean Air Act, US EPA 2011). When discussing prior applications (p. 15), 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. Presumably, the EPA will use the resulting estimates to inform the economic analysis of PFOA and PFOS treatment in public water systems. The Panel concludes that an outline is needed explaining how the CVD risk reductions captured using this approach will be monetized, as well as clarifying the differences from previous EPA analyses that have monetized cardiovascular impacts. For example, in the calculation of Clean Air Act benefits (US EPA, 2011), the cardiovascular impacts monetized include premature mortality, myocardial infarction, and cardiovascular hospital admissions, whereas the current application examines fatal and non-fatal myocardial infarction, fatal and non-fatal ischemic stroke, and other coronary heart disease mortality.

An advantage of the life table approach is that it takes into consideration the timing of the treatment and the aging of the population, which captures the impact of treatment on partially treated populations (i.e., those born before treatment occurred). The proposed methodology, however, involves many assumptions and modeling decisions 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. These modeling assumptions are likely to omit components of CVD benefits. The Panel recommends that these assumptions be clearly listed (e.g., in Table 7).

While the overall approach in Section 5.1 was clear, Table 3 could benefit from reorganization to avoid confusion. Specific suggestions for Table 3 include the following: (1) add two additional columns that separate CVD and Non-CVD calculations under "Baseline Calculations" and "Treatment Scenario Calculations" or split Table 3 into calculations specific to baseline *vs*.

treatment scenarios, (2) "linearize" steps in Table 3, as the steps are not consecutively ordered, (3) add equation labels from Appendix B (B.3.2, B.3.3, B.3.4) in the Table 3 steps, and, where possible, map steps in Table 3 to steps in Figure 3.

Additionally, a list of specific areas in the EPA draft CVD document and the EPA appendix that need clarification/revision has been provided in Appendix C.

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).

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 clearlydescribed.

The ASCVD pooled cohort equation (PCE) risk model is a scientifically valid approach to estimating the probability of first CVD events. A limitation is that it is estimated only for non-Hispanic white and Black populations.

Many studies have examined the discrimination and calibration of the PCE risk model, and both under-estimation and over-estimation of risk have been demonstrated depending on the characteristics of the study sub-population, in particular related to age, race and socioeconomic factors. For example, epidemiologic data have shown that ASCVD risk is overestimated among Black and white adults with less social deprivation, defined on the basis of income, education and living alone (Colantonio et al., 2017). Among those with more social deprivation, the PCE has good calibration or underestimates CVD risk. Thus, 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 PCE was derived from cohort data among individuals aged 40-79 years, and the model does not allow for changes in risk associations with increasing age (i.e., it assigns fixed weights to each risk factor regardless of age). A recent study (Dalton et al., 2020) among individuals aged

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

Because the ASCVD model is estimated only for non-Hispanic whites and non-Hispanic Blacks, EPA needs to choose how to estimate risk for other populations. Although Goff et al. (2014) suggested using the coefficients for non-Hispanic whites, EPA found that projections using the coefficients for non-Hispanic Blacks provide a closer match to population prevalence and mortality for Hispanic and non-Hispanic other populations, and appropriately adopted these coefficients in its analysis.

The accuracy of the ASCVD model seems modest, even for the populations for which it is estimated. Table B-12: Summary of ASCVD Model Validation reports the population-weighted average (over sex and race/ethnicity categories) of the absolute value of the proportional deviation of reported incidence from modeled incidence. For females, the value of this statistic is 2.00 for non-Hispanic whites and 1.37 for non-Hispanic Blacks. For subpopulations to which the model was not fitted (Hispanic and non-Hispanic other), the statistic is better, between 0.9 and 1.5. For males, model accuracy is substantially better, with the statistic ranging between about 0.2 and 0.6 for all population subgroups. The Panel concludes that the accuracy of the model predictions deserves more discussion. Moreover, it is important to test and report the average bias between model predictions and population incidence, overall and for population subgroups, in addition to the measure of spread.

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.

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

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.

The ASCVD model is calibrated to data from epidemiological studies that establish a relationship between total cholesterol and CVD risk. Such studies do not by themselves provide evidence that a change in total cholesterol will change CVD risk, nor do they provide information about whether the effect of a change in total cholesterol on CVD risk depends on the

source of the change. Intervention studies provide evidence about the joint effects of an intervention (e.g., statins) on total cholesterol and CVD risk. However, such studies are unlikely to provide evidence about the causal effect on CVD risk of a change in total cholesterol due to reducing exposure to PFOA/PFOS in drinking water. It appears the validity of using the model in this context can be assessed only by scientific judgment about the plausibility that a change in total cholesterol due to a change in drinking water exposure has the same relationship to CVD risk as a change due to sources that have been evaluated by intervention or observational studies. Whether or not all components in the ASVCD risk model reach the threshold for POD derivation (e.g., blood pressure) or statistical significance in a meta-analysis (e.g., HDLC), the decision not to consider PFOA and PFOS effects on other parameters in the ASCVD model when estimating avoided CVD risk as a result of the reduction of PFOA and PFOS in drinking water requires further justification. This is of particular concern for HDL cholesterol, which has been shown to have similar discrimination for ASCVD risk in certain populations when included in the model. The Panel recommends that EPA evaluate whether inclusion of HDLC would influence the results of the modeling.

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.

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?

Appendix E of the draft CVD document describes EPA's planned approach to uncertainty analysis, which focuses on uncertainties in each step of the CVD risk reduction model (PFOA/PFOS serum concentration inputs, PWS population size and demographics, impacts of PFOA/PFOS on TC, risk of CVD event given TC and non-TC predictors, life tables, CVD mortality and non-fatal events), most of which will be modeled directly using Monte Carlo simulation. Section 7 of the EPA draft CVD document describes many sources of uncertainty that will not be modeled directly and suggests the likely direction of the effect for each source on CVD outcome estimates (i.e., underestimate, uncertain, or overestimate).

The Panel agreed that EPA was generally clear in describing the individual contribution of sources of uncertainty in Section 7 and Appendix E of the Analysis of CVD Risk Reduction document, and the approach to characterizing some uncertainties using Monte Carlo analysis in Appendix E of the EPA draft CVD document. Additional specific areas needing further clarification are listed in Appendix A of this report.

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).

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APPENDIX A

The Panel provided comments regarding specific areas requiring more thorough and clearer descriptions and revisions and additions for both the draft EPA PFOA and PFOS MCLG documents.

General comment: In tables that present data from specific publications throughout the document, suggest adding the citation for the publication to the title or as a footnote. It is important for the reader to be able to see which study the data comes from without going back into the text.

p. 4, Step 2. Suggest clarifying that BMD modeling is performed on data for internal doses (serum/plasma levels) rather than administered doses. As written, this is not clear. Additionally, some of the tables of dose-response data in Appendix B do not state that the doses are blood serum/plasma levels, and the text refers to "blood levels" rather than serum or plasma levels. This should be clarified.

p. 5, last paragraph. What is a "screening MCLG"? This is not mentioned elsewhere in the document. Also, the intent of the following sentence is not clear: "In the event that one of the identified toxicological assessments includes an updated RSC based on new literature, the updated RSC will be considered for use in deriving the screening MCLG on a case-by-case basis."

p. 8-9. The units for the drinking water occurrence data (mg/L) are incorrect throughout. The correct units are µg/L.

p. 23, last paragraph. page 24, first partial paragraph. It should be noted that the reports of the NTP (2019) 28 day studies are not currently available online.

p. 25, first paragraph. Much of the information regarding receptor activation included here appears to be relevant to topics other than ADME.

p. 26, first paragraph. The cited study (Ruggiero et al., 2021) discusses uptake into hepatocytes, and it does not appear to be relevant to uptake from the gut, as stated here and in several other places in the document. It is about Na+/taurocholate co-transporting polypeptide (NTCP), a transporter for bile acids into hepatocytes.

p. 27. If possible, suggest expanding the discussion of dermal and inhalation absorption to discuss the magnitude of absorption via these routes as compared to the oral route. When drinking water is contaminated with PFOA and PFOS, one of the most frequent questions from residents is whether there is exposure when showering and bathing.

p. 29, last paragraph. The discussion of effects of PPAR-alpha agonists on lipid metabolism and transport does not appear to be relevant to tissue distribution of PFOA and PFOS.

p. 30, last paragraph. The relevance of exposure sources needs clarification. It is unclear how exposure source can affect the maternal:cord serum ratio, unless this refers to the proportion

exposure to branched vs. linear isomers, which can vary with exposure sources. If that is what is meant, it should be so stated.

p. 33, first full paragraph. It is unclear why the term "C8" (a "nickname" for PFOA) is used.

p. 33, first paragraph of Section 3.2.1.4.4. Two sentences beginning with "Second, ..." and ending with "...epidemiological data." Suggest clarifying that the information presented here supports the idea that menstruation is an important elimination route.

p. 35, last paragraph. It should be clarified that the PFOA half-life value of 0.53 years in young females is for one specific branched isomer, not branched isomers of PFOA in general. The half-lives of other branched isomers were reported as several years, similar to linear PFOA. Additionally, the relative importance of exposure to branched vs. linear isomers of PFOA should be clarified. The most recent NHANES data show that exposure to branched isomers of PFOA is much lower than for linear PFOA in the U.S. general population. See PDF pages 266-271 of https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume2_Mar2021-508.pdf

p. 37. Regarding: "First, sex related differences with males exhibiting somewhat longer half-lives compared to females." "Females" should be revised to "females of childbearing age."

Also, the following sentence is unclear: "This variability in serum and urine concentrations may reflect the role of non-urinary routes of excretion and the difficulty in measuring renal resorption."

p. 38. "One of the largest challenges in the estimation of half-life is the problem of estimating exposure to PFOA. Russell et al. (2015, 2851185) addressed this problem by estimating the amount of bias in elimination half-life that is introduced when the ongoing background exposure is not taken into account, with application to PFOA as an example." It should be noted that Russell found that considering background exposure had little impact on half-life estimates from retired workers or populations with elevated exposures from local sources of environmental contamination. This comment also applies to the sentence citing Russell et al. (2015) in the first paragraph of p. 44.

p. 44, First paragraph. "In studies that calculate the half-life in a population with greatly decreased PFOA levels, …" Suggest revising "greatly decreased PFOA levels" to "greatly decreased PFOA exposures."

"On the other hand, a half-life value determined from a population with very high exposure may not be informative of the half-life in typical exposure, because of non-linearities in PK that may occur due to the saturation of PFAS-protein interactions." Can a citation be provided for this statement?

p. 90, first paragraph. When comparing developmental effects of PFOA in mice versus rats, it is important to note that female rats excrete PFOA very rapidly (half-life of several hours), while it is slowly excreted in female mice. This difference has a large impact on the difference in sensitivity to PFOA's developmental effects in rats vs. mice.

p. 92, Figure 43. This figure is confusing because for some endpoints, an increase is adverse (e.g., offspring mortality) while for other endpoints, a decrease is adverse (e.g., fetal survival). Is it possible to adjust the way the data are displayed to avoid this issue?

p. 95, 3.3.1.2.5. Were any studies that evaluated whether PFOA causes structural malformation identified other than Lau et al. (2006)? If this is the only such study, it appears to be an important data gap. Additionally, suggest distinguishing structural malformations (e.g., limb and tail defects) from developmental delays (e.g., delayed ossification) in this writeup.

p. 96. The potential impact of delayed mammary gland development caused by PFOA on lactational function has only been evaluated in one small study. There is insufficient information to make firm conclusions about this issue.

p. 98, first paragraph. The following sentence needs revision: "At PND22, the mammary glands of all PFOA-exposed P0 dams, including the control dams receiving 5 ppb PFOA in drinking water, resembled glands of mice at or near the peak of lactation (~PND10)." The dams receiving 5 ppb PFOA in drinking water were not "control" dams; they were dosed with PFOA. It appears that the information in the sentence above was based on the following text from White et al. (2011): "As evidenced by significantly elevated histological scores at PND22, normal weaning-induced mammary involution was compromised among **all PFOA-treated P0 dams, including those with only low-dose exposures via drinking water** (Table 1). **In contrast with the extensive gland regression observed in control dams at weaning, glands in PFOA-treated dams at PND22** demonstrated structural similarity to normal dam mammary tissue at or near the peak of lactation at PND10, including the presence of functional lobuloalveolar units…"

p. 97, third paragraph. As discussed in DWQI (2017, https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf), the strain differences regarding doses at which delayed mammary gland development occurred in Tucker et al. (2015) may have been due to toxicokinetic differences (higher PFOA serum levels from the same administered dose in CD-1 mice than C57BL/6 mice) rather than differences in sensitivity to the effects of PFOA.

p. 101, last paragraph above beginning of Section 3.3.2. It is stated that delayed eye opening from Lau et al. (2006) was considered for POD derivation. However, a POD for this effect was developed later in the document for Wolf et al. (2007), not Lau et al. (2006).

p. 101-102. First paragraph of Section 3.3.2.1.1.1. The first part of the paragraph discusses effects on sperm parameters in general population studies. This is followed by a statement that occupational studies observed "minimal effects in male employees." It appears that the occupational studies looked at hormone levels, not sperm parameters discussed for general population above. If this is the case, this should be clarified.

p. 102, second paragraph, first sentence. Did the 21 studies mentioned evaluate endocrine effects related to male reproduction, or endocrine effects in general?

p. 131, 176, 186 and elsewhere. It is not clear why Olsen et al. (2012) and Wang et al. (2012) are included as new human studies since they were published before the 2013 end date of the literature search for the 2016 HESD.

p. 133, last paragraph. It is stated that Jin et al. (2020) (medium confidence study) found increased odds of nonalcoholic steatohepatitis in a medium confidence study. It is unclear why it is then stated that Darrow (2016) is the only medium confidence study of liver disease, since nonalcoholic steatohepatitis, reported by Jin et al. (2020), is a liver disease.

p. 142, Section 3.3.3.2.3. Suggest adding discussion of steatosis caused by PFOA reported by Das et al. (2017, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5994610/). This effect may be relevant to the potential for PFOA to impact fatty liver in humans. It is also suggested that Quist et al. (2015, https://journals.sagepub.com/doi/full/10.1177/0192623314551841), which reported histopathological changes in mouse liver that are observable only with electron microscopy at very PFOA low doses, be discussed.

p. 142, Section 3.3.3.2.3, second sentence. It appears that "hyperplasia" refers to bile duct hyperplasia in the studies discussed below. Suggest clarifying this.

p. 144, second and third paragraphs, and p. 148, last full paragraph. Can the difference between single cell death and single cell necrosis be clarified?

p. 148, last paragraph continuing onto p. 149. The incidence of focal cell and individual cell hepatic necrosis data in male mice from Loveless et al. (2009) was used as the basis for a POD. As the Office of Water is aware, the criteria for evaluation of rodent liver histopathology have changed since Loveless et al. (2008) was published. In the EPA Office of Water toxicity assessment for GenX that was recently finalized (EPA, 2021), histopathological changes in mouse liver was the critical effect, and the liver histopathology from the key studies was reevaluated by the National Toxicology Program (NTP, 2019) using the updated criteria (Elmore et al., 2016, https://journals.sagepub.com/doi/10.1177/0192623315625859). Is the fact that the Loveless et al. (2008) histopathology results are based on the older criteria an issue for using these data for POD development?

p. 146, first paragraph. Should "cystoid" be "cystic"?

p. 151, first full paragraph beginning with "Of the studies, …", and last paragraph, three sentences beginning with "The Faroe Islands studies {Grandjean et al., 2012, 1248827; Grandjean et al., 2017, 3858518; Grandjean et al., 2017; 4239492; Mogensen, 2015, 3981889} observed associations…" and ending with "…at later time periods from children at age 5 years, age 7 years, and age 13 years." In the four studies from the Faroe Islands cited here (Grandjean et al., 2012; 2017a, 2017b; Mogensen et al., 2015), two different cohorts were evaluated, and associations of maternal and/or child (at different age points) serum PFAS levels and antibody response to vaccination at different ages are reported. The text here is difficult to follow, and does not clearly and accurately indicate that, among these studies, maternal serum PFAS were measured late in pregnancy or two weeks after delivery (depending on the cohort), offspring

serum PFAS levels were measured at 18 months, 5 years, 7 years, and/or 13 years (depending on the cohort), and vaccine antibodies were measured at 5 years pre-booster, 5 years post-booster, 7 years, and 13 years (depending on the cohort). Add a table of the cohort(s), age points for serum PFAS, and age points for vaccine response analyzed in each study to organize this information. This information is important because these studies and this endpoint were selected as the critical study and endpoint for RfD.

p. 151, last paragraph continuing on p. 152. Suggest clearly stating that Timmermann et al. (2020) and Abraham et al. (2020) did not study residents of the Faroe Islands and were each conducted in a different location.

p. 153, first paragraph. "This study was rated low confidence." Two studies (Zeng et al., 2019 and 2020) are mentioned above. It should be clarified that both studies were rated low confidence.

p. 162, last paragraph, third sentence. "PFOS" should be replaced with "PFOA".

p. 165, third full paragraph beginning with "Alterations in the serum levels of globulin can be associated with decreases in antibody production {FDA, 2002, 88170}."

Suggest qualifying this statement by adding that globulin consists of several components, most of which are not immunoglobulins. "The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells." See: Busher (1990).[2] Therefore, a decrease in total globulin does not necessarily indicate decreased immunoglobulin. Also relevant to this point, U.S. Food and Drug Administration Guidance for Industry Immunotoxicology Evaluation of Investigational New Drugs (FDA, 2002)[3] states: "Decreases in serum globulin levels (often detected, where seen, as an increase in the serum albumin/globulin ratio) may indicate impairment of immunoglobulin production. However, decreased basal serum globulin level is a relatively insensitive indicator, because under normal circumstances the immune system should be challenged with antigen and a particular antibody response evaluated to detect immunosuppression. When decreased serum globulin level is observed, the protein components affected should be determined using appropriate assays (Duncan et al., 1994; Hall, 2001; Weingand et al., 1996)."

p. 164, last paragraph, and 165, first paragraph (or elsewhere where immune effects reported by Loveless et al., 2008) are discussed. Suggest mentioning that, while Loveless et al. (2008) concluded that immune effects are secondary to increased corticosterone, corticosterone was not increased at the LOAEL for increased relative spleen weight, the study by DeWitt et al. (2009) in sham-operated and adrenalectomized mice demonstrated that immune system of toxicity of PFOA is not secondary to increased corticosterone.

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[2] https://www.ncbi.nlm.nih.gov/books/NBK204/#:~:text=The%20globulin%20fraction%20includes%20hundreds,into%20four%20groups%20by%20electrophoresis.

[3] https://www.fda.gov/media/72228/download

p. 167, Section 3.3.4.4 on evidence integration for immune effects.

“The antibody results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFOA were associated with suppression of at least one measure of the anti-vaccine antibody response to common vaccines in two well-conducted (though overlapping) birth cohorts in the Faroe Islands, supported by a low confidence study in adults. Thus, antibody response to vaccination in children was considered for POD derivation.”

The older and newer studies from other locations should also be mentioned here (e.g., Granum et al., 2013; Timmermann et al., 2020).

Also, why are the two cohorts described as “overlapping”? The subjects in the two cohorts were born during different time periods that do not overlap.

p. 173, first paragraph. It is not clear how "very high PFOA levels" are defined, and why 6.19 ng/ml is described as "very high." Does this mean that this level is very high in the context of other general population studies?

p. 173, third full paragraph. Should it be mentioned that both of the NHANES-based studies (Shankar et al., 2012; Huang et al., 2018) are based on self-reported CVD?

174. “Ten studies examined other CVD-related outcomes including CHD, CVD, stroke, carotid artery atherosclerosis, angina pectoris, C-reactive protein, CHF, peripheral artery disease (PAD),
microvascular disease, CIMT, and mortality.” Does “CVD” in the list of CVD-related outcomes mean combined occurrence of all of the specific endpoints listed here?

p. 176, Section 3.3.5.1.2.1, 2nd paragraph. Suggest stating the number of occupational, high exposure community, and general population studies included in the 2016 HESD.

p. 177, 2nd full paragraph. There is a typographical error in the first sentence.

p. 184, first partial paragraph. In discussion of Convertino et al. (2018), suggest mentioning that serum PFOA concentrations associated with reduced total cholesterol are similar to the serum PFOA concentrations that caused decreased cholesterol in rodents. Different mechanisms may be involved with PFOA’s effect on serum cholesterol at these high exposures compared to the low exposures relevant to environmental contamination including drinking water contamination.

p. 186, second paragraph on 3.3.5.1.2.6 and p. 187, second full paragraph (last paragraph of section). Suggest also mentioning the results of the older occupational studies included in the 2016 HESD. The conclusion about an association between PFOA and TC in workers should be based on all of the available studies.

p. 189, last sentence. It is suggested that the differences in the effect of PFOA on serum lipids in rodents versus humans may arise from the fact that the animals were not fasted in some studies

before serum collection. Other potentially important factors include differences in exposure levels (see comment on Convertino et al., 2018, above) and differences in the fat content of human diets versus rodent lab diet. This is discussed in studies reviewed in DWQI (2017 https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf) including Tan et al. (2012, https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0061409) and Rebholz et al. (2016, https://www.sciencedirect.com/science/article/pii/S2214750015300822?via%3Dihub) as well as in newer studies such as Schlezinger et al. (2020, https://www.sciencedirect.com/science/article/abs/pii/S0041008X20303306?via%3Dihub).

p. 191, first paragraph. Similar to the comment above, it is stated that associations of PFOA and serum lipids (for the three low confidence studies of PFOA and serum lipids in workers that are not included in the 2016 HESD) are "generally low" and that this differs from the conclusion in the 2016 HESD. Earlier in this section, it is stated that there is "relatively consistent and robust associations" of PFOA and serum lipids in worker studies considered in the 2016 HESD, although the number of worker studies is not stated. This a good illustration of why the epidemiology evidence for each effect should be considered as a whole, including studies included in the 2016 HESD and newer studies.

p. 192, first paragraph. Although not mentioned here, Dong et al. (2019) was the human study selected for POD derivation for serum lipid changes. It is not clear how Dong et al. (2019) was selected from the large number of possible studies.

p. 192, Section 3.3.6. Although this section is called "Endocrine," only thyroid effects in humans and animals and adrenal effects in animals are discussed. It should be noted that other endocrine effects are discussed in other sections (e.g., in discussing reproductive and metabolic effects).

Additionally, this section is especially difficult to follow, particularly the discussion of human studies. It is suggested that a summary table be added.

p. 199, third paragraph. It should be noted that Convertino et al. (2018) is not relevant to RfD development because of the high doses used and because it was conducted in advanced cancer patients.

p. 204, Figure 78. Here and throughout, TSH is not a thyroid hormone, but rather a pituitary hormone that affects thyroid hormone levels. Suggest revising to "thyroid-related hormones."

p. 255. It is unclear why the studies cited in this paragraph are not included in the discussion of neurodevelopmental outcomes in the first full paragraph on p. 254 above.

p. 272, 3rd paragraph. It is stated here that kidney lesions were not considered for POD development, and issues with use of increased kidney weight as an endpoint for RfD development are discussed above. However, BMD modeling was performed for increased relative kidney weight in male rats from NTP (2019), as shown in Section B.2.10.3, indicating that kidney lesions were considered for POD development.

p. 273, last paragraph. Again, it should be noted that Convertino et al. (2018) is not relevant to RfD development because of the high doses used and the fact that it was conducted in advanced cancer patients.

p. 289, third paragraph, and p. 290, second to last paragraph. If Onishchenko et al. (2011) is discussed, Koskela et al. (2011) should also be included (Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. https://pubmed.ncbi.nlm.nih.gov/27068293/)/
This study looked at effects on bone in the same group of mice used in Onishchenko et al. (2011). This study is the basis for the ATSDR MRL for PFOA, although it included only one dose level.

Also note here, in last paragraph of p. 156, and in second to last paragraph on p. 290, Onishchenko et al. (2011) is not a "single dose study." It is a repeated dose study that included only one dose level.

p. 308, Figure 123. Steenland et al. (2015) is mentioned as one of 13 recent studies in the paragraph above, but it is not included in the table.

p. 311, first paragraph. When discussing lower responses to PFOA in female rats than male rats, suggest mentioning that internal doses of PFOA are lower in female rats because of their very rapid excretion rates.

p. 314, first paragraph and/or p. 316, second paragraph. Strongly suggest mentioning that malignant tumors as well as benign tumors were increased by PFOS in NTP (2020), in contrast to earlier chronic rat studies in which only benign tumors were increased by PFOA.

p. 318, Table 15. It is not clear why decreased offspring survival is supported by prenatal loss in other studies. These two effects occur at different stages of development and are not necessarily related.

p. 322, Table 15, increased cholesterol. "No information is readily available that allows for determining a minimally biological significant response." Some studies, including some included in 2016 HESD, evaluate increased incidence of clinically defined high cholesterol. Could this clinical endpoint be considered as the basis for POD development?

p. 323, Section 4.1.3.1.1, first sentence. "The model predictions from Wambaugh et al. (2013, 2850932) were evaluated by comparing each predicted final serum concentration to the serum value in the supporting animal studies (Table 17)." However, this does not appear to be shown in Table 17.

p. 330. "The purpose of the animal PBPK model is to make predictions of internal dose in lab animals used in toxicity studies or in humans." This is the model used to predict serum levels from administered dose in lab animal studies. It is unclear what is meant by "and in humans" at the end of the sentence.

p. 331. “For these reasons, EPA selected the model structure published by Verner et al. (2016, 3299692), which is a one compartment developmental models for humans.” As stated in Verner et al. (2016) and charge question 1.B on the human toxicokinetic model, this model has “two compartments: one for the mother and one for the child.”

p. 333, last sentence. "PFOS" should be "PFOA."

p. 334, first paragraph, last sentence. It is stated that additional details are provided in footnotes to Table 21. However, there are no footnotes that provide information about individual PODHEDs, except for one footnote stating that a NOAEL/LOAEL approach was used. A footnote regarding the PODHED for decreased antibody response to vaccines at age 7 and serum levels at age 5 is included in the analogous Table 21 in the PFOS document, but it was omitted in Table 21 of the PFOA document.

p. 336, Table 21. The terminology for the BMDLs (e.g, "5RD", "10RD") is not consistent with Table 15 and is unclear.

p. 336, Table 21, increased placental lesions. It appears that "BMDL – 1 SD" is a typo and that it should say BMDL -10. Table 16 states that the BMR used for this effect is a 10% change, and the BMD modeling results shown in Appendix B are for changes of 5% and 10%, not 1 SD.

p. 344, first partial paragraph. Guyton et al. (2009, https://pubmed.ncbi.nlm.nih.gov/20049115/) is a more relevant citation than Felter et al. (2018) for the point being made here.

p. 345, Table 25. Suggest that the units used for CSFs for human data in this table be the same ($mg/kg/day^{-1}$) as for the CSFs from animal data in Table 24.

Table C.4. Grandjean et al. (2017). Maternal blood was sampled two weeks after term date, not infant blood two weeks after term date as stated here.

p. D-3. Section D.1.4. Dermal Exposure. An additional study relevant to dermal absorption of PFOA is: Fairley, K.J., Purdy, R., Kearns, S., Anderson, S.E., Meade, B.J. (2007). Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. Toxicol. Sci. 97: 375-383.

p. D-7, second full paragraph. "an assortment of peroxisome proliferators." Does this mean xenobiotics that activate PPAR?

p. D-8, first sentence of Section 2.3.1.1. While it is true that human blood is a major site of PFOA accumulation, it is not at all clear how the example provided demonstrates this.

p. D-9, first full paragraph, third line. Should "blood fractions" be "blood serum/plasma fractions since blood cells are not mentioned?

p. D-10, last paragraph, first sentence. The intended meaning of "as well as toxicokinetics" here is unclear.

p. D-10, last paragraph, second sentence. "regiments" should be "regimens."

p. D-10, last paragraph, third sentence. "male animals accumulate more PFOA…" The species to which this statement applies should be stated.

p. D-11, last paragraph. For "presumably due to increased urinary elimination in the 30 mg/kg/day group." Suggest clarifying that saturation of reabsorption is likely occurring at the higher dose, if this is what is meant.

p. D-11, last paragraph. "Interestingly, female rats exhibited only 5, 14, and 27% of PFOA in serum when compared to male concentrations at 3, 10, and 30 mg/kg/day doses, respectively. These low levels of absorption were also seen in solid tissue as liver and kidney measurements were ~10 and 30% of levels detected in males, respectively. In females, there was a dose-related increase in tissue levels and serum." This discussion needs to be clarified. It is unclear whether percentages of total administered PFOA or absolute concentrations of PFOA in blood serum and tissues are being compared in males and females. It is well established that female rats excrete PFOA much more rapidly than male rats, so it is expected that concentrations in blood and tissues would be much lower in females.

p. D-12, first full paragraph. It should be stated that Kawabata et al. (2017) detected PFOA in brains, not that they measured it. The LOQ is the level below which quantitation (measurement) is not possible.

p. D-15, second paragraph, first sentence. "indicating that excretion may play a role" is unclear. What does excretion play a role in?

p. D-15, second paragraph. "Based on the timing of the measurements and the results, females appear to absorb and excrete PFOA more rapidly than males, however, the samples were collected at 1.25 and 4 hours in females and 10.5 and 171 hours in males, providing more time for absorption in the males." The rationale for this statement is unclear and appears to be incorrect. The sampling timepoints in females and males were based on previous toxicokinetic experiments that were designed to determine the Tmax and Tmax/2 in males and females.

p. D-18, second sentence. It should be stated that this study was conducted in male mice.

p. D-20, Section D.2.4, Distribution during Reproduction and Development. As a general comment, many of the parameters discussed in this section (e.g. maternal:cordblood ratio; maternal blood:breastmilk ratio) were used in the PBPK model. How does the discussion here tie in with the choices made in the model that was used?

p. D-21, third paragraph. The statement “however, all were below the level of detection in maternal blood and placentas except linear PFOA and 3m-PFOA” does not appear to be correct, because Table S5 of Chen et al. (2017) shows that iso-PFOA was detected in all maternal blood serum samples.

p. D-21, last paragraph. "Despite the reduced proportion of branched PFOA within each biological compartment, the proportion of maternal branched PFOA that accumulated in the placenta was significantly higher than the proportion of linear PFOA." This is not true for iso-PFOA, since it was found in maternal blood but not in the placenta. Also, the authors state: No obvious structure−activity relationship of R_{CM} and R_{PM} (plasma:maternal serum ratio) was observed for PFOA isomers (Figure 4), consistent with results of Beesoon et al. (2011, https://ehp.niehs.nih.gov/doi/10.1289/ehp.1003265).

p. D-22, third paragraph. "Linear PFOA is more is detected at higher frequency and at higher levels in maternal serum than branched isomers likely due to different binding affinities in plasma." This statement does not appear to be supportable. A primary factor that impacts the proportion of linear versus branched PFOA in maternal serum is the relative exposure to linear versus branched isomers. Differences in toxicokinetics (e.g., binding affinities that impact excretion rates) is a secondary factor.

p. D-23, third paragraph. The following discussion does not appear to be correct: "…show consistently higher levels of PFOA in maternal serum versus cord serum regardless of the gestational age. Moreover, for studies with participants of similar gestational ages, the PFOA concentrations in both maternal and cord serum varied substantially across studies that were reflected in RCM ratios from 0.57 to 1.33." If PFOA levels are consistently higher in maternal serum than cord serum, all cord:serum ratios (RCM) should be <1.

D-23, third paragraph. "Factors such as exposure sources, parity, and other maternal demographics can potentially account for these variations [in cord:serum ratio]. For example, nulliparous mothers generally have significantly higher serum PFOA than parous women (Kato et al., 2014, 2851230). Conversely, younger women tend to have lower serum PFOA than older women (Kato et al., 2014, 2851230). Therefore, studies with high percentages of young, multiparous women may report lower levels of PFOA in maternal and cord blood." The rationale for this statement is not clear. While young, nulliparous women may have lower levels of PFOA in maternal and cord blood, the topic here is the cord: maternal blood ratio, and it is unclear why it would differ in women with lower maternal and cord blood levels than in women with higher levels. "A summary of recent studies examining RCM is presented in Table D-10. The percentages of maternal PFOA that accumulate in cord blood ranged from 57 to 133% and did not strictly correlate to maternal serum values. This variability suggests that TTE may differ across populations likely due to maternal characteristics or differing levels of exposure." Again, it is not clear why the ratio between maternal and cord blood would be expected to correlate with maternal serum levels and why differing exposure levels would impact the TTE.

p. D-24, second paragraph. Another relevant publication is: Beesoon, S., Webster, G.M., Shoeib, M., Harner, T., Benskin, J.P., Martin, J.W. (2011). Isomer profiles of perfluorochemicals in matched maternal, cord and house dust samples: manufacturing sources and transplacental transfer. *Environ. Health Perspect*. 119, 1659–1664.

p. D-28, first paragraph. "PFOA is highly soluble in water relative to PFOS (solubilities of 3.4 g/L and 0.68 g/L, respectively). Since amniotic fluid is 94% water, the solubility properties may account for the observation that the PFOA concentration (0.044 ng/mL) was twice as much

as PFOS (0.02 ng/mL) in this matrix." This explanation does not appear to be plausible, since the concentrations in amniotic fluid mentioned here are many orders of magnitude below the water solubilities of PFOA and PFOS. Therefore, it is highly unlikely that solubility is the limiting factor here.

p. D-28, second paragraph. In the phrase "…of comparing the portioning of PFOA from mother to fetus across studies," replace portioning with partitioning.

p. D-28, third paragraph, first sentence. With respect to "range of concentrations," it appears that "concentrations" is being referred to when ratios are what is meant in the discussion in this paragraph.

p. D-28, last paragraph, last sentence, continuing on p. D-29. It is not clear why PFOA and PFOS are being compared here, but they are not compared elsewhere in this document.

p. D-33, D.2.4.1.4. The maternal serum:infant serum data from Fromme et al. (2010) should be discussed in this section and included in Table D-12.

p. D-33, second paragraph. Is the small difference in GM maternal serum levels between breastfeeding and non-breastfeeding mothers in Mondal et al. biologically or statistically meaningful?

p. D-36, last sentence. Suggest that discussion of relationship between drinking water exposure and serum PFOA levels in children be expanded.

Section 2.4.2.1, pp. D-37 and D-38. When discussing toxicokinetics of PFOA in female rats during gestation and lactation (and in general), it is important to mention that PFOA does not bioaccumulate in the serum of female rats or in the rat placenta, amniotic fluid, and embryo/fetus in the same way as in mice because PFOA is very rapidly excreted in female rats, with a half-life of only 2-4 hours. In the cited studies (e.g., Hinderliter et al., 2005), the pregnant rats were dosed once daily, and each daily dose is therefore virtually completely excreted before the next dose is administered. As such, studies in which the female rat is dosed once per day are not ideal for evaluation of potential developmental effects of PFOA in humans. In contrast, the half-life of PFOA is much longer (17 days) in female mice and makes mice a more suitable species for evaluation of PFOA's developmental effects from steady state exposure.

p. D-41, last full sentence ending with "…at the time of peak lactation." The explanation provided by the authors of Fenton et al. (2009) about the reason that dam serum levels increased after peak lactation, although there was only one dose several weeks earlier, should be included here.

p. D-42, Table D-22. For these data to be meaningful, it should be stated that there was a single dose of PFOA on GD17.

p. D-46, first full paragraph. The Vd used by Thompson et al. (2010) and Lorber and Egeghy (2011) does not come from NHANES data, as stated here. It comes from data from the C8 study

population in West Virginia and Ohio, a population with exposure to drinking water contaminated with PFOA by releases from an industrial facility.

p. D-46, last paragraph. Suggest mentioning that the highest value mentioned (200 ml/kg) is only 18% higher than the lowest value mentioned (170 ml/kg), and that the choice of any of these values will not have a substantial impact on the resulting Reference Dose.

p. D-61, last paragraph continuing onto p. D-62. The effect of probenecid on PFOA excretion in the cited study should be discussed.

p. D-64, first full paragraph. This paragraph is unclear, in that it states that "similarities" between the sex differences in the excretion of PFOA in rats and the excretion rate of PFOA in humans. However, it is well established that the half-life of PFOA is long in humans in both males and females. The intent of the comparison of rats and humans here is not clear, and this should be clarified.

p. D-65, fourth full paragraph. For "…conjugated metabolites of toxic chemicals, including PFOA," this information appears to be incorrect. PFOA is not metabolized to conjugated metabolites (or other metabolites).

p. D-66, first full paragraph, third sentence citing Ruggiero et al. (2021). The NTCP transporter is relevant to uptake into hepatocytes, not the gut, and Ruggiero et al. (2021) discusses uptake into hepatocytes, not the gut.

p. D-68, first paragraph of Section D.4.4. The sentence stating that Zhang et al. (2013) reported longer PFOA half-lives in older females than young females and males should be corrected to state that PFOA half-lives were longer in males and older females than in young females (i.e., females of childbearing age).

p. D-68, last paragraph. Regarding discussion of Lorber et al. (2015), the following sentence appears to be out of context: "These authors suggested that factors other than blood loss, such as exposure to or disposition of PFOA/PFOS, may explain the differences in elimination rates between males and females." If this sentence is included, it should be mentioned that the authors also concluded that their data and modeling support blood loss as an explanation for the sex-specific differences in human PFOA elimination as follows: "Overall, this study provides data and modeling that supports the initial hypothesis that ongoing blood loss explains lower PFAA concentrations in humans."

p. D-69, second full paragraph, first sentence and Table D-35 footnote. Units should be mg/L, not mg/mL.

p. D-70, Section D. 4.5.1, first paragraph. The intended meaning of the following sentence is not clear, and it is suggested that it be clarified: "The calculation of PFOA half-lives reported in the literature vary considerably posing challenges in predicting both the routes and rates of excretion."

p. D-70, Section D.4.5.1, paragraph 1. Is there evidence that the half-life of PFOA is shorter in populations exposed to contaminated drinking water or occupationally? Any studies relevant to this issue should be evaluated and discussed here. Also, it is unclear why exposure to contaminated drinking water is referred to as occurring "under acute conditions," since exposure to contaminated drinking water generally has occurred for a considerable period of time (years) before it is discovered.

p. D-75, Table D-36, first row. The units for drinking water concentrations under “Exposure” are ng/L, not ng/µL.

p. D-77, first paragraph, second sentence. The range of 0.53 - 22 years does not appear to be appreciably "more defined" than the range of 0.61 - 60.9 years. More importantly, the value of 22 years from Glynn et al. is not an excretion half-life and does not appear to be appropriate for inclusion in this discussion. This value is based on biomonitoring data from the general population over a period of 14 years, not declines in levels in individuals over time. The value of 22 years represents changing exposures over time, and it is not an accurate measure of biological half-life. For some other PFAS evaluated in Glynn et al., serum levels increased over the 14-year period, indicating increased exposure over time. Finally, it should be clarified that the PFOA half-life value of 0.53 years in young females is for one specific branched isomer. The half-lives of other branched isomers and linear PFOA were reported as several years. The most recent NHANES data shows that exposure to branched isomers of PFOA is much lower than for linear PFOA in the U.S. general population. See PDF pages 266-271 of https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume2_Mar2021-508.pdf

p. D-82. Table D-40. The title of the table should be revised to indicate that data from males, as well as females, are shown.

p. D-83, Table D-41. Additional citations for PFOA half-life in the mouse and the rat are provided in Section 17.2 of the ITRC Technical & Regulatory document for PFAS at https://pfas-1.itrcweb.org/17-additional-information/#17_2

APPENDIX B

The Panel provided comments regarding specific areas requiring more thorough and clearer descriptions and revisions and additions for the draft EPA Mixtures document.

p. 3, last paragraph and Table 1-3. Although the TSCA (2020) Federal Register notice cited here defines only long-chain PFCAs and does not define long-chain PFSAs, EPA (2009) has previously defined long-chain perfluorinated carboxylate and sulfonates in the same way as mentioned here for the OECD definition. See p. 2 to 4 of EPA (2009) Long-Chain Perfluorinated Chemicals (PFCs) Action Plan posted at https://www.epa.gov/sites/default/files/2016-01/documents/pfcs_action_plan1230_09.pdf.

p. 4, last paragraph. Suggest adding that PFAS salts that 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. 7, last paragraph. Suggest citing a more general source of information on elevated serum PFAS levels in locations with PFAS contamination. For example, ITRC Table 17-6 at https://pfas-1.itrcweb.org/17-additional-information/#17_2 and ATSDR PFAS exposure assessment results at https://www.atsdr.cdc.gov/pfas/communities/factsheet/Community-Level-Results-Factsheet.html.

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?

APPENDIX C

The Panel provided comments regarding specific areas requiring more thorough and clearer descriptions and revisions and additions for both the draft EPA main CVD document and CVD Appendix.

CVD risk reduction main draft document

- P. 11. EPA assumed drinking water accounts for 20% of total daily PFOA/PFOS dose under the baseline scenario, and that the other 80% is independent of drinking water PFOA/PFOS concentration. It tested the effect of alternatively assuming that drinking water accounts for 80% of the total and found "the assumption about drinking water source contribution does not affect the estimated changes in serum PFOA/PFOS." The Panel suggests that EPA explain why the effect of a change in drinking-water PFOA/PFOS concentration on serum PFOA/PFOS should not be four times as large in the alternative case.
- P. 14. "EPA adjusts the modeled population cohort to exclude individuals with pre-existing conditions." Row 1 in Table 3 cites national level CVD prevalence statistics, but the Panel finds that more detail is needed, as well as a reference to the Appendix (if applicable) for how the adjustment is done. Specifically, by how much does this adjustment reduce overall population numbers and which pre-existing conditions are excluded?
- P. 16. the Panel concludes that the EPA should report the calibration factors used in Table 3 and discuss how reasonable these magnitudes appear. Do these calibration factors inform how reasonable the projections from the ASCVD model are?
- P. 21. "ASCVD-based estimates may be inconsistent with the recent CVD prevalence statistics." The Panel seeks clarification as to the level of inconsistency.

EPA Appendix B

- P. 28. How is integer age (a) defined? There are multiple statements like "at the beginning of integer age a and calendar year t." Is a the age an individual reaches in the calendar year? Obviously, age and year do not change on the same date, unless everyone is born on New Year's Day.
- P. 28. The definition of $l_{b,a,s,r,t,p}$ should presumably say "we assume that people who have just been born do NOT have CVD history by definition" (the word "not" is missing).
- P. 41. Table B-8 Post-Acute All-Cause Mortality After the First Myocardial Infarction shows all-cause death risk is lower for men than women who are non-Hispanic Blacks aged 45-64, but higher for men than women in all other age/race groups. Is this correct?
- P. 46. In "The uncalibrated number of persons experiencing their first hard CVD event in year t, $i_{b,a,s,r,t}$" should the $i_{b,a,s,r,t}$ be $\tilde{n}_{b,a,s,r,t}$?
- P. 46. "EPA applies a constant baseline annual probability of first hard CVD event estimated at age 80 to those currently aged 81–89 years." What is known about baseline probability for ages 81-89? If the probability rises with age, this assumption will lead to an underestimate.
- P. 47. Should the first term in brackets in eqn. (B-12), $\left[1 + \sum_{f \in F}\left(\mu_{a,s,r,f,0} - 1\right) \cdot \gamma_{a,s,r,f}\right]$ be $\sum_{f \in F}\left(\mu_{a,s,r,f,0} \cdot \gamma_{a,s,r,f}\right)$, i.e., the sum over types of nonfatal CVD events of the

product of the mortality rate and share by type? It would be useful to explain the logic of the term as written. The same question applies to eqn. (B-23) on p. 48.

- P. 48. How is the term $\rho_{b,a,s,r}$ calculated? Is it a rate of post-acute CVD mortality?
- P. 49. Equations B-34 and B-36 may contain a sign error. As written, avoided deaths $\Delta m_{b,a,s,r,t}$ and ΔM_t are both less than zero (when treatment is associated with lower incidence of first CVD events, as stated).

Suggestions for revision

1. 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. It is not clear whether the confidence intervals reported in Table E-1 are based on the variance of the error term for individual observations or include some contribution for between-model variation (e.g., model-specific random error τ^2). Moreover, uncertainty about the functional form of the relationship seems likely to be important. The meta-regressions use only linear models, but the central estimates using all studies or using only linear-log models for TC both fall below the lower end of the confidence interval for the linear models for PFOA (Table A-2), though not for PFOS (Table A-3).

2. Organization of Table 7 – In the presentation of Table 7 and the discussion in Section 7 of the report, clarify which sources of uncharacterized uncertainty are likely to be most significant in terms of the likely magnitude of their impact on the estimates. Some row entries include a fairly detailed discussion (with sources cited), and others are much sparser. Ideally, Table 7 would list the biggest concerns with respect to uncharacterized uncertainty first and offer the most detail for those, with smaller concerns listed later.

3. Requested specific clarifications

a. Why are systolic blood pressure and other CVD-related outcomes excluded? Row 7 in Table 7 notes their exclusion but does not explain it.
b. In row 10 of Table 7, why does non-linearity in the ASCVD model lead to underestimation of CVD impacts when EPA uses the fraction of population that smokes and has diabetes (compared to when the model uses binary values for an individual patient)? In addition, a paper has recently been published in *JAMA Cardiology*, using the Framingham cohort study data, showing that inclusion of former smoking status, pack-years and years since quitting smoking improves ASCVD risk prediction among White individuals over the reference model with 2013 PCE variables (Duncan et al, doi:10.1001/jamacardio.2021.4990). The results require replication in other racial and ethnic groups, but EPA may want to consider this approach to inclusion of detailed smoking variables in their implementation of the ASCVD model.
c. We suggest adding some detail to row 4 of Table 7 to explain what is meant by "high-quality data." (We realize that the study selection for the meta-analysis is described in detail in Appendix A, but a very brief list of selection criteria for "high-quality" studies would be useful to include in Table 7.)
d. Is there empirical evidence supporting the assumption of independent effects of PFOA/PFOS on TC, and if so, can sources be cited in row 6 of Table 7?

4. Requested specific additions

a. Row 7 of Table 7 notes that the exclusion of non-TC CVD-related outcomes results in an "underestimate" of the CVD impacts of reducing PFOA/PFOS exposure. However, this is not correct with respect to any impacts of PFOA/PFOS on HDLC which, as noted in the discussion on pp. 11-13, is negatively associated with CVD events. Because EPA included HDLC as one of only two predictors of CVD events in its meta-analyses, this is an important omission. The exclusion of HDLC effects should be added as a separate row in Table 7 (with "overestimate" entered in the "effect on estimate" column), or should be listed in the existing row 7, changing "effect on estimate" to "uncertain," and explaining this in the "details" column.
b. Table E-1 in Appendix E notes that EPA is "currently evaluating including the PK model in the uncertainty analysis." Panel members encourage this evaluation, because uncertainty around baseline (pre-policy) PFOA/PFOS concentrations seems important to model. However, column 3 of this row entry in Table E-1 ("will the source be analyzed") says "No." By performing the uncertainty analysis, EPA can change the column 3 entry to "Yes."
c. Add a row to Table 7 describing the likely impact of excluding the population of individuals with pre-existing conditions.
d. Add a row to Table 7 describing the likely impact of having assumed that no PWS households will engage in averting behavior (e.g., bottled water purchases) to reduce PFOA/PFOS exposure.
e. Add a row to Table 7 describing the uncertainty introduced by linking county FIPS codes with PWS locations (given the lack of a spatial match between county borders and PWS service territories). This is discussed in Appendix E (Table E-1), but it would be helpful to mention it in Table 7 as well.
f. Some reviewers felt that EPA should describe cardiovascular impacts that are monetized in other EPA analyses but not included here. For instance, EPA (2011) cited on page 15 monetizes cardiovascular hospitalizations. Excluding these impacts would be a limitation of the current study.