



## American Water Works Association

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December 30, 2021

Dr. Weihsueh A. Chiu, Ph.D.  
Chair  
Science Advisory Board PFAS Review Panel  
Environmental Protection Agency  
1300 Pennsylvania Ave NW  
Washington, DC 20460

### **SUBMITTED ELECTRONICALLY**

RE: AWWA Comments for Science Advisory Board PFAS Review Panel Consideration

Dear Dr. Chiu,

The American Water Works Association (AWWA) appreciates the public service provided by the Science Advisory Board (SAB) PFAS Review Panel members. The documents the Panel is reviewing will lay the foundation for the national primary drinking water regulation (NPDWR) for at least two per- and polyfluoroalkyl substances (PFAS) in drinking water and perhaps other PFAS through EPA's current effort and future rulemakings. AWWA looks forward to the SAB Panel's feedback. As the Panel is aware, the Safe Drinking Water Act (SDWA) and Executive Orders set a clear bar for transparent analysis and use of sound science in setting NPDWRs. AWWA appreciates the Panel's assistance to EPA in this process. AWWA offers the following comments for the Panel's consideration in its deliberation and for the Agency as it addresses the Panel's review.

### **Sufficient Resources are Needed to Ensure Scientific Integrity**

Under the SDWA, the EPA has a responsibility to use the best-available science in accordance with sound and objective scientific practices. Doing so is imperative in ensuring that drinking water contaminants are addressed in a meaningful way that protects the public and can be implemented feasibly. As EPA has already presented and discussed during the Panel's Dec. 16 meeting, there are five significant draft documents for your consideration relating to the draft approaches for developing a perfluorooctanoic acid (PFOA) MCLG and a perfluorooctanesulfonic acid (PFOS) MCLG, evaluating cardiovascular health effects of PFAS, and evaluating non-cancer health risks associated with PFAS mixtures. This represents a substantial body of work for the Panel to review and on which to provide feedback.

The SAB PFAS Review Panel's report of recommendations is anticipated to be completed in May 2022. If EPA adheres to the schedule set in its PFAS Strategic Roadmap, this leaves less than six months for the Agency to review these recommendations, implement appropriate changes, incorporate resulting differences into its cost analysis, craft a regulatory proposal, and complete the associated procedural

requirements for proposing rulemakings. Normally one-third of that six-month period would be taken up by inter-Agency review. This schedule is well within EPA's statutory deadline for proposal of the rule. AWWA urges the EPA Administrator to afford the SDWA program staff sufficient resources to act on your review so that the proposal is based on the best available science and that the analyses are sound. This is a critical rulemaking for the program, and the Administrator must be sure that EPA employs a defensible premise for benefits anticipated to be accrued through this rulemaking.

### **Charge Questions and Ensuring Recommendations Reflect Purpose of Documents**

On Dec. 16, EPA presented the Panel with its intended use of the documents being reviewed. The Agency made clear why it included five economists in a Panel charged with reviewing scientific assessments of health data. Unfortunately, the final charge questions distributed to the Panel do not address the key questions EPA must answer or make best use of the expertise of the economists on the Panel.

To inform its deliberations, AWWA recommends that the Panel request a briefing from the National Center for Economic Analysis on the construction of benefits analyses to support SDWA regulatory standard setting. Such a briefing would provide the Panel members a common understanding of the task before EPA using the materials the Panel is reviewing. With that basis, the panelists with expertise in national benefit analysis would be able to speak to the strengths and weaknesses of the Agency approach for purposes of the benefits analysis. For example, the Agency is positing in one of its analyses that a fraction of cardiovascular disease in the United States is attributable to PFOA and PFOS. The U.S. Centers for Disease Prevention and Control currently estimates that the mortality rate for diseases of the heart in the United States is 200.8 per 100,000. There are recognized risk factors, some of which have marked socio-economic correlations. It is important that EPA (1) neither grossly over- or under-estimate benefits from risk reduction in its rulemakings and (2) understand and communicate how assumptions and uncertainties in its analysis impact use of the risk reduction model. The plausibility of the Agency's analytical approach being adequate to underpin an economic analysis for an SDWA primary standard warrants discussion.

### **In-Depth Review of the Documents Provided for Panel Review**

AWWA contracted with Ramboll U.S. Consulting, Inc. (Ramboll) to prepare a review of the documents before the Panel and compile comments relative to the charge questions posed to the Panel. The scientists at Ramboll included experts in both cancer and non-cancer health risk assessments, physiologically based pharmacokinetic modeling, and epidemiological research. A summary of the review by Ramboll scientists is attached and is organized to align with the Agency's charge to the Panel. Some key points are:

1. EPA did not apply the Agency's current systematic review process to all the studies it utilized; studies central to its quantitative analysis were likely not held to the expectations applied in the current systematic review process.
2. The evidence for a causal relationship between PFOA or PFOS exposure and cardiovascular disease is weak. It is plausible that PFOA and PFOS exposure is associated with higher cholesterol levels but without an increased risk of cardiovascular disease. Because epidemiological evidence of increased risks of cardiovascular disease in relation

to PFOA and PFOS exposure is weak, it is currently speculative to assume that the small increases in total cholesterol or low-density lipoprotein cholesterol (LDL-C, the “bad” cholesterol) are causally related to increased incidence of cardiovascular disease.

- EPA utilizes studies based on an apparent association between PFOA and PFOS exposure and total cholesterol/ LDL-C when the weight-of-evidence is limited and there are scientific reasons to suspect the association is not meaningful for EPA’s analysis.
  - Furthermore, if PFOA and PFOS are associated with small increases in high density lipoprotein cholesterol (HDL-C), it is biologically plausible that the risks of cardiovascular disease remain unchanged. HDL-C is considered to be the “good” cholesterol and higher levels of HDL-C are associated with a decreased risk of cardiovascular disease. The EPA should consider this as part of the analysis.
  - The analysis presented in the cardiovascular disease risk reduction document concludes that PFOA and PFOS levels lead to an increase in cholesterol. This is not a certainty, and it is possible that the correlation between PFOA and PFOS levels and cholesterol levels is more likely related to the transport mechanisms of cholesterol and PFOA and PFOS within the body.
3. Shearer et al. (2021), one of the key studies in EPA’s analyses, should not be used as a basis for either cancer characterization or dose-response assessment. Despite analyses that adjusted for estimated glomerular filtration, there is the possibility of additional confounding by effects of the underlying cancer induction processes on other aspects of kidney function, such as the renal transporters that are required for control of PFOA excretion, that could also lead to higher PFOA blood concentrations. In addition, the maximum latency in the study was 18 years since the blood collection, which is generally inadequate for kidney cancer, which has a long disease latency. Separately, there is an apparent anomaly between the number of cases and controls in the referent category of exposure (<4 ng/mL) which may lead to under- or over-estimated odds ratios in the higher quartiles of exposure.
  4. EPA’s analyses for PFOA and PFOS rely upon a half-life from a study of retired workers exposed occupationally (Olsen et al., 2007) and thought to have been exposed intermittently since retirement. Recent work by the Alliance for Risk Assessment concluded that the most appropriate studies support a much lower half-life. EPA acknowledges the study’s shortcomings but does not provide reasoning for nonetheless using the higher half-lives.
  5. EPA’s analyses rely on human epidemiological studies with published findings of reduced vaccination efficacy based on cohorts in the Faroe Islands. There are several concerns associated with these studies relating to the level of clinical protection, inconsistencies regarding study subjects included in various studies, and confounding resulting from contaminants (e.g., polychlorinated biphenyl, methyl mercury) anticipated to be high in

the Faroese diet. Should the EPA decide to use these studies, the data should be obtained from the study investigators and independently evaluated prior to finalizing the Agency's analysis.

6. Several key aspects of these analyses are not presented for the SAB PFAS Review Panel and the public to review and verify, including:
  - Neither EPA's animal nor human pharmacokinetic model files were made available for public verification. Without these model files, the model cannot be verified. Lack of access to files also limits the ability to evaluate the pregnancy and lactation model. All model files (including R scripts) should be made available for review by the SAB PFAS Review Panel, as well as the public, to provide scientific transparency.
  - The source for the milk ingestion for animal pups was not fully documented, nor was it peer reviewed. EPA should fully describe the basis for milk ingestion in the PFOA and PFOS documents to adequately support the Panel's review.
7. The draft framework for assessing non-cancer health effects of PFAS mixtures is a significant improvement upon previous approaches applied by other regulatory agencies. However, the document relies on the conclusion that dose additivity occurs for chemicals with a similar toxic endpoint. This begs the question of "How similar?". While chemicals may share a common toxicity endpoint, if the health effects vary, then it becomes necessary to define sub-classes of chemicals for which dose-additivity is appropriate.

If you have any questions regarding this correspondence or if we can be of assistance in some other way, please contact Chris Moody (202.326.6127, [cmoody@awwa.org](mailto:cmoody@awwa.org)).

Best regards,

FOR THE AMERICAN WATER WORKS ASSOCIATION

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Attachment (1)

***Who is AWWA***

*The American Water Works Association (AWWA) is an international, nonprofit, scientific and educational society dedicated to providing total water solutions assuring the effective management of water. Founded in 1881, the Association is the largest organization of water supply professionals in the world. Our membership includes more than 4,500 utilities that supply roughly 80 percent of the nation's drinking water and treat almost half of the nation's wastewater. Our 50,000-plus total membership represents the full spectrum of the water community: public water and wastewater systems, environmental advocates, scientists, academicians, and others who hold a genuine interest in water, our most important resource. AWWA unites the diverse water community to advance public health, safety, the economy, and the environment.*

ATTACHMENT 1  
Technical Expert Review of  
Agency Review Documents Relating to PFAS Health Risks in Drinking Water

**Technical Expert Review of  
Agency Review Documents Relating to PFAS Health Risks in Drinking Water**

*Prepared by*  
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*December 23, 2021*

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## PROPOSED APPROACHES TO THE DERIVATION OF A DRAFT MAXIMUM CONTAMINANT LEVEL GOALS FOR PFOA AND PFOS IN DRINKING WATER

**General Comment:** *As noted in the background sections for both documents, because PFOA and PFOS are listed on the Fourth Drinking Water Contaminant Candidate List (USEPA 2021), EPA made a determination to regulate PFOA and PFOS with a National Primary Drinking Water Regulation. While there are separate documents to discuss the approach for the development of the Maximum Contaminant Level Goal (MCLG) for each compound, there is a lot of information focused on the combination of these compounds or additional PFAS as a class of compounds. For example, in the Occurrence Summary (Section 1.4), examination of the occurrence relies upon the data from the third Unregulated Contaminant Monitoring Rule (from 2013-2015; Section 1.4) and is focused on the occurrence of PFOS and PFOA in aggregate, summing concentrations. This concept, as well as others throughout the documents, should be focused on each compound separately when considering data in the estimation of the individual MCLGs for each compound.*

### Study Identification and Inclusion

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

**Comment:** *As noted in both the PFOA and PFOS documents, EPA has built upon the data included and analyses conducted as part of the 2016 Health Effects Support Documents (HESD) for the Health Advisories for each compound. In the identification of relevant studies, EPA conducted broad literature searches focused on the chemical name/synonyms with no limitations on lines of evidence (Appendix A of the documents). Therefore, any relevant study published since the 2016 HESDs should have been identified.*

*EPA has also noted in both the PFOA and PFOS documents that all studies relied upon for quantitative analysis were not put through the same systematic review process. Many of the epidemiological and animal studies are qualitatively incorporated into this assessment based on the HESD. Specifically, EPA notes that only the animal studies supporting the candidate Reference Doses (RfDs) derived in the 2016 HESDs were incorporated into the systematic review methods outlined in the current SAB External Peer Review Draft MCLG documents. EPA notes that all other studies referenced from the 2016 HESD adhered to the specific criteria for inclusion in the 2016 HESDs, but study confidence between the studies included in the 2016 HESD and this assessment cannot be compared. Therefore, only the animal studies supporting the candidate RfDs derived in the 2016 HESD were considered quantitatively in this assessment.*

*It is important that all of the studies relied upon quantitatively be put through the same evaluation process to determine study confidence and quality. Because processes have changed since the 2016 HESDs, the requirements for a high-quality study may have changed. EPA (2021a, b) indicates that the current systematic review processes have been applied to the animal studies being used quantitatively and this would also be important for the epidemiological studies. The epidemiological studies were the focus and played an integral role in the assessment, with the animal studies*

*providing support. In reviewing all of the studies considering quantitatively, it is also important for EPA to confirm that the majority of the epidemiological studies relied upon for quantitative analysis in the current SAB External Peer Review Draft documents are more recent than the 2016 HESDs and therefore, should have been put through the same systematic review processes. Because there are a limited number of epidemiological studies that are pre-2016, it would not be a large effort to put these studies through the same critical review to ensure that all confidence ratings are comparable.*

## Noncancer Hazard Identification

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

**Comment:** *The most striking aspect of the EPA review of the health effect/outcome categories identified from the PFOA and PFOS literature is that, while the evidence is characterized as suggestive for many endpoints, there is only one health outcome where the EPA characterizes the evidence as strong: an apparent association of PFOS exposure and Total Cholesterol/LDL-C (but not directly with CVD). Even in this case, the PFOA document does not characterize the association as strong. These equivocal characterizations reflect the fact that, despite the large number of studies that have been carried out on PFOA, the evidence from animal studies is almost exclusively from studies conducted with dosing in the range where effects may be associated with activation of PPAR-mediated disruption of lipid metabolism, which is not relevant to the much lower exposures experienced by human populations. On the other hand, the evidence from epidemiological studies is highly susceptible to confounding by pharmacokinetic interactions between the health outcome being studied and the transport/excretion of PFAS (Andersen et al. 2021a,b, open access).*

*Reduced birthweight provides an example of the potential for unrecognized confounding in epidemiological studies with PFAS. As cited by EPA, numerous studies of human populations have reported small decreases in birth weight in relation to increasing PFOA and PFOS blood concentrations. However, the C8 Science Panel evaluated the epidemiological evidence in 2011 and concluded that there was not a probable link between exposure to PFOA and low birth weight (C8 Science Panel 2011; Stein et al. 2009; Savitz et al. 2012; 2011b). Since then, additional epidemiological studies have reported small reductions in infant birth weight (less than 20 grams per ng/ml increase of PFOA or PFOS) (ATSDR 2021). Steenland et al. (2020) recently reviewed literature published since 2011. They attributed the association between PFOA or PFOS and decreases in birth weight as possibly due to reverse causality or confounding. Studies with insufficient exposure contrast (i.e., low exposures with little variability), such as those in the general population (in the absence of drinking water contamination) are particularly prone to distorted effects due to reverse causality and confounding. Stronger associations with birth weight are seen in studies when PFOA is measured later in pregnancy (Steenland et al. 2018; Apelberg et al. 2007; Chu et al. 2020). When PFOA is measured earlier in pregnancy, the associations with birth weight are largely null (Darrow et al. 2013; Manzano-Salgado et al. 2017; Steenland et al. 2018). In addition, most epidemiological studies that specifically evaluated the risk of low birth weight (that is, birth weight <2500 grams) have reported null associations with increased concentrations of PFOA or PFOS (Savitz et al. 2012a; 2012b; Darrow et al. 2013; Stein et al. 2009; Chen et al. 2012; Manzano-Salgado et al. 2017).*

*These findings are consistent with the hypothesis that the apparent association between PFOA and birth weight is confounded by the magnitude of plasma volume expansion during pregnancy and glomerular filtration rate (Steenland et al. 2018; Verner et al. 2015). Evaluations of potential pharmacokinetic bias have demonstrated that associations between prenatal exposure to PFAS and lower birth weight in epidemiological studies may actually be driven by changes in glomerular filtration, which increases by about 50% during the first half of pregnancy followed by a slight decline in the second half (Andersen et al. 2021a). Studies have shown that women with less of an increase in GFR tended to have smaller babies (Gibson, 1973; Morken et al., 2014). Verner et al. (2015) used a PBPK model to run simulations of a study population and to generate pairs of predictions for PFAS level and birth weight. Results obtained from simulated PFAS levels and birth weights were compared with published epidemiological studies to evaluate how much of this association might be attributable to the influence of GFR. The analysis used a detailed PBPK model of PFOA and PFOS during pregnancy (Loccisano et al., 2013) that was modified to describe the association of GFR with birth weight. The model was then used to simulate study populations exposed to PFOA or PFOS and to predict the resulting distributions of concentrations in maternal and cord plasma. Results from Monte Carlo PBPK model simulations (of longitudinal data) indicated that even controlling just for the effect of GFR changes accounted for the majority of the association of PFOA and PFOS with reduced birth weight.*

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

***Comment:*** EPA concerns regarding the clinical relevance of the small increases in ALT reported in the epidemiological literature and the non-specificity of ALT as a biomarker of liver injury/disease are justified. They would not be appropriate as a basis for setting a quantitative exposure guideline.

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

***Comment:*** The decision not to select ALT for dose-response modeling is appropriate due to the questionable clinical relevance of the small increases in ALT reported in the epidemiological literature and the non-specificity of ALT as a biomarker of liver injury/disease particularly. The relevance of these observations of small changes in ALT is of particularly questionable in the case of PFAS, due to the likelihood that the associations may be secondary to a reverse relationship between altered liver function and the control of PFAS transport/excretion, where individuals with impaired liver function may have reduced transport/clearance of PFAS, resulting in relatively higher blood

*concentrations compared to healthy individuals (similar to the case of impaired kidney function, described in the comment on cancer classification below).*

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

**Comment:** *No.*

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

**Comment:** *No.*

## Cancer

### 1. Cancer classification for PFOA/PFOS

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

**Comment:** *The EPA’s proposed change in the categorization of PFOA from “suggestive evidence” to “likely carcinogen” is not justified. The EPA’s determination appears to be based on an epidemiology study reporting an association between PFOA concentrations and incidence of renal cell carcinoma (Shearer et al. 2021). PFOA (median 5.5 ng/mL sampled during 1993-2001) was measured in blood serum at least 2 to 18 years before diagnosis of kidney cancer; given the half-life of <2 years (see comments under Toxicokinetics section), a single PFOA measurement is unlikely to accurately portray the exposure relevant to the development of disease. In epidemiological studies of higher exposures, there is inconsistent evidence of increased kidney cancer risk. Epidemiological studies of residents exposed to PFOA and other PFOS in contaminated drinking water have reported modest increases in kidney cancer (Li et al. 2022; Vieira et al. 2013). Studies of occupational cohorts have been inconsistent, with one cohort showing decreased risk of kidney cancer (Raleigh et al. 2014) and another cohort showing increased risk of kidney cancer (Steenland and Woskie, 2012); however, the number of kidney cancer cases or deaths in the occupational cohorts have been relatively few, and the investigators have cited low statistical power to draw conclusions regarding the reported associations. Nevertheless, if there were a strong causal association between PFOA or PFOS exposure and kidney cancer, it would be expected that much higher estimates of relative risk (a magnitude of 3-fold or more) would be seen in occupational cohorts who were exposed to PFOA at much higher concentrations than the general population and who were followed for more than 30 years on average (Raleigh et al. 2014; Steenland and Woskie 2012). Separately, kidney cancer is frequently associated with impaired kidney function. Lower renal function (calculated as estimated glomerular filtration rate (eGFR)) is likely to result in decreased PFOA excretion and a consequent increased concentration in serum. Cross-sectional analyses of adults exposed at background levels (Shankar et al. 2011) and of children exposed at high levels (Watkins et al. 2013) found a positive*

association between lower kidney function (i.e., lower eGFR) and higher measured serum PFOA. Dhingra et al. (2016), performed an analysis of cross-sectional studies reporting associations between PFOA and renal function, and concluded that reverse causation led to the observed associations. Shearer et al. (2021) reported that a higher percentage of cases (9%) than controls (5.6%) had diminished kidney function; however, the overall difference in kidney function between cases and controls was not statistically significant when kidney function was stratified by normal ( $\text{eGFR} \geq 90 \text{ mL/min/1.73 m}^2$ ), mild loss ( $\text{eGFR} 60\text{--}89 \text{ mL/min/1.73 m}^2$ ) or diminished kidney function ( $\text{eGFR} < 60 \text{ mL/min/1.73 m}^2$ ). In sensitivity analyses, Shearer et al. (2021) stratified by kidney function and separately restricted analyses to study subjects with high kidney function. In both analyses, the odds ratios for kidney cancer were statistically significantly increased for PFOA exposure; however, it is not clear that eGFR based on a single sample collected 2 to 18 years before the diagnosis of kidney cancer falls within a relevant time window for kidney cancer induction and latency associated with impaired kidney function. Steenland and Vieira (2021) reviewed these studies (including the Shearer et al. 2021) and concluded that the evidence from epidemiologic studies of PFAS in relation to cancer “remains limited.”

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

**Comment:** The decision to continue the ‘suggestive’ categorization is appropriate. Although Shearer et al. (2021) also reported statistically significantly increased odds ratios for kidney cancer when PFOS was measured as a continuous variable, the odds ratios did not increase with increasing exposure when PFOS exposure was categorized. After adjusting for PFOA and PFHxS, there was a non-significantly decreased odds ratio for kidney cancer (OR 0.92, 95% CI 0.60–1.42).

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

**Comment:** The EPA relies extensively (and exclusively, for epidemiological studies generated since the 2016 HESD) on the Shearer et al. (2021) study. However, there are earlier studies of occupational groups exposed to much higher exposures that were discounted largely because of small numbers of cases and questions about whether the studies had adequate power to detect an excess cancer risk if one existed (Steenland and Woskie et al. 2012; Raleigh et al. 2014). See response to Cancer Classification.

- (i) Use of Shearer et al. (2021) study: For purposes of deriving the cancer slope factor, the EPA estimated the dose-response between PFOA and kidney cancer using a weighted linear regression of the quartile-specific odds ratios where the weights were inverse variance of each OR. Although Shearer et al. (2021) reported a statistically significant increased risk of renal cell carcinoma when exposure was modeled as a continuous variable. In a separate categorical analysis, an exposure-response relationship was not seen, that is, the odds ratios did not increase with increasing exposure. In the analysis that adjusted (OR 1.71, 95% CI 1.23–2.37) after

adjusting for body mass index, smoking status, history of hypertension, estimated glomerular filtration rate, previous freeze-thaw cycle, and calendar year of blood draw, the OR was statistically significant for the highest quartile of PFOA exposure only.), a separate categorical analysis did not show an exposure-response relationship. The odds ratios did not increase with increasing exposure, although the OR was increased for PFOA in blood serum concentrations >7.2 to 27.2 ng/mL when compared to concentrations <4.0 ng/mL. After further adjustment for exposure to other PFAS, the OR for the 4th quartile was attenuated, and was not statistically significant. (see Table below). The p-for-trend was 0.13 (not significant). The Shearer et al. (2021) study should not be used to derive a POD for calculating a cancer slope factor.

*Shearer et al. (2021)*

Cases / Controls	ng PFOA/mL blood	OR	95% CI
47 / 81	<4.0	1.00	Reference
83 / 79	≥4.0 – 5.5	1.41	0.69–2.90
69 / 83	>5.5 – 7.3	1.12	0.52–2.42
125 / 81	>7.3 – 27.2	2.19	0.86–5.61
	Continuous	1.68	1.07 – 2.63

Study participants were 55-74 years at time of blood draw. Serum samples were collected at a single point in time (before diagnosis of kidney cancer). Kidney cancers were diagnosed on average 8.8 years after the blood draw (range, 2-18 years). In comparison, Steenland and Woskie (2012) studied mortality among 5,791 workers exposed to PFOA during 1952 to 2004. These workers had mean duration of employment of 19 years, a mean duration of follow up of 30 years, and an estimated mean annual serum concentration of 350 ng/mL. When kidney cancer mortality was compared to other workers in the same region, there was no exposure-response relationship when PFOA was categorized according to quartiles of cumulative PFOA exposure. Although an excess of kidney cancer deaths was seen for workers exposed to ≥1,819 ng/ml-years (the 4th quartile) when exposure was lagged by 20 years to account for latency, the SMR was decreased for the second quartile and there were no kidney cancer deaths that occurred in the third quartile of exposures (Steenland and Woskie, 2012). Although some have argued that the results from the worker studies are limited because workers are healthier than adults in the general population, the healthy worker effect is not a significant source of bias when evaluating cancers with long latencies (Checkoway et al, 2014).

Under a hypothetical assumption of 30 years of exposure to 15 ng/mL (which represents a mid-range estimate from the highest quartile of exposure (>7.3 to 27.2 ng/mL) in Shearer et al. (2021), the cumulative exposure would be 450 ng/mL-years for cases and controls in the highest quartile. These cases from the Shearer et al. (2021) population would fall within the lowest occupational exposure category from the Steenland and Woskie (2012) workers (1st quartile, 0 to <515 ng/mL-years, when exposure was lagged 20 years to allow for an appropriate induction and latency for kidney cancer). Among workers exposed to PFOA, three kidney cancer deaths were reported (compared to 2.2 expected, SMR 1.34, 95% CI 0.28 – 3.91) (Steenland and Woskie et al. 2012). Raleigh et al. (2014) relied on a job-exposure matrix to estimate individual concentrations of APFO in micrograms per cubic meter of air, rather than drawing blood for analysis. Although these estimates of inhalation exposure reported by Raleigh et al. (2014) cannot be directly converted into serum PFOA concentrations, other studies of workers that overlapped with the Raleigh et al. (2014) cohort (Olsen et al. 2003; Olsen and Zobel, 2007) reported very high serum concentrations of PFOA in the blood of workers (>1,000 ng/mL) and at concentrations higher than those reported by Steenland and Woskie (2012). In contrast to

*Steenland and Woskie (2012), Raleigh et al. (2014) did not find an increased risk of kidney cancer in the highest category of exposure (4th quartile, hazard ratio (HR) 0.73, 95% CI 0.21–0.48) when compared to the non-exposed population based on four incident kidney cancers. There was also no increased risk of kidney cancer when the 3rd and 4th quartiles were combined (8 kidney cancers, HR 0.85, 95% CI 0.36–2.06).*

*Shearer et al. (2021) chose quartile cut-points based on the serum PFOA exposure in the controls. There was, however, a substantial difference in the number of kidney cancer cases (n=47) and controls (n=81) for the lowest exposures (1st quartile, <4.0 ng/mL). This concentration is similar to the geometric mean concentration for PFOA (4.8 ng/mL for participants age 60 years and older) in a nationally representative sample (NHANES) collected during 1999–2000. It seems unlikely that there would be a discrepancy in kidney cancer incidence in a population exposed at background levels and there is no logical explanation for a deficit in kidney cancers at these low concentrations. The clear discrepancy between the number of cases and controls in the referent category may potentially create a spurious association between PFOA and kidney cancer when higher quartiles of exposure are compared to the referent group (1<sup>st</sup> quartile of exposure).*

*The Shearer et al. (2021) study does not consider cancer induction and latency, which is a study limitation, especially considering population-level exposures. Presumably, high exposures in occupational groups would result in a shorter latency than population level exposures; however, inconsistent associations between PFOA and kidney cancer have been reported in workers exposed to much higher concentrations and followed for 30 years or more (Raleigh et al. 2014; Steenland and Woskie, 2012). Solid-tumor cancers are unlikely to have cancer induction and latency periods that are shorter than 20 years at higher exposures. For example, Smith et al. (2018) reported latency periods for kidney cancer were at least 20 years following exposure to arsenic in drinking water and remained elevated after 40 years of follow up. Because the time to diagnosis of kidney cancer ranged from 2 to 18 years since blood in Shearer et al. (2021), an inadequate latency for cancer development raises the possibility of reverse causality or confounding, i.e., impairment of kidney function associated with renal cell cancer induction results in increased concentrations of PFOA. Dhingra et al. (2017) reported that decreased renal function (lower glomerular filtration rate) led to higher PFOA concentrations. Decreased renal function is expected as kidney cancer develops, although it may not be noticed before clinical manifestation of kidney cancer. Although Shearer et al. (2021) adjusted their results for estimated glomerular filtration rate (eGFR), the estimate was based on a single blood serum sample, which is inadequate for an evaluation of glomerular filtration rate. Moreover, glomerular filtration is only one of the many kidney functions that control the excretion of PFOA; multiple renal transporters, including the basolateral and apical organic acid transporters and the urate transporter, are also involved in the regulation of PFOA excretion, and hence blood levels.*

- (i) Use of peak exposure: Peak exposure was not discussed as an exposure metric in Shearer et al. (2021), and a search of the EPA PFOA MCLG document did not identify any statement to indicate that peak exposure was used in the EPA analysis. While the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) used peak concentration in their derivation of a Public Health Goal (PHG) for PFOA, and it seems likely that the EPA followed a similar approach, there is no citation to the OEHHA PHG document in the EPA MCLG document. The*

*uncertainty regarding the approach taken by EPA represents a serious lack of transparency in the document.*

*The use of peak exposure is not appropriate for a chemical like PFOA, which has a half-life on the order of a year or more. With such a long human half-life, it can take as much as a decade of continued exposure at a given intake to produce a corresponding change in target tissue concentration, so the peak internal exposure would not be well represented by the peak intake. There is also no rationale for using peak exposure as the dose metric for renal cell carcinoma from human exposures to PFOA on the basis of potential nonlinear pharmacokinetics or pharmacodynamics of PFOA over the range of human exposures. Therefore, the time-weighted average exposure should be used as the dose metric for the analysis, or cumulative exposure (that is, the product of intensity and duration) as used by Steenland and Woskie (2012).*

## Toxicokinetic Models

### 1. Human model –

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

***Comment:*** *The use of steady-state to calculate HED is an acceptable approach for cross-species extrapolation of the key model derived points of departure in this document. For longer half-lived PFAS compounds like PFOA and PFOS, it is unlikely that event-based approaches would yield different results. This may not be the case for PFAS with shorter half-lives.*

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

***Comment:*** *The use of Thompson et al. (2010) for the Volume of Distribution (VD) for PFOA and PFOS is appropriate for human and the animal. However, the half-lives for humans used by EPA for the clearance calculation was 3.8 years for PFOA and 5.4 years for PFOS, derived from studies of retired workers (Olsen et al. 2007). Given the extensive discussion in the MCLG document of additional studies reporting the half-life of PFOA in environmentally exposed populations, it is unclear why the half-life from an occupational study that included*



*only a small number of retired workers was chosen over other reported values for larger populations exposed at environmental levels, including the more accepted half-life value of 2.3 years reported by Bartell et al. (2010) study. The Zhang et al. (2013) study, which included the collection of urinary data to support an estimate of renal clearance, and which was reviewed in the EPA's half-life discussion, also appears to support the results of the Bartell that the 3.8 year half-life is incorrect. These studies provide a strong argument against using the half-life reported in Olsen et al. (2007), which is thought to include subjects that intermittently had occupational level exposures after retiring.*

*A recent review of studies with human PFOA half-life information by an international collaboration sponsored by the Alliance for Risk Assessment (Campbell et al., 2022) concluded that the results from the most appropriate studies support a PFOA half-life in the range of 0.5 to 1.7 years, indicating that the half-life of 3.8 years (Olsen et al. 2007) is much too high. Verner et al. (2016) did not justify the use of 3.8 years nor did they evaluate the impact of the shorter estimated half-lives in their analysis and noted that the "much greater ratio of estimated intakes of PFOA may be partially due to the half-life we used (3.8 years); others have suggested a lower value (e.g., 2.3 years)." The Agency discusses this in their half-life review but does not provide reasoning as to why they nevertheless used the 3.8-year half-life from the model in Verner et al. (2016). A broader evaluation of the half-lives reported for PFOA would indicate that, when accounting for continued exposure, the half-life is likely to be below 2 years. The Olsen et al. (2007) study also serves as the basis for the PFOS half-life used in the Verner et al. (2013) model by the EPA; it is likely that the correct half-life for PFOS is also much shorter than the value used by EPA.*

- 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.

**Comment:** *The use of the serum to milk and serum to fetus ratios is a default approach that, while increasing uncertainty in the fetal/lactational modeling, is not unreasonable. The application of the model to the available time-course data in the rat during lactation indicates that the approach was valid for PFOA and PFOS.*

## 2. Animal Model –

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

**Comment:** *The choice of a simple PK model that accounts for saturable resorption is reasonable, and the approach taken (e.g., choice of dose metrics) in deriving the animal dose metrics, as well as the PODs in humans, are reasonable. Based on the model fits presented in the PFOA and PFOS documents, the models provide acceptable fits to the data overall. However, neither the animal nor the human PK model files were available to allow verification of the EPA's implementation of the published models that they actually used to derive the dose metrics. The EPA routinely verifies the code of submitted models, and the same possibility should be provided to the public for agency model code. All model files (including R scripts) should be available for review by the SAB, as well as the public, to provide full disclosure.*

- 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?

**Comment:** *Median values from Bayesian inference using a PK model are reasonable to the extent that the PK model adequately captures the kinetic data used in the calibration and the implementation of the models by EPA are error-free. (see previous).*

- 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?

**Comment:** *While it is reasonable to suggest that the kinetics of PFOS in male and female rat are similar, the impact of that assumption on the simulations of the Chang et al (2012) study presented in Wambaugh et al. (2013), and of the Kim study shown in supplemental E (Figure E-7 left panel) should be presented. Wambaugh et al. (2013) did not show the model fit to the female rat and the agency has not included simulations using the Wambaugh et al. (2013) model in Appendix E. Wambaugh et al. (2013) did not discuss their use of gender-specific parameters for PFOS in the rat, which had not been required in the previous modeling efforts. It is not possible to fully evaluate whether there is an error in the approach taken with the PFOS animal modeling, because there is a less than full documentation of the EPA model.*

- 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.

**Comment:** *The model structure for the nursing pup is reasonable. The potential issues noted for the adult animal; however, could lead to issues with the prediction of fetal dose metrics. Given the adult model cannot be fully evaluated due to limited documentation, the pregnancy and lactation model can also not be fully evaluated to determine whether or not the results are correct.*

- 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.

**Comment:** *The source for the milk ingestion for animal pups was not fully documented and has not been peer reviewed – cited as in prep (Kapraun et al. 2021). The PFOA and PFOS MCLG documents should provide the information on milk ingestion by mouse and rat pups, describe whether the values are consistent or different from those used in previous lactational modeling in mouse and rat, and justify the choice of intakes. There was no documentation provided on the lactational transfer in humans and very little information is provided regarding the Verner model. While Verner et al. (2016) includes a model file in his manuscript, this does not preclude the agency from providing their model files for the SAB review, as well as for the public, since the parameters actually used in the model scripts determine the output, not the parameters in the publication.*

- 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.

**Comment:** *Sex differences in PFOA clearance have only been observed for adult rat where the female rat exhibited a much shorter half-life than the male rat. The evidence supports an assumption that PFOA and PFOS clearance in neonatal animals is similar to adults, and that it is similar to the adult female rat for PFOA.*

## Epidemiological Study RfD Derivation

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

**Comment:** *The EPA appropriately evaluated confounding as a risk of bias domain when evaluating the quality of an individual study. Based on a visual review of the heat maps, it appears that a study can be deemed deficient with respect to confounding and still be judged to be a “medium” quality study. It is not clear if each of the risk of bias domains were considered of equal weight when judging the study confidence level (high, medium, low, or uninformative) or if there were certain domains that were given greater weight when assessing study confidence. A written protocol would provide guidance for the evaluation of study quality.*

*In the overall synthesis of evidence from epidemiological studies, the EPA appeared to focus primarily on co-exposures to other PFAS. Potential confounding from exposures to other environmental contaminants is only discussed by citing statements in the study publications that exposures to a particular compound (e.g., PCBs) were not highly correlated with PFAS exposure. However, an evaluation of the correlation between the two exposures is not a substitute for including the co-exposures in the analysis as an additional covariate. Previous studies for other compounds by some of the same investigators have failed to include important covariates out of concern that there might no longer be a significant association for the main effect. Given the importance of these studies for the risk assessment, the EPA should not finalize this document without obtaining the data from the critical studies and performing their own analysis, so that it would be available for public scrutiny.*

*Another potential mechanism of confounding is referred to as pharmacokinetic (PK) bias. PK bias due to confounding arises when a confounding factor affects both the biomarker (e.g., PFAS blood concentration) and the health outcome (e.g., decreased birthweight). PK bias can also result from reverse causation, that is, when the health outcome alters biomarker levels. Importantly, PK bias analysis, whether for reverse causality or effects of confounding factors, can readily be conducted with pharmacokinetic models to examine the influence of confounding factors or health outcomes on pharmacokinetic processes and the resulting epidemiological associations (Andersen et al. 2021a). EPA has the capability to review published studies of PK bias and to perform PK bias analysis themselves, as evidenced by their previous applications of PBPK and BBDR modeling.*

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

**Comment:** *The EPA stated that human epidemiological studies consistently reported decreases in antibody response following vaccination and recommended antibody response to vaccination in children as an outcome for POD derivation. For the POD derivation, the EPA relied an analysis of response to tetanus and diphtheria vaccination in two birth cohorts of children in the Faroe Islands (Budtz-Jorgensen and Grandjean, 2018). Should the EPA decide to use the Faroe Island studies as the basis of the RfD, the data should be obtained from the study investigators and independently evaluated. The analysis and interpretation of the results from the series of studies deserves further review due to inconsistencies within and between the studies regarding participants and methods. Additional concerns are provided below.*

Level of clinical protection: Grandjean et al. (2012) reported 2-fold and 4-fold increased ORs for falling below a clinically significant protective level for tetanus and diphtheria antibodies at age 5 years and age 7 years, respectively. The clinically protective level used by the study investigators was 0.1 IU/mL. Grandjean et al. (2012) reported: “[s]erum concentrations of antibodies against the tetanus toxoid were measured in coded samples by the Statens Serum Institut using enzyme-linked immunosorbent assay (Hendriksen et al. 1988).” Hendriksen et al. (1988) describes the toxin binding inhibition test (ToBI), an assay which is a modified ELISA, for which clinical protection is achieved at 0.01 IU/mL (WHO, 2018; WHO, 2017).

The WHO (2017) reports:

“There is no definitive immunological correlate of protection for tetanus. The minimum amount of circulating antibody that, in most cases, ensures immunity to tetanus is assay-specific. Using in vivo neutralization tests or modified enzyme-linked immunosorbent assays (ELISA), concentrations exceeding 0.01 IU/ml are usually considered protective, whereas antibody concentrations of at least 0.1–0.2 IU/ml are defined as protective when using standard ELISA techniques. [p. 61]”

The WHO (2018) states:

“A toxin binding inhibition (ToBI) assay has been reported and demonstrated to show good correlation with the neutralization assay (correlation coefficient = 0.95) (Hendriksen et al., 1988). The assay determines the level of inhibition of binding of TT to a polyclonal antitoxin by tetanus antibodies in the test sera. The ToBI assay has been subsequently demonstrated to be able to measure tetanus antibody levels below 0.01 IU/ml, making the test attractive for assessing tetanus immunity (van Gageldonk et al., 2008). [p.7]”

For vaccination against tetanus and diphtheria, the children in the Faroe Islands cohorts followed a vaccination schedule of 3 shots in the primary series (at ages 3 months, 5 months, and 1 year) and one booster at age 5. The current recommendation by the WHO for primary vaccination and booster doses in children recommends a primary series of 3 doses of TTCV (similar to the Faroe Islands schedule for primary series) and a boosting regime of 3 doses of TTCV for a total of 6 doses in order to achieve long-term immunity (WHO, 2017):

“The 3 TTCV booster doses should be given at: 12–23 months of age; 4–7 years of age; and 9–15 years of age. Ideally, there should be at least 4 years between booster doses.”

The duration of protection and requirements for booster is further illustrated in the WHO Immunological Basis for Immunization Series, Module 3: Tetanus (WHO, 2018):

“To illustrate the kinetics of immunity among children  $\geq 1$  year, adolescents and adults following primary and booster vaccination with TTCV, Figure 2 provides a schematic diagram of the typical response. A single dose of TT in the absence of priming induces little, if any, protection. Two to four weeks after the second dose, the mean level of tetanus antitoxin typically exceeds the minimum putatively protective level of 0.01 IU/mL. One year after the second dose, the mean antibody levels are expected to decline and may fall to the protective threshold level. After each subsequent dose of vaccine, immunity is boosted, then persists above the protective threshold for a time, and then wanes over time. Putatively protective levels of immunity are induced by a primary series of three TTCV doses and immunity typically persists for at least 5 years. After the third dose, each additional booster dose given after at least a one-year interval increases tetanus antitoxin levels and further prolongs the duration of immunity. Immunity may persist for approximately 10 years after the fourth dose of TTCV and for at least 20 years after the fifth dose. [WHO, 2018, pp. 14-15].”

Figure 2: Schematic diagram of the antibody response to tetanus toxoid (TT) among children  $\geq 1$  year, adolescents and adults

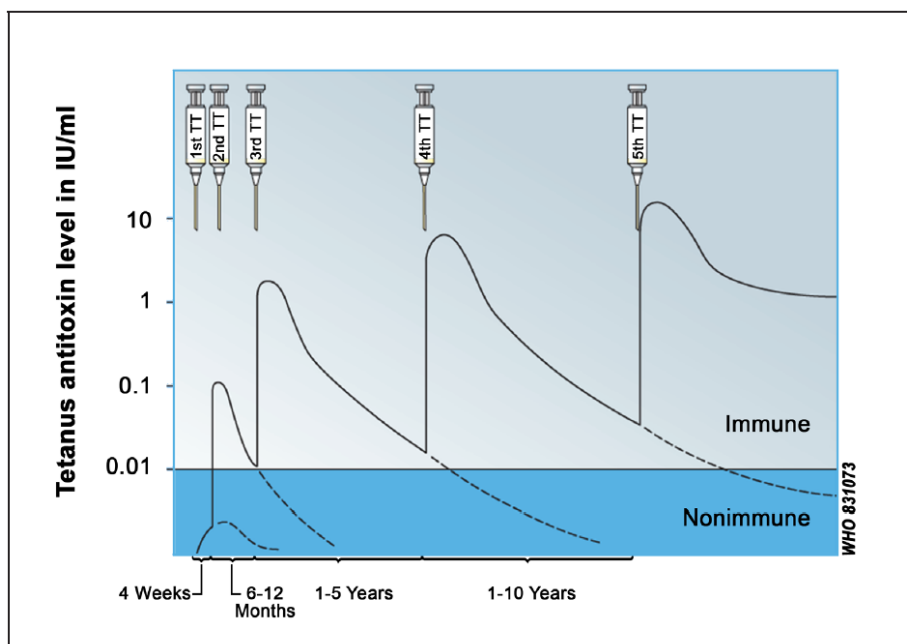


Figure reproduced from: Plotkin S, Orenstein W, Offit P, Edwards KM. Plotkin's vaccines, seventh edition. New York (NY): Elsevier; 2017: Chapter 58, Tetanus toxoid, p. 1071, reproduced by permission.

"Five properly spaced doses [given to children  $\geq 1$  year, adolescents and adults provides] protection lasting at least 20 years and probably substantially longer for most recipients. (Threshold of protection of 0.01 IU/mL applies to values measured by in vivo neutralization, modified ELISA [or bead-based immunofluorescence]; for standard ELISA, protection is [usually defined between 0.10–20 IU/mL, based on the assay])." (Modified source: Borrow R, Balmer P, Roper MH. The immunological basis for immunization. Module 3: Tetanus (update 2006). Geneva: World Health Organization; 2007.)

Although the WHO (2012) Guidelines for Immunotoxicity Risk Assessment recommend measures of vaccine response as a measure of immune effects, the guidelines refer to EHC 180 (UNEP, 1996):

"A description of biomarkers in epidemiological studies is provided in EHC 180: Principles and methods for assessing direct immunotoxicity with exposure to chemicals (IPCS, 1996). The risk assessor should refer to the assay descriptions in EHC 180 for immunotoxicity endpoints contained in the data set for the chemical in question to provide specific context, cautions and information that may assist in the interpretation of immunosuppression data for risk assessment. In addition, it is recommended that the risk assessor consult an expert in immunotoxicology or clinical immunology to help interpret the biological plausibility of the study results. [p.51]"

EHC 180 Principles and methods for assessing direct immunotoxicity with exposure to chemicals (UNEP, 1996) described assays recommended by WHO (UNEP, 1996) and the National Research Council (NRC) of the National Academy of Sciences (NRC, 1992) for preliminary assessment of individuals exposed to immunotoxicants, including secondary antibody responses to proteins (e.g., diphtheria, tetanus, poliomyelitis) and polysaccharides (e.g., pneumococcal, meningococcal). Specifically, the WHO (1996) describes the tests for antibody response to immunization:

*“In order to test for T cell-dependent antibody responses, commercially available diphtheria-tetanus vaccine can be given in recommended doses. Blood is taken two weeks after each injection and tetanus and diphtheria antibodies are determined. In patients who have been immunized with diphtheria-tetanus or diphtheria-pertussis-tetanus vaccine, one booster injection is given before determination of antibodies. In testing for T cell-independent antibody responses, commercially available pneumococcal vaccine can be given in recommended doses. Three doses of killed poliomyelitis vaccine (10 ml intramuscularly, at intervals of two weeks) can also be used as the immunogen. Blood is taken two weeks after the last injection, and antibody is usually determined by virus neutralization. [WHO, 1996, pp. 238-239]”*

*Therefore, it seems that the most relevant findings from the studies in the Faroe Islands are the associations between 5 year post-booster responses compared to PFAS serum measured at age 5 years pre-booster, four weeks earlier. None of the studies of the Faroe Islands reported whether any person had a post-booster concentration that fell below 0.1 IU/mL four weeks after receiving a booster.*

*Although there is no information on the number of individuals who received a booster but failed to mount an appropriate response within four weeks, there is evidence that the booster worked overall. For tetanus, the post-booster median concentration (35 IU/mL, interquartile range (IQR) 16-96) in 456 children was 159-fold higher than the pre-booster median concentration based on 532 children (0.22 IU/mL, IQR 0.10 – 0.51) (Grandjean et al. 2012, Table 1). There is approximately the same variability in post-booster and pre-booster concentrations based on the IQR (6-fold difference from 75th to 25th percentiles post booster and 5-fold difference from 75th to 25th percentiles in antibody concentrations pre-booster). For diphtheria, the post-booster median concentration (13 IU/mL, IQR 6.4–26) was 108-fold higher than the pre-booster median concentration (0.12 IU/mL, IQR 0.05 – 0.40). There was a 4-fold difference between the 75th to 25th percentiles after boosting, and an 8-fold difference between the 75th and 25th percentiles before boosting.*

*Inconsistencies regarding study subjects included in various analyses: Although the Faroe Islands are four separate studies, they each address one or two birth cohorts (1997-2000 and 2007-2009) from a single hospital in the Faroe Islands. There are inconsistencies reported in relation to the number of participants at different ages in these cohorts. For the 1997-2000 birth cohort, Grandjean et al. (2012) and subsequent studies reported “[a] total of 587 children (89% of the cohort) participated in 1 or more of the examinations, which took place at age 5 years pre-booster, approximately 4 weeks after the booster, and at age 7 years.” Separately, Grandjean et al. (2012) reported “[a] lower antibody response was observed in 2 groups of 173 and 168 children, who had been inoculated with combination booster vaccines containing pertussis, polio, or both, as compared with the 151 who received diphtheria and tetanus toxoids only.” The total of 492 children receiving a booster dose does not match with the 532 children attending the age-5 pre-booster examination or the 456 children attending the age-5 post-booster exam (Grandjean et al. 2012, table 1) or the 537 children included in the 5-year pre-booster analysis or the 440 children included in the 5-year post-booster analysis (Grandjean et al. 2012, table 2).*

*It is also not clear that the children examined at the 5-year post-booster examination were restricted to the same children that participated in the 5-year pre-booster exam. In fact, it seems more likely that there were children who participated in the 5-year post-booster exam that were not part of the study population that had attended the 5-year pre-booster exam (and therefore, had not provided a PFAS serum sample 4 weeks earlier). Grandjean et al. (2012) reported that “[f]or the 5-year post-*

booster data, we adjusted for the time since vaccination, using a restricted cubic spline (Heilmann 2006).” There would be no need to make this adjustment for time since last vaccination if all of the 5-year post-booster results were restricted to the same individuals who attended the 5-year pre-booster exam 4 weeks earlier. Potentially, some of the children at the 5 year post-booster exam were sampled for PFAS serum and antibody concentration immediately after they had their booster shot. If the 5-year post-booster serum samples described mixed populations (individuals who were last vaccinated four weeks earlier as well as individuals who were last vaccinated at one year), the analyses of 5-year post-booster data would have significant variability in levels of circulating antibodies. Regardless of PFAS serum concentrations, some proportion of individuals included in the post-booster analysis would have had relatively high levels of circulating antibodies (that is, individuals who were boosted 4 weeks earlier) while others would have had low levels of circulating antibodies (those who had last received a vaccination at age 1) due to the well-known effect of waning antibodies 1 to 3 years after the primary series (WHO, 2017; 2018). The analysis of post-booster results should be restricted to the individuals who provided serum four weeks earlier so that the boosting effect is not diluted.

Confounding: Many factors affect humoral immunity and vaccine response. Possible confounding due to co-exposures to PCBs and other persistent organic compounds, as well as methyl mercury (MeHg), existing in studies of the Faroese Island populations. The Faroese diet includes a high proportion of whale meat, which contains high levels of PFAS (Weihe et al. 2008), but it also contains high levels of PCBs, polybrominated flame retardants and MeHg. (Andvik et al. 2021). The PFAS exposure from eating whale meat is significant: “On a relative scale, a high intake of two pilot whale dinners per month is associated with increases in the 14-year serum concentrations of PFOS, PFNA, and PFDeA by almost 25%, 50%, and 100%, when compared to concentrations in subjects eating little or no whale at all (Table 3). Fish dinners had a much weaker effect, although each weekly fish dinner augmented the PFHxS concentration by about 10%.” (Weihe et al. 2008)

Budtz-Jorgensen and Grandjean (2018) cited Grandjean et al. (2012) when they reported that confounding by methyl mercury and polychlorinated biphenyls was unlikely because of their weak correlations with serum concentrations of PFAS. The evaluation of methyl mercury as a potential confounder should be independently confirmed by EPA. Although Grandjean et al. (2012) reported that PCBs were weakly correlated with PFAS, methyl mercury was not discussed in these analyses and was presumably not part of the evaluation. The IPCS (1996) reported that MeHg decreased humoral immunity in mice in a study conducted by Blackley et al. (1980).

Conclusion: It seems more likely than not that the results of this study are merely consistent with heterogeneity in vaccine response. Furthermore, reverse causality and/or confounding are likely to be an issue in studies with little variation in exposure and low exposure contrasts. Exposure varied little in the Faroe Island studies; the exposure contrasts were low based on measurements of PFAS in serum. Although the study investigators did not provide minimum or maximum concentrations, they reported interquartile ranges. In children measured for PFAS in serum at age 5, the IQRs were: PFOA, 3.33 to 4.96 ng/mL (median 4.06); PFOS, 13.5 to 21.1 ng/mL (median 16.7); PFHxS 0.45 to 0.88 ng/mL (median 0.63) ; PFNA 0.76 to 1.24 ng/mL (median 1.00); and PFDA, 0.21 to 0.38 ng/mL (median 0.28). Grandjean et al. (2012) and subsequent evaluations of the Faroe Islands cohort used log base 2 transformations of these exposure data (with low exposure contrasts) and found that the strongest effect was at the lowest concentrations, which seems biologically implausible.



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

***Comment:*** As stated in the comments on the previous question, the level of circulating antibody that correlates with clinical protection is assay-specific. Based on the ToBI assay used in the Faroe Islands, the relevant clinical level of protection for the population in that study would be 0.01 IU/mL, not the value of 0.1 IU/mL used by the EPA. Furthermore, none of the studies of the Faroe Islands population provided information on whether the prevalence of failure to respond to secondary immunization was beyond that expected from natural variation in vaccine response. In addition, epidemiological studies of PFOA and/or PFOS and common infectious diseases and their symptoms (including otitis media, common colds, gastroenteritis, respiratory tract infections, fevers) have reported inconsistent associations (Granum et al. 2013, Impinen et al. 2018, Dalsager et al. 2016; Looker et al. 2014; Okada et al. 2012), bringing into question the validity of the EPA's assumptions regarding the clinical relevance of the antibody titer endpoint.

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

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

***Comment:*** Yes. Since the RfDs are derived from data in human populations, an uncertainty factor of 10 to consider human interindividual variability is more than adequate to properly account for uncertainty in the derivation of the RfDs.

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

***Comment:*** Yes. Since the RfDs are derived from data in human populations, no uncertainty factors are required apart from one for human interindividual variability.

## Relative Source Contribution

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

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

**Comment:** *The basis for the recommended RSCs of 50% for infants/children and 20% for adults is adequately supported.*

## EPA'S DRAFT FRAMEWORK FOR ESTIMATING NONCANCER HEALTH RISKS ASSOCIATED WITH MIXTURES OF PER- AND POLYFLUOROALKYL SUBSTANCES

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

**General Comment:** *The EPA Draft Framework is a very impressive document that proposes a scientifically sound approach and applies widely accepted practices for mixture risk assessment. It also provides informative examples that help to clarify the proposed approach. It represents a major improvement over some approaches that have been used by regulatory agencies, such as applying the PFOA RfD to total PFAS concentrations. It is important to emphasize, however, that these approaches are best suited for site- or source-specific assessments, where the composition of the mixture at the site or source has been determined. While the approaches described in the framework are sound risk assessment tools, it should be made clear that they are not as well suited for use by states or other entities in the promulgation of drinking water standards, where the composition of the mixtures of PFAS compounds in drinking water may not be consistent across locations and sources.*

### Charge questions

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

**Comment:** *Grouping chemicals for dose-additivity on the basis of similarity in toxicity/health effects begs the question of "how similar". For example, dose additivity is not necessarily appropriate for a chemical that causes centrilobular hypertrophy and a chemical that causes single cell hepatocellular necrosis. In the specific case of PFAS, if the effects in a tissue differ somewhat, it is critical that the assumption of dose-additivity be restricted to compounds that also have sufficient structural similarity to support the likelihood that the key elements of the Mode of Action are similar (that is, similar interactions of the compounds with the key proteins controlling the pharmacokinetics and pharmacodynamics of PFAS). As in the case of pyrethroids and PCBs, it may be necessary to define sub-categories of PFAS for which dose-additivity can be applied. Two examples of the potential considerations are short vs. long chain length, and linear fluoroalkyl acids vs. branched-chain fluoroether acids.*

- 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).

**Comment:** *Common toxicity endpoint/health effect is an acceptable similarity domain for those PFAS with limited or no available MOA-type data. However, the concerns raised in the comment on the previous question need to be adequately addressed as part of the evaluation of similarity.*

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

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

**Comment:** *The proposed approach is reasonable and is consistent with EPA practice.*

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

**Comment:** *The proposed HI methodologies in the framework are adequately supported for use in preliminary site-specific risk assessments for a specific PFAS mixture composition.*

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

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

**Comment:** *The proposed RPF approach is a reasonable methodology for estimating risk associated with specific mixtures of PFAS and is consistent with EPA practice.*

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

**Comment:** *The proposed RPF methodology is scientifically supported as an approach for a specific PFAS mixture during a site- or source-specific assessment, but it should be made clear that it is not well suited for the development of general standards that are intended to be applied across sites or sources with different mixing-ratios of component chemicals.*

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

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

**Comment:** *The Mixture BMD approach is a reasonable methodology for estimating what is in essence a point-of-departure for a specific mixture composition, but the resulting POD should not be applied across mixtures with different compositions.*

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

**Comment:** *The proposed Mixture BMD methodology in the framework is scientifically supported for a PFAS mixture with a specific mixing-ratio of component chemicals, and can be applied during a site- or source-specific assessment. It is important that the EPA make it clear that it should not be applied in the development of a single standard that is intended to be applied across sites or sources with different mixing-ratios of component chemicals.*

## ANALYSIS OF CARDIOVASCULAR DISEASE RISK REDUCTION AS A RESULT OF REDUCED PFOA AND PFOS EXPOSURE IN DRINKING WATER

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

### Charge Questions

1. Section 4.2 presents EPA's meta-analysis for the total cholesterol dose-response function.
  - A. Please provide specific feedback on the extent to which the study selection criteria, the identified studies, and the methodological approach of the meta-analysis are complete and capture up to date scientific literature.

**Comment:** *Although the EPA describes the study selection criteria, there is no apparent integration of study quality criteria in the meta-analysis. Several of the identified studies were judged by reviewers to be of low (or deficient) quality. Separately, there was no information on the risk of bias analysis for five studies (of 14 total studies considered in the meta-analysis) conducted before 2018. Meta-estimates should be derived for studies which were considered higher quality and/or studies that adjusted for certain confounding factors (lipid-lowering medication). In addition, the EPA meta-analysis did not address the associations between PFOA or PFOS and LDL-C, which were discussed in the draft documents. LDL-C is well established as a causal risk factor for CVD; it is recommended that individuals with high LDL-C (and not high TC) take LDL-C lowering medication to manage CVD risk (Grundy et al. 2019). Presumably, EPA excluded LDL-C from the meta-analysis because the ASCVD risk calculator does not include LDL-C as a predictor. The majority of cholesterol in human lipid profiles is LDL-C so the exclusion of LDL-C does not appear to be a fatal flaw. However, the ASCVD risk model is intended solely for patients with LDL-C <190 mg/dL, without ASCVD, and not on LDL-C lowering therapy. In contrast, studies in the general population did not consistently adjust for lipid-lowering medication and did not exclude individuals with LDL-C  $\geq$  190 mg/dL.*

*An international scientific panel (Andersen et al. 2021b) concluded that correlated net absorption or excretion of bile salts and PFAS in the gut enterocytes could give rise to the apparent associations of cholesterol and PFAS in blood observed in epidemiological studies. It has been demonstrated that several bile acid transporters expressed in enterocytes and hepatocytes can also transport PFAS, suggesting that PFAS could be entrained within the enterohepatic recirculation of bile acids. Co-modulation of the kinetics of bile acids and PFAS at these specific transporters by cholesterol has been shown in the rat. Correlated uptake/biliary excretion of PFAS and bile salts could serve as a confounding link between cholesterol homeostasis and PFAS kinetics, leading to an apparent association between Total Cholesterol (TC) and PFAS concentrations in serum. Importantly, if PFAS and cholesterol kinetics were both correlated with a common confounding process (e.g., bile acid recirculation), the fractional change in TC (compared to the average) in a given study would be expected to be the same as the fractional change in PFAS serum (compared to the average) in a same study. Moreover, this expected relationship would hold whether the exposures were at low or high PFAS*

concentrations. In fact, this relationship is consistently seen in studies of PFAS exposures, whether the study subjects are exposed to low environmental concentrations or are occupationally exposed to high concentrations. In other words, a similar fractional change in PFAS concentration (rather than the concentration itself) is associated with a fractional change in TC across many orders of magnitude of exposure concentrations. However, cross-sectional epidemiological studies have evaluated the concentration of PFAS in relation to TC concentrations (using cross-sectional study designs). When evaluating the body of evidence, the apparent effect on cholesterol is stronger at lower concentrations than higher concentrations; however, it is biologically implausible that the potency of PFAS on cholesterol homeostasis decreases as PFAS concentration increases. Therefore, it is more likely that the association is not causal, and that bile acid recirculation distorts the association between PFOA or PFOS and TC.

A recent review of epidemiological studies and other scientific evidence published since 2012 (Steenland et al. 2020) discussed confounding by enterohepatic cycling of PFAS and bile acids as a possible explanation for the positive association between PFOA and total cholesterol. Genius et al. (2014) discussed supporting evidence that cholestyramine, a bile acid sequestrant, also reduced PFOS, PFOA, and PFHxS serum concentrations in humans and rats. Epidemiological studies have not shown increased risks of cardiovascular disease in relation to PFOA or PFOS, even among workers with the highest exposures (Steenland et al. 2015; Steenland and Woskie, 2021; Alexander et al, 2014) or community members exposed to PFOA in contaminated drinking water (Winquist and Steenland, 2014).

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

**Comment:** Small increases in HDL cholesterol have also been reported in relation to PFOA and/or PFOS concentrations in blood serum in cross-sectional studies. In addition to a small, non-statistically significant increase in total cholesterol represented in the pooled estimate seen in Figure A-4 of the Serum Cholesterol Dose-Response Function Appendix, the meta-analysis also showed a small, non-statistically significant increase in HDL cholesterol (Figure A-4 in relation to PFOA and Figure A-8 in relation to PFOS). Presumably, a pooled estimate for the total cholesterol dose-response function reduces the random error associated with relying on a dose-response estimate from a single study. Nevertheless, the pooled estimates showed significant heterogeneity in the underlying studies ( $I^2$  values >70% for both the PFOA (Table A-2) and the PFOS meta-analyses (Table A-3)). This also suggests a single dose-response study should not be relied upon. In any case, the meta-analysis did not address the issue of systematic error and it included studies judged by reviewers to be low quality and/or deficient.

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

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

**Comment:** *The life table approach is clearly described and is a standard life table approach. Because there is substantial uncertainty introduced in using the ASCVD model to predict CVD events in general (see Comment below, Question 2), and substantial uncertainty regarding the risk of CVD in relation to PFOA and PFOS exposure, a simpler approach to the quantifiable impacts of a potential reduction in cholesterol in relation to reductions in PFOA and PFOS exposure is preferred. Instead of attempting to quantify the impacts of avoided CVD events in relation to reduced total cholesterol, the EPA could consider quantifying avoided health care costs associated with treating high LDL-C (for example, avoided prescriptions for cholesterol-lowering medication) or measuring a reduction in the population eligible for treatment with cholesterol-lowering medication. There are uncertainties with this suggested approach as well; however, it avoids the potential amplification of uncertainty associated with the EPA approach.*

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

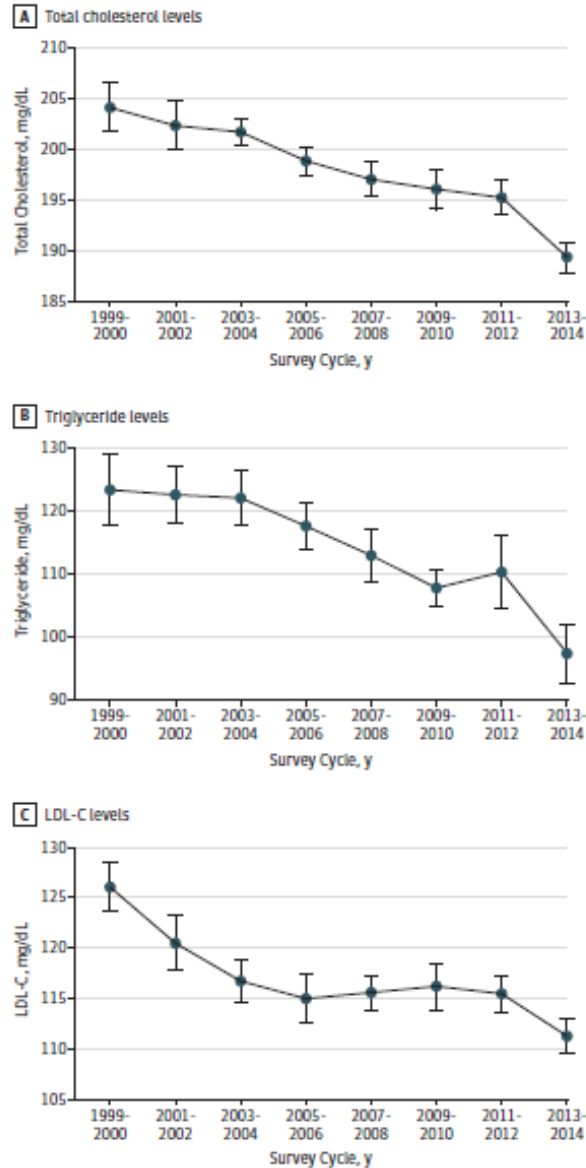
- A. Please comment on the scientific validity of the ASCVD model application for estimating the probability of first time CVD events in various sub-populations and the extent to which it is clearly described.

**Comment:** *The ASCVD risk model was developed for use in clinical practice to provide a quantitative risk score for an individual based on an estimate of the 10-year probability of an initial CVD event based on several inputs. The calculator uses age, total and HDL-cholesterol levels, systolic blood pressure, antihypertensive therapy status, history of diabetes and current smoking and assumes that the individual has LDL-C <190 mg/dL, is free of CVD, and is not taking lipid-lowering medication. The pooled cohort equations that form the basis for the risk estimates are based on older population studies that enrolled volunteers (for example, the Framingham Cohort study, and the Atherosclerosis Risk in Communities (ARIC) study including other cohorts). Although the data may be useful for informing a clinician-patient discussion of managing potential CVD risk for a particular patient, it is not valid for estimating current population-level risks of CVD. Several validation studies have reported that the ASCVD model has overestimated the rate of CVD (DeFilippis et al. 2015; Cook and Ridker, 2014; Rana et al. 2016). The ASCVD model does not account for changes in CVD risk predictors (including cholesterol) over time at a population level. For example, Rosinger et al. (2017) reported that mean cholesterol levels (both TC and LDL-C) and mean triglyceride levels decreased from 1999 to 2014, based on estimates using eight NHANES cycles (see figure below). The decreases over time were similar when stratified by lipid-lowering medication use and are possibly related to the reduction and elimination of artificial trans fats in foods.*



Rosinger et al. 2017:

Figure. Age-Adjusted Total Cholesterol, Triglyceride, and Low-Density Lipoprotein Cholesterol (LDL-C) Trends for US Adults Aged 20 Years and Older, 1999 to 2014



A, Predicted total cholesterol levels and 95% confidence intervals in a sample size of 39 049. B, Predicted log-transformed triglyceride levels and 95% confidence intervals; log-transformed values were exponentiated after the regression, sample size of 17 406. C, Predicted LDL-C levels and 95% confidence intervals in a sample size of 17 096. Figure generated using marginal standardization from age-adjusted linear regression models. Data source: Centers for Disease Control and Prevention/National Center for Health Statistics, the National Health and Nutrition Examination Survey.

SI conversion factors: To convert LDL-C to micromoles per liter, multiply by 0.0259; to convert total cholesterol to micromoles per liter, multiply by 0.0259; to convert triglycerides to micromoles per liter, multiply by 0.0113.

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

**Comment:** *Avoided CVD events should not be the basis for the regulatory impact assessment. It is unclear whether the EPA approach and assumption of a uniform first CVD event over a 10-year period is sufficiently robust. Moreover, the link between PFAS exposure and CVD is too tenuous to support a meaningful cost-benefit comparison.*

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

**Comment:** *The evidence for a causal relationship between PFOA or PFOS exposure and cardiovascular disease is weak, and it is plausible that PFOA and PFOS exposure is associated with higher total cholesterol levels but without an increased risk of cardiovascular disease (Steenland et al. 2020). It is highly uncertain that reduced PFOA or PFOS exposure will lead to lower cholesterol concentrations that can then be quantified as avoided CVD events. As stated previously, the ASCVD model has not accounted for decreases in total cholesterol over the past 20 years or more and is intended for individuals with LDL-C < 190 mg/dL. A more direct and relevant regulatory cost analysis that requires fewer assumptions (and less uncertainty) is preferred. A regulatory analysis of quantified health risk reduction that focuses on cholesterol reduction (and specifically LDL-C reduction) as the quantified endpoint rather than CVD events avoided would reduce uncertainties, such as use of ASCVD to calculate avoided CVD cases when the epidemiological literature does not show increased risks of CVD in relation to PFOA or PFOS exposure. For example, the EPA could consider avoided use of cholesterol-lowering medication or the reduction in number of adults eligible for treatment for high cholesterol. However, this approach would still not address the possibility that confounding by enterohepatic cycling of PFAS and bile acids is actually responsible for the positive association between PFOA and total cholesterol (Steenland et al. 2020).*

5. 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?

**Comment:** *If PFOA and PFOS are associated with small increases in TC and small increases in HDL-C, it is biologically plausible that the risks of cardiovascular disease remain unchanged (Steenland et al 2020). The uncertainties described in the ASCVD model included whether PFOA and PFOS potentially impact other risk factors in the ASCVD model (diabetes and systolic blood pressure, for example); however, the ASCVD model does not include all identified or important risk factors (e.g., elevated LDL-C and elevated C-reactive protein (CRP) levels, for example). Although the association between PFOA or PFOS and CRP levels is not well studied, Genser et al. (2015) reported that CRP levels decreased with increasing serum PFOA concentration in an analysis of adults older than 18 years who resided in water districts contaminated with PFOA (the C8 Health Study).*

## REFERENCES

- Alexander BH, Olsen GW, Burris JM, Mandel JH, Mandel JS. 2003. Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. *Occup Environ Med*, 60(10), 722–729.
- Andersen ME, Mallick P, Clewell HJ 3rd, Yoon M, Olsen GW, Longnecker MP. 2021a. Using quantitative modeling tools to assess pharmacokinetic bias in epidemiological studies showing associations between biomarkers and health outcomes at low exposures. *Environ Res*. 197:111183
- Andersen ME, Hagenbuch B, Apte U, Corton JC, Fletcher T, Lau C, Roth WL, Staels B, Vega GL, Clewell HJ 3rd, Longnecker MP. 2021b. Why is elevation of serum cholesterol associated with exposure to perfluoroalkyl substances (PFAS) in humans? A workshop report on potential mechanisms. *Toxicology*. 459:152845.
- Andvik C, Jourdain E, Lyche JL, Karoliussen R, Borgå K. 2021. High Levels of Legacy and Emerging Contaminants in Killer Whales (*Orcinus orca*) from Norway, 2015 to 2017. *Environ Toxicol Chem*. 2021 Jul;40(7):1850-1860.
- Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, Goldman LR. 2007. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ Health Perspect*, 115(11), 1670–1676.
- Budtz-Jørgensen E, Grandjean P. (2018). Application of benchmark analysis for mixed contaminant exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity. *PLoS ONE* 13: e0205388.
- Campbell J, Clewell H, Cox T, Dourson M, Ethridge S, Forsberg N, Gadagbui B, Hamade A, Naidu R, Pechack N, Peixe TS, Pruitt R, Prussia A, Rachamalla M, Rhomberg L, Smith J, Verma N. (2022). The conundrum of the PFOA human half-life: An international collaboration. *Regulatory Toxicology and Pharmacology*. Submitted. Available from: [https://tera.org/Alliance%20for%20Risk/Projects/PFOA%20Groups/ARA\\_2021\\_PFOA\\_Summary\\_December\\_9.pdf](https://tera.org/Alliance%20for%20Risk/Projects/PFOA%20Groups/ARA_2021_PFOA_Summary_December_9.pdf)
- Checkoway H, Pearce N, Kriebel D. *Research Methods in Occupational Epidemiology*, Second Edition. Oxford University Press, New York, New York. 2004
- Chen MH, Ha EH, Wen TW, Su YN, Lien GW, Chen CY, Chen PC, Hsieh WS. 2012. Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. *PLoS One*, 7(8), e42474.
- Chu C, Zhou Y, Li QQ, Bloom MS, Lin S, Yu YJ, Chen D, Yu HY, Hu LW, Yang BY, Zeng XW, Dong GH. 2020. Are perfluorooctane sulfonate alternatives safer? New insights from a birth cohort study. *Environ Int*, 135, 105365.
- Cook NR, Ridker PM. Further insights into the cardiovascular risk calculator: the role of statins, revascularization, and underascertainment in the Women's Health Study. *JAMA Intern Med*. 174: 11964-1971.

- Dalsager L, Christensen N, Husby S, Kyhl H, Nielsen F, Høst A, Grandjean P, Jensen TK. 2016. Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1–4 years among 359 children in the Odense Child Cohort. *Environ Int*, 96, 58–64.
- Darrow LA, Stein CR, Steenland K. 2013. Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. *Environ Health Perspect*, 121(10), 1207–1213.
- DeFilippis AP, Young R, Carrubba CJ, McEvoy JW, Budoff MJ, Blumenthal RS, Kronmal RA, McClelland RL, Nasir K, Blaha MJ. (2015). An analysis of calibration and discrimination among multiple cardiovascular risk scores in a modern multiethnic cohort. *Ann Intern Med*. 2015; 162:266–75.
- Genser B, Teles CA, Barreto ML, Fischer JE. (2015). Within- and between-group regression for improving the robustness of causal claims in cross-sectional analysis. *Environ Health* 14: 60.
- Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbaek K, Weihe P, Heilmann C. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA* 307: 391-397.
- Granum B, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, van Loveren H, Løvik M, Nygaard UC. 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotoxicol*, 10(4), 373–379.
- Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tommasino J, Forman DE, Goldberg R, Heidenreich PA, Hlatky MA, Jones DW, Lloyd-Jones D, Lopez-Pajares N, Ndumele CE, Orringer CE, Peralta CA, Saseen JJ, Smith SC Jr, Sperling L, Virani SS, Yeboah J. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*. 2019;139:e1082–e1143.
- Impinen A, Nygaard UC, Lødrup Carlsen KC, Mowinckel P, Carlsen KH, Haug LS, Granum B. 2018. Prenatal exposure to perfluoroalkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. *Environ Res*, 160, 518–523.
- Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, Fletcher T. 2014. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. *Toxicol Sci*, 138(1), 76–88.
- Manzano-Salgado CB, Casas M, Lopez-Espinosa MJ, Ballester F, Iñiguez C, Martinez D, Costa O, Santa-Marina L, Pereda-Pereda E, Schettgen T, Sunyer J, Vrijheid M. 2017. Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort. *Environ Int*, 108, 278–284.
- National Research Council. 1992. *Biologic Markers in Immunotoxicology*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/1591>.
- Office of Environmental Health Hazard Assessment (OEHHA), California Environmental Protection Agency. 2021. FIRST PUBLIC REVIEW DRAFT. Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water. July 2021. <https://oehha.ca.gov/water/report/perfluorooctanoic-acid-pfoa-and-perfluorooctane-sulfonic-acid-pfos-drinking-water#FirstPubDraft>

Okada E, Sasaki S, Saijo Y, Washino N, Miyashita C, Kobayashi S, Konishi K, Ito YM, Ito R, Nakata A, Iwasaki Y, Saito K, Nakazawa H, Kishi R. 2012. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. *Environ Res*, 112, 118–125.

Olsen GW, Burris JM, Burlew MM, Mandel JH. 2003. Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. *J Occup Environ Med*, 45(3), 260–270.

Olsen GW, Zobel LR. 2007. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. *Int Arch Occup Environ Health*, 81(2), 231–246.

Raleigh KK, Alexander BH, Olsen GW, Ramachandran G, Morey SZ, Church TR, Logan PW, Scott LL, Allen EM. 2014 Mortality and cancer incidence in ammonium perfluorooctanoate production workers. *Occup Environ Med*, 71(7), 500–506.

Rana JS, Tabada GH, Solomon MD, Lo JC, Jaffe MG, Hee Sung S, Ballantyne CM, Go AS. 2016. Accuracy of the Atherosclerotic Cardiovascular Risk Equation in a large contemporary, multiethnic population. *J American Coll Cardiol* 67:

Rosinger A, Carroll MD, Lacher D, Ogden C. 2017. Trends in total cholesterol, triglycerides, and low-density lipoprotein in US Adults, 1999–2014. *JAMA Cardiol*. 2: 339–341.

Savitz DA, Stein CR, Bartell SM, Elston B, Gong J, Shin HM, Wellenius GA. 2012. Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposed community. *Epidemiology*, 23(3), 386–392.

Savitz DA, Stein CR, Elston B, Wellenius GA, Bartell SM, Shin HM, Vieira VM, Fletcher T. 2012. Relationship of perfluorooctanoic acid exposure to pregnancy outcome based on birth records in the mid-Ohio valley. *Environ Health Perspect*, 120(8), 1201–1207.

Steenland K, Fletcher T, Stein CR, Bartell SM, Darrow L, Lopez-Espinosa MJ, Ryan PB, Savitz DA. 2020. Review: Evolution of evidence on PFOA and health following the assessments of the C8 Science Panel. *Environ Int* 145: 106125. Elsevier Ltd. <https://doi.org/10.1016/j.envint.2020.106125>

Steenland K, Barry V, Savitz D. 2018. Serum Perfluorooctanoic Acid and Birthweight: An Updated Meta-analysis With Bias Analysis. *Epidemiology*. 29:765–776.

Steenland K, Zhao L, Winkquist A. 2015. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). *Occup Environ Med*, 72(5), 373–380.

Steenland K, Woskie S. 2012. Cohort mortality study of workers exposed to perfluorooctanoic acid. *Am J Epidemiol*, 176(10), 909–917.

Stein CR, Savitz DA, Dougan M. 2009. Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. *Am J Epidemiol*, 170(7), 837–846.

United Nations Environment Programme; World Health Organization; International Labour Organisation (1996) International Programme on Chemical Safety (IPCS), World Health Organization. 1996. Environmental Health Criteria 180: Principles and Methods for Assessing Direct Immunotoxicity Associated

with Exposure to Chemicals. World Health Organization: Geneva.

<https://wedocs.unep.org/20.500.11822/29544>

Weihe P, Kato K, Calafat AM, Nielsen F, Wanigatunga AA, Needham LL, Grandjean P. 2008. Serum concentrations of polyfluoroalkyl compounds in Faroese whale meat consumers. *Environ Sci Technol*. 2008 Aug 15;42(16): 6291-5.

Winquist A, Steenland K. 2014. Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. *Environ Health Perspect*, 122(12), 1299–1305.

World Health Organization & International Programme on Chemical Safety. (2012). *Guidance for immunotoxicity risk assessment for chemicals*. World Health Organization.

<https://apps.who.int/iris/handle/10665/330098>

WHO. (2018). *WHO Immunological basis for immunization series module 3: tetanus update 2018*.

Retrieved from <http://www.who.int/immunization/documents/ISBN9789241513616/en/>

WHO. (2017). *Tetanus Vaccines: WHO Position paper – February 2017*. Retrieved from

<https://www.who.int/publications/i/item/tetanus-vaccines-who-position-paper-february-2017>