



February 10, 2022

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**Re: Supplemental Comments on Meeting Materials for Public Meetings of the  
Science Advisory Board Per- and Polyfluoroalkyl Substances (PFAS) Review  
Panel**

The 3M Company (“3M”) writes to follow up on its prior submission of written comments on the meeting materials published in association with the Environmental Protection Agency (“EPA” or the “Agency”) Science Advisory Board’s (“SAB”) public meetings to review data and analysis prepared by EPA as it considers setting Maximum Contaminant Level Goals (“MCLGs”) and National Primary Drinking Water Regulations (“NPDWR”) for Perfluorooctanoic Acid (“PFOA”) and Perfluorooctanesulfonic Acid (“PFOS”).

As noted in its prior submission, 3M is providing these supplemental comments because it was unable to provide the full scope of its technical comments in its December 30, 2021 submission due to the inadequate comment period. This document includes certain of 3M’s comments on some aspects of the meeting materials, specifically toxicokinetic models (human and animal), EPA’s proposed cancer classification for PFOA, potential non-cancer effects such as cardiovascular disease and birth-weight, and EPA’s mixtures framework.<sup>1</sup> 3M is continuing to review the lengthy meeting materials and may provide additional supplemental technical comments on the meeting materials given the limited time provided by SAB. 3M incorporates by reference its prior comments related to the inadequacy of the comment period and further notes that even with the additional time taken to provide these supplemental comments, the opportunity for public review has been wholly insufficient.

Overall, consistent with other commenters, 3M has observed a number of broad, persistent issues that are prevalent across the SAB PFAS Panel’s meeting materials from EPA. These themes are presented here and detailed examples of each are discussed in 3M’s December 30, 2021 submission as well as in the supplemental technical comments below.

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<sup>1</sup> 86 Fed. Reg. 62526 (Nov. 10, 2021).

- **Failure to Comply with Health Risk Assessment Principles, Guidelines, and Policies**

For more than 40 years, EPA has adopted and followed certain health risk assessment and risk management policies as a basis for assessing scientific evidence for public health protection. From 1976 until today, the assessment of potential health risk associated with any agent is essentially a two-step process: (1) assessment of the weight of evidence that a substance can cause health effects, considering all evidence, including human, animal, and mechanistic studies; and (2) on the assumption that the agent can cause harm, describe quantitatively the levels at which harm might be induced (dose response).

All of EPA's draft reports currently under review by the SAB PFAS Panel fall short of this established health risk assessment process. The draft reports do not clearly consider all lines of evidence, both positive and negative studies from human, animal, and mechanistic information, to provide a weight of evidence assessment for each endpoint for which EPA presents a point of departure ("POD"), in a two-step process. EPA's failure to carry out these assessment steps and presentation of results raises concerns that EPA is simply searching for the lowest theoretical POD, without regard for whether the endpoint being assessed poses a real risk.

Also, there has been a typical practice in cancer potency/slope factor development to avoid quantitative assessment when the weight of evidence is weak or where data are too poor on which to base a quantitative assessment. EPA's 2005 cancer guidelines state that "[w]hen there is suggestive evidence, *the Agency generally would not attempt a dose-response assessment*, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." P. 3-1 (emphasis added). Non-cancer health endpoint assessment in EPA's draft reports should adhere to similar approaches.

- **Lack of Clarity and Transparency**

Repeatedly throughout EPA's draft reports, EPA has failed to be clear and transparent in its approach. Numerous examples are described in 3M's initial and supplemental submissions, as well as by many other commenters and by SAB Panel members themselves.<sup>2,3</sup>

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<sup>2</sup> 3M's initial and supplemental submissions and those of other commenters may be found on the SAB website at: [https://sab.epa.gov/ords/sab/f?p=100:19:5099513780240::RP,19:P19\\_ID:963#materials](https://sab.epa.gov/ords/sab/f?p=100:19:5099513780240::RP,19:P19_ID:963#materials).

<sup>3</sup> *Revised and Preliminary Individual Comments SAB PFAS Review Panel*. January 24, 2022, [https://sab.epa.gov/ords/sab/apex\\_util.get\\_blob?s=31551561024923&a=100&c=5659346460770746&p=19&k1=5934&k2=&ck=eQOnA\\_wpq7AMDmRHHV6wbKbqVujmVHVZrA3Z1rldaBKMZxSP361uDnP-xKmDgE\\_4EeXDyt0SKANMAGPvXzL9okw&rt=IR](https://sab.epa.gov/ords/sab/apex_util.get_blob?s=31551561024923&a=100&c=5659346460770746&p=19&k1=5934&k2=&ck=eQOnA_wpq7AMDmRHHV6wbKbqVujmVHVZrA3Z1rldaBKMZxSP361uDnP-xKmDgE_4EeXDyt0SKANMAGPvXzL9okw&rt=IR).

In the SAB Charge questions, EPA asked the SAB Panel whether there is agreement with specific labels for both carcinogen and non-carcinogen evidence but no definitions for the non-carcinogen classification are provided. The draft MCLG Documents do not clearly define strong versus suggestive or any weaker weights of evidence.<sup>4</sup> In its health assessments, EPA uses various terms such as “association,” “impact,” and “effect” somewhat interchangeably and inconsistently, which creates confusion and hampers the ability to judge the different conclusions.

- **Inconsistencies in Analysis and Approach**

EPA’s draft reports include a number of significant and unexplained inconsistencies. For example, EPA identified cholesterol as a critical endpoint despite noting that verification of cardiovascular disease is negative in human studies. Nonetheless, EPA’s documents call for a benefit analysis of cardiovascular disease cases avoided by prescribed reduction in PFAS exposure. This is but one example of circular and inconsistent analyses that plague the draft reports.

As mentioned above, EPA should also ensure it uses consistent, well-defined protocols for non-cancer weight of evidence characterization. Only where there is clear evidence, and where studies of sufficient quality are available, should EPA proceed to the next step of evaluating protective quantitative toxicity levels from a POD. Selection of studies for PODs should be based on the strength of and confidence in the potential hazard, not just the availability of studies that are amenable to dose-response (and vice versa) or provide the lowest POD.

We also note, for POD selection (notwithstanding the quality of the studies involved) EPA mixes clinically relevant disease endpoints (liver necrosis) with changes in response, biomarker levels of potential exposure but not of effect. The latter include candidate PODs based on vaccine response, not on infections, birth-weight, but not for later life problems arising from thyroid hormone levels, but not thyroid disease, and increased total cholesterol, but not CVD. 3M’s December 30, 2021 comments, as well as those included here, discuss EPA’s use of PODs that are derived for elevated cholesterol, and antibody response to vaccines. Attention should be focused on the evidence for the actual endpoints to provide support to any POD derivations based on changes in clinical biomarkers of exposure.

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<sup>4</sup> The charge question does not provide a term for weaker than suggestive evidence: “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?”

- **Consideration of All Relevant Evidence**

EPA's draft reports do not consistently use or evaluate all relevant published studies, nor do they explain why certain studies were not included in its analysis.

- **Failure to Identify Relationship Between Current and Prior Assessments**

EPA's draft reports represent a significant departure from its 2016 health risk assessments of PFOA and PFOS. Consistent with past practice, EPA should present the relationship of the current assessment of evidence and quantification recommendations to its earlier 2016 health risk assessments. EPA should describe the basis for reaching different conclusions in the current draft reports, particularly focusing on differing interpretations and weight given to what are in most cases essentially the same sets of studies as in 2016 and explain the differing approaches that have led to the significant changes in EPA's current draft reports.

3M encourages SAB to consider the information presented in its December 30, 2021 submission and the comments below when providing EPA with SAB's technical input on the meeting materials. As indicated in 3M's December 30, 2021 submission, EPA's approach is deeply scientifically flawed, substitutes non-scientific judgments for science, and employs unprecedented approaches to reach an illogical outcome. SAB should make these technical deficiencies clear to EPA in its response and should recommend that the Agency use scientifically sound approaches in considering these important regulatory levels and meaningfully engage relevant stakeholders in any future actions.

## **SUPPLEMENTAL TECHNICAL COMMENTS**

Given the extremely limited comment period and the complex nature of the meeting materials published by EPA, 3M supplements it previously submitted comments with the comments below. These comments address EPA's Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid ("PFOA) in Drinking Water, and EPA's Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanesulfonic Acid ("PFOS") in Drinking Water (collectively, the "Draft MCLG Documents"), as well as EPA's Analysis of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water ("CVD Risk Analysis"), and EPA's Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of PFAS ("Mixtures Framework").

### **I. SUPPLEMENTAL COMMENTS ON DRAFT MCLG DOCUMENTS**

#### **A. Toxicokinetic Models**

##### **1. Human Modeling: Fetal to maternal partitioning and partitioning to breastmilk**

In the charge questions posed to the SAB, the PFAS Panel was asked to "comment on the choice to assume that fetal to maternal partitioning and partitioning to breastmilk did not vary in time [and] describe whether there are other methods you would recommend to account for these changes over time and across development."<sup>5</sup> 3M believes that it is imperative that SAB provide input on EPA's assumption that the partitioning does not vary over time and must ask the Agency to look at the timing of collection of samples across available literature as well as whether or not a constant partitioning is consistent with the data on cord blood, milk, and maternal and infant serum samples that were analyzed at different times during gestation. In addition, the SAB should provide input on the duration of breastfeeding that EPA assumed. Finally, SAB should encourage EPA to evaluate and discuss whether there are other modeling approaches that are more fit for this purpose than a constant dose, including whether a drinking water concentration is more appropriate.

In particular, the SAB should focus EPA's attention on data in the scientific literature on the volume/amount of breastmilk that is typically consumed by an infant during lactation and the average duration for breastfeeding in the US. In fact, the 2011 US EPA Exposure Factors Handbook (chapter 15) has an entire section on human milk intake.<sup>6</sup> This shows that as a child grows the volume/amount consumed per kg of body weight decreases over time. EPA's analysis does not account for this fact, nor does it adequately describe why it was discounted. Likewise, EPA assumed that breastfeeding lasted 1 year, and that weaning was an immediate process (i.e., EPA assumed the child's sole diet for 1 year was breastmilk and then immediately stopped). The SAB should suggest that EPA incorporate data on the decrease in consumption relative to body weight and weaning to better reflect actual potential exposure. As the SAB's PFAS Panel itself

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<sup>5</sup> Toxicokinetic Models, Charge Question 1.C.

<sup>6</sup> <https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252>

recognized during its public meetings, EPA failed to adequately discuss its the calculation of early life stage exposures and the uncertainty with EPA's approach.

Failure to consider these factors is an important gap in the EPA analysis and is something the SAB should recommend EPA correct.

## **2. Animal Modeling: EPA's selection of the Wambaugh et al. (2013) model**

SAB should recommend that EPA clarify why it believes a single compartment model is a better choice than one of the available PBPK models. As the PFAS Panel itself noted in response to charge questions<sup>7</sup> related to use of the Wambaugh et al. (2013) model, EPA's discussion in the Draft MCLG Documents is cursory at best and does not provide the necessary detail to allow adequate public input. The Draft MCLG Documents state:

"Typically, PBPK models are preferred because they can provide individual tissue information and have a one-to-one correspondence with the biological system which can be used to incorporate additional features of PK including tissue specific dosimetry and local metabolism. In addition, though PBPK models present a great increase in complexity, many of the additional parameters are chemical-independent and have widely accepted values. The decision to not use one of the PBPK models for PFOA/PFOS was motivated in part by previous issues identified when evaluating the application of PBPK models to other PFAS compounds for the purpose of risk assessment.... However, while these errors usually don't substantially alter the results of the model, correction of the free-fraction error was judged to result in a significant impact which could not be easily resolved" (EPA Document No. 822D21001, p. 332; EPA Document No. 822D21002, p. 303).

The Agency does not explain what this error is and why this could not be "easily resolved." The PFAS Panel should recommend that EPA use a PBPK model (which EPA acknowledges is generally a better approach) and explicitly state why it finds that the "error" could not be easily resolved. With sufficient time, outside commenters could conduct these modeling efforts and provide assistance to EPA in an effort to fully understand the differences and consequences of model choice. No final decisions should be made before taking account of these flaws.

## **3. Animal Modeling: EPA incorrectly assumed no sex differences in clearance in neonatal animals**

In response to charge questions posed to SAB regarding the validity of EPA's assumption that there were no sex differences in clearance in neonatal animals<sup>8</sup>, SAB should recommend that EPA review Hinderliter et al. 2006, where the study authors looked at PFOA clearance in post-weaning rats. This study demonstrates that contrary to EPA's assumption, there are some sex-

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<sup>7</sup> Toxicokinetic Models, Charge Question 2.A.

<sup>8</sup> Toxicokinetic Models, Charge Question 2.C.

and age-dependent differences, at least for PFOA in rats. The PFAS Panel should recommend that EPA should modify their models accordingly. At a minimum SAB should recommend that EPA more adequately describe the uncertainty associated with its assumptions.

#### **4. Animal Modeling: Transfer of chemical from the mother to her pup and from the mother to the fetus**

It is unclear whether the parameters EPA used are appropriate (both the values and whether more description is needed—i.e., is the description of maternal-pup transfer sufficient). It is also unclear on what literature EPA relied (for the cord blood: maternal serum ratio, apparently EPA used only reports where the ratio was actually reported in the study. It is unclear how many measurements EPA actually used and how many were disregarded. In this regard, the Agency assessment lacks transparency. The gaps in the Agency assessment could be filled by outside experts and results submitted to assist the Agency unless the Agency undertakes this effort itself. Either way, the PFAS Panel should recommend that EPA clearly disclose the parameters, measurements, and literature it relied on in reaching its conclusions. EPA should be encouraged to engage with knowledgeable stakeholders to help ensure a transparent, scientifically, valid approach.

#### **B. RfD Derivation: Decreased Birthweight is not a Causal Effect of PFOA and PFOS**

EPA identifies decreased human birth-weight as a candidate critical effect for development of RfDs for both PFOA and PFOS.<sup>9</sup> In doing so, EPA ignores the foundational problem that decreased birth weight is not established as a causal effect of PFOA or PFOS. Again, without a known causal link to a given health outcome, an RfD will not serve its purpose of protecting against the risk of that health outcome. SAB should recommend that EPA reevaluate this RfD analysis.

For PFOA:

EPA uses equivocal language in summarizing the epidemiology of PFOA and fetal growth, concluding that “there is *suggestive* evidence that PFOA *may* impact fetal growth restriction across a variety of [birthweight]-related measures” (EPA Document No. 822D21001, p. 100; emphasis added). EPA acknowledges that there is even “less consistent evidence” of an effect of PFOA on postnatal growth (EPA Document No. 822D21001, p. 100), and that “evidence for any association with PFOA and metabolic outcomes,” including body size in childhood or adulthood, is “inconsistent” (EPA Document No. 822D21001, p. 240). Regarding fetal growth and other developmental outcomes, EPA adds: “Collectively, across these various endpoints there is *moderate* evidence of developmental effects related to PFOA based on the more recent epidemiological literature. However, as noted previously there is some uncertainty as to what degree the evidence may be impacted by pregnancy hemodynamics and sample

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<sup>9</sup> Reduced birth weight is one of only two health effect endpoints selected by EPA for candidate RfDs, the other being vaccine response. (See Table 23 of the Draft MCLG Documents).

timing differences across studies as this may result in either confounding or reverse causality {Steenland, 2018, 5079861}. Additional uncertainty exists due to the potential for confounding by other PFAS” (EPA Document No. 822D21001, pp. 100–101; emphasis added).

For PFOS:

EPA’s language regarding the epidemiology of PFOS and fetal growth is similarly ambivalent: “As noted in the epidemiological fetal growth restriction summary, there is *suggestive* evidence that PFOS may impact fetal growth restriction in humans” (EPA Document No. 822D21002, p. 90; emphasis original). Regarding postnatal growth, EPA identifies “inconsistent evidence of PFOS impacts” (EPA Document No. 822D21002, p. 90), and the Agency notes that for endpoints such as body size after early childhood, “evidence for any association with PFOS and metabolic outcomes was inconsistent” (EPA Document No. 822D21002, p. 230). The following language regarding associations between PFOS and developmental outcomes, such as fetal growth, is nearly identical to that used for PFOA: “Collectively across the various endpoints outlined in the human epidemiological sections, there is *moderate* evidence of developmental effects related to PFOS based on the more recent epidemiological literature. As noted previously there is some uncertainty as to what degree the available evidence may be impacted by pregnancy hemodynamic and sample timing differences across studies, as this may result in either confounding or reverse causality {Steenland, 2018, 5079861}. Additional uncertainty exists due to the potential for confounding by other PFAS” (EPA Document No. 822D21002, p. 90; emphasis original).

In response to the charge question posed to it about approaches to addressing potential confounding, as mentioned during the public meeting, SAB should recommend that EPA review or conduct a meta-analysis of the literature relating to birthweight effects. Conducting such an analysis of certain bodies of literature but not others is not scientifically sound. Such an analysis should consider that the interpretation of associations between PFAS and measures of fetal growth is complicated because these associations are susceptible to confounding by maternal physiological mechanisms, such as glomerular filtration rate (“GFR”), (i.e., the flow rate of fluid being filtrated by the kidneys), glucose metabolism, and plasma volume expansion, as well as maternal nutrition, which can produce spurious, non-causal associations with fetal growth (Savitz 2007, Morken et al. 2014, Verner et al. 2015). Such relationships with shared physiological mechanisms can distort results even in studies with prospective exposure assessment; that is, the bias is not limited to cross-sectional or retrospective studies. In particular, circulating PFAS levels are dependent on GFR, since these chemicals are eliminated by the kidneys (Han et al. 2012). GFR generally increases by about 50% during the first half of pregnancy and declines slightly during the second half of pregnancy, and insufficient GFR during pregnancy has been shown to be associated with poorer fetal growth (Verner et al. 2015). Lower maternal GFR may also contribute to greater placental transfer of PFAS (Pan et al. 2017). Thus, maternal GFR can be responsible for a spurious association between higher fetal PFAS exposure and impaired fetal growth. Maternal plasma volume also typically expands during pregnancy, and greater plasma volume expansion is associated with lower circulating PFAS



levels and lower risk of fetal growth restriction (Salas et al. 1993, Salas et al. 2006), again biasing the observed association toward an association between PFAS and poorer fetal growth.

Few epidemiological studies of fetal growth adjusted for maternal estimated GFR or plasma volume expansion, leaving nearly all of the results susceptible to confounding by these physiological factors. The expected bias toward an association between higher PFAS levels and lower fetal growth would be magnified in studies that measured maternal PFAS levels later in pregnancy (Verner et al. 2015, Steenland et al. 2018b). One study that reanalyzed the association between PFOA exposure and birth weight in a prospective Danish cohort found that the observed association was attenuated by 66% after adjustment for maternal eGFR in the second trimester of pregnancy (Morken et al. 2014). Four other studies (including two from the same cohort) did not detect a strong positive confounding effect of maternal eGFR estimated in the first trimester of pregnancy (Manzano-Salgado et al. 2017a, Rokoff et al. 2018, Sagiv et al. 2018, Costa et al. 2019), and another did not observe substantial confounding by maternal eGFR estimated three weeks after delivery (Gyllenhammar et al. 2018). However, these authors and others acknowledged that measurement of PFAS and estimation of eGFR at other times, especially later during pregnancy, might reveal a greater impact of confounding. Additionally, the single measurement of PFAS used in nearly all studies, and the variability across studies in the timing of exposure assessment, limits the ability of these studies collectively to capture the true relationship between PFAS exposure during gestation and fetal growth.

While EPA acknowledges in the Draft MCLG Documents the potential confounding between the timing of the maternal blood sampling and its role in the inverse associations with birth weight and measured maternal serum PFOS/PFOA concentrations, EPA needs to justify why it did not consider Sagiv et al. 2018, which attempted to reduce confounding bias due to pregnancy hemodynamics by measuring maternal PFOS and PFOA only during the 1<sup>st</sup> trimester, in keeping with its own assessment (highlighted above) as well as the recommendations of Steenland et al. (2018) and Dzierlenga et al. (2020). On a more extensive scale and as noted above, 3M recommends EPA conduct a meta-analysis of all studies, including those that met EPA's systematic review of *high quality* epidemiologic studies that measured maternal serum PFOS and PFOA concentrations only during the first trimester (to minimize pregnancy hemodynamic bias) in its modelling assessment on the clinical outcome of low birthweight which has not been shown to be an adverse health outcome associated with maternal PFOS or PFOA measurements determined in the individual epidemiologic studies that have been published to date.

Another consideration EPA failed to address is that GFR varies through the day and can be affected by age, sex, diet, timing of a meal, medication use, and other factors. eGFR is an approximation of actual kidney function, derived from regression equations that in some cases were developed on small numbers of subjects using broad assumptions (Stevens et al. 2008, Soares et al. 2009, Fesler and Mimran 2011). Therefore, it is well known that the accuracy of eGFR as an estimate of kidney function varies by person, and adjustment for eGFR therefore may not sufficiently account for individual-level kidney function.

Likewise EPA does not address the separate issue relating to control for confounding by gestational age or preterm birth. Although the appropriateness of adjusting for gestational age

has been debated (Wilcox et al. 2011), the majority of studies of PFAS and fetal growth adjusted for gestational age through statistical adjustment, standardization of fetal growth measures by gestational age, or restriction of analyses to full-term births, sometimes in secondary analyses. Several other studies, however, did not report any results adjusted for gestational age, thus failing to address concerns about confounding by this strong determinant of fetal growth. At a minimum, SAB should recommend that EPA discuss why it did not need to consider this issue.

A 2018 meta-analysis of 24 studies that EPA should review, co-authored by two of the three members of the C8 Science Panel, found no significant association between PFOA and birth weight after restricting the analysis to studies where PFOA was measured early in pregnancy or shortly before conception, when pregnancy-related changes in maternal GFR and plasma volume expansion (in women with otherwise healthy kidney function) would have little influence (Steenland et al. 2018b). By contrast, a significant inverse association was found in studies where blood sampling was conducted late in pregnancy, when the confounding impact of maternal GFR would be greater. Moreover, inclusion of a large study that used estimated instead of measured serum PFOA levels in Mid-Ohio Valley pregnant women (Savitz et al. 2012b) led to a statistically non-significant association in the combined analysis of all 24 studies. The authors concluded: “Present human evidence provides only modest support for decreased birthweight with increasing PFOA. Studies with a wide range of exposure, and studies with blood sampled early in pregnancy, showed little or no association of PFOA with birthweight. These are studies in which confounding and reverse causality would be of less concern” (Steenland et al. 2018b).

Similarly, SAB should encourage EPA to review Dzierlenga et al. (2020) where the authors conducted a meta-analysis on maternal serum PFOS concentrations and birth weight and their findings were consistent with the conclusion offered by Steenland et al. (2018) on maternal serum PFOA and birth weight. Dzierlenga et al. (2020) conducted a meta-analysis of 29 published studies and reported the random effects summary was  $-3.22$  g/ng/mL PFOS (95% confidence interval [CI] =  $-5.11, -1.33$ ). In a subgroup analysis stratified by when in pregnancy the PFOS concentration was measured, the summary for the “Early” group was  $-1.35$  g/ng/mL PFOS (95% CI =  $-2.33, -0.37$ ) and for the “Later” group was  $-7.17$  g/ng/mL PFOS (95% CI =  $-10.93, -3.41$ ). “Early” group included prepregnancy, first trimester, or first and second trimester; and “Later” group included second trimester, third trimester, second and third trimester combined, or cord blood). In a meta-regression model including a term for timing of blood draw, the intercept was slightly positive but essentially zero ( $0.59$  g/ng/mL, 95% CI =  $-1.94, 3.11$ ). Thus, the model indicated that when blood was drawn at the very beginning of pregnancy, there was no relation of birth weight to maternal serum PFOS. Dzierlenga et al. (2020) concluded the evidence was weakly or not supportive of an association between a reduction in birth weight and maternal serum PFOS concentrations.

The essential message from these meta-analyses indicates physiological aspects of pregnancy, including plasma volume expansion, its role in maternal GFR, and the timing when the maternal PFOS/PFOA measurements are made during pregnancy, are critical points to evaluate in the associations between birth weight and maternal serum PFOA and PFOS concentrations. Both Steenland et al. (2018) and Dzierlenga et al. (2020) concluded the least amount of confounding bias, as a consequence of pregnancy hemodynamics, would require the

examination of the association between birth weight and that of the maternal serum PFOS/PFOA measured early in the pregnancy.

Following the publication of the Steenland et al. (2018) meta-analysis, the C8 Science Panel and co-authors reviewed an additional 12 studies of PFOA and birthweight, and found that “[m]ore recent studies continued to generate mixed findings, some suggesting a reduced birthweight associated with elevated PFOA and others not finding evidence for such an effect” (Steenland et al. 2020). Again they cautioned that “[r]everse causality or confounding would be most likely to affect studies with low exposure contrasts,” and that “studies with PFOA measurement later in pregnancy show stronger associations with birth weight than those with measurements earlier in pregnancy, consistent with the possibility that the overall association is distorted by the magnitude of plasma blood volume expansion and glomerular filtration rate” (Steenland et al. 2020). With respect to preterm birth, the authors stated that there were “few studies,” and that “[w]hat studies do exist provide little indication of an adverse effect of PFOA” (Steenland et al. 2020).

### **C. EPA’s Proposed Cancer Classification of PFOA is Not Supported by Human Epidemiological Evidence**

SAB should recommend that EPA reevaluate its proposed cancer classification for PFOA. As discussed below, EPA does not adequately describe the rationale for its designation and the support identified is not adequate. EPA’s failure to provide this information leaves the public in the dark on its analysis and unable to provide appropriately thorough input.

EPA reaches the conclusion that “PFOA is considered *Likely to Be Carcinogenic to Humans*” “based on the evidence of kidney and testicular cancer in humans,” combined with tumor studies in rats (EPA Document No. 822D21001, p. 344). In its summary of the available epidemiologic evidence on PFOA and cancer, EPA notes that “one human epidemiological study identified since the 2016 assessment adds support to the previous evidence of an association between PFOA and kidney cancer (Shearer, 2021, 7161466). No new epidemiological studies on testicular cancer were identified” (EPA Document No. 822D21001, p. 315). Thus, EPA appears to have relied entirely on the results of Shearer et al. (2021) to advance the state of the epidemiologic evidence past its status in 2016, when EPA concluded that “*there is suggestive evidence of carcinogenic potential of PFOA in humans*” (EPA 822-R-16-003, p. 3-159; emphasis original).

Such heavy reliance on Shearer et al. (2021), however, is misplaced. Shearer et al. (2021) is a nested case-control study of 324 renal cell carcinoma cases and 324 matched controls identified from a cohort of approximately 148,000 U.S. adults aged 55–74 years participating in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. PFOA exposure was classified based on a single prediagnostic serum sample collected at study enrollment in 1993–2001, an average of 8.8 years (range: 2–18 years) prior to case diagnosis. Contrasts in PFOA levels in this study cohort were modest – comparing a top quartile of >7.3 µg/L PFOA to a lowest quartile of <4.0 µg/L PFOA - and substantially smaller than exposure contrasts in more highly exposed populations that showed no significant difference in kidney cancer risk.

Although Shearer et al. (2021) reported positive associates between PFOA and risk of renal cell carcinoma, the results are undermined by the study's reliance on PFAS exposure measured at a single point in time less than a decade prior to cancer diagnosis among cases; and the insufficient adjustment for confounding by key risk factors including smoking history (classified as never, former, or current), hypertension history (classified as no or yes), and body mass index (classified using standard cutoffs for underweight, normal weight, overweight, or obese and above).

In this matched case-control study, according to Shearer et al, the category cut points were assigned based on quartiles of serum concentrations of each PFAS among controls. By standard definition the odds ratio of the least exposed category (referent) is set at 1.0. However, there were only 47 cases in this reference group with the least exposure to PFOA (< 4.0 ng/mL). This distribution seems rather odd where there are 81 controls and only 47 cases in the referent group. One would expect more similar distribution among the least exposed. Neither Shearer et al. nor EPA commented on this referent group which becomes the main driver in the subsequent or calculations for the other 3 exposure categories.

Besides Shearer et al. (2021), most of the remaining body of scientific literature indicates no association between PFOA exposure and kidney cancer risk. No significant association or exposure-response trend was observed between PFOA exposure and kidney cancer mortality (Lundin et al. 2009, Raleigh et al. 2014) or incidence (Raleigh et al. 2014) among highly exposed workers at the 3M chemical plant in Cottage Grove, Minnesota. The initial study of workers at the DuPont chemical plant in Parkersburg, West Virginia, also found no significant association between PFOA exposure and kidney cancer mortality (Leonard et al. 2008). The only study of highly PFOA-exposed workers that found a positive association with kidney cancer was an updated cohort mortality study of DuPont plant employees (Steenland and Woskie 2012). Furthermore, there were no new or additional kidney cancer deaths identified in the study by Steenland and Woskie (2012) because the prior study by Leonard et al. (2008)<sup>10</sup> on the same population had already identified these 12 kidney cancer deaths by the year 2002.

EPA also did not mention that the second highest exposure category in Steenland and Woskie study had zero kidney cancer deaths (SMR = 0.0; 95% CI 0.0 – 1.48). Combining the upper two exposure categories, Raleigh et al. reported an HR for kidney cancer of 0.85 (95% CI 0.36 – 2.06). Steenland and Woskie did not report the combined upper two quartiles of exposure for an SMR but it can be readily calculated from Table 1 of the Steenland and Woskie study. There was a total of 9.4 expected deaths for all quartiles combined. These calculations can then be made for the 1<sup>st</sup>, 2<sup>nd</sup>, and 4<sup>th</sup> quartiles which resulted in approximately 0.9, 2.2, and 3.0 expected deaths which yields 3.3 expected deaths occurring in the 3<sup>rd</sup> quartile (compared to the 0 observed deaths). Therefore, combining the upper two quartiles in Steenland and Woskie, there were then 8 observed kidney cancer deaths and approximately 6.3 expected deaths (SMR = 1.27; 95% CI 0.39 – 1.76) for estimated cumulative exposure of PFOA ≥ 1500 ng/mL-years. Thus, there appears to be no substantial differences between estimates of the magnitude of risk between the upper two exposure categories (albeit different measurements of exposure) in Raleigh et al. study for kidney cancer incidence and Steenland and Woskie study for kidney cancer mortality.

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<sup>10</sup> Leonard et al. 2008 Ann Epidemiol 18 15-22

A reasonable question for the EPA to have considered is why were there no observed kidney cancer deaths in the second highest exposure category in Steenland and Woskie. Was it chance or could there have been some degree of exposure misclassification? Given the fact there were 8 kidney cancer deaths in this 4<sup>th</sup> quartile, three of these deaths would have had to been misclassified from the 3<sup>rd</sup> quartile to make the SMR estimate for the 4<sup>th</sup> quartile not statistically significant.

Inconsistent findings were reported in studies of the Mid-Ohio Valley population where no significant association or exposure-response trend was observed between PFOA exposure and kidney cancer incidence in analyses restricted to workers or combining community members and workers (Barry et al. 2013). In addition, an apparent non-monotonic trend was found between estimated serum PFOA and kidney cancer incidence in a semi-ecologic geographic analysis (Vieira et al. 2013). Thus, among three occupational analyses (Raleigh et al. 2014; Barry et al. 2013; and Steenland and Woskie et al. 2012), which likely represent the highest exposed individuals based on overall reported biomonitoring data, only one analysis showed a statistically significant association with kidney cancer. However, that association was not seen when the two highest exposure categories were used. And there remains the confusing possibility of overlapping of kidney cancer cases between Steenland and Woskie (2012)<sup>11</sup>, Vieira et al. (2013)<sup>12</sup>, and Barry et al. (2013). This was acknowledged by Steenland and Winquist (2021)<sup>13</sup> but they did not provide any insights as to the percentage. And the Shearer et al. (2021) single serum PFOA concentrations measured at general population levels are inconsistent with the other 4 studies. Even though an excess of kidney cancer incidence (which, unlike mortality, is not influenced by non-causal prognostic factors) was found among residents of Ronneby, Sweden, who had elevated drinking water exposure to primary to PFOS and PFHxS, and to a much less degree of PFOA, however, this was not based on measured serum PFOA levels amongst those who were diagnosed with kidney cancer in this timeframe study by Li et al. (2022).

Taken together, epidemiological findings on PFOA exposure and kidney cancer are inconsistent: notably, no excess risk was detected among highly exposed workers in several studies (Leonard et al. 2008, Lundin et al. 2009, Barry et al. 2013, Raleigh et al. 2014). The positive association in Shearer et al. (2021) is therefore unexpected, especially given that the study population consisted of adults with background-level exposure to PFOA (CDC 2021), orders of magnitude below that among occupationally exposed workers. Moreover, several established risk factors for kidney cancer, such as cigarette smoking, overweight/obesity, hypertension, and chronic kidney disease, were not controlled for in the Mid-Ohio Valley/Parkersburg studies, and several studies classified PFOA exposure imprecisely, thereby limiting the ability to draw firm causal conclusions based on these results.

In light of the methodological limitations of Shearer et al. (2021), the inconsistent findings across the epidemiological literature as a whole, and the biological implausibility of an effect of background PFOA exposure but not high-level occupational PFOA exposure on kidney cancer, up-classification of the potential human carcinogenicity of PFOA from “suggestive” to “likely” is not scientifically justified.

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<sup>11</sup> Steenland and Woskie 2012 *Am J Epidemiol* 176 909-917

<sup>12</sup> Vieira et al. 2013 *Environ Health Perspect* 121 318-323

<sup>13</sup> Steenland and Winquist 2021 *Environ Res* 194 110690

#### **D. The Proposed Cancer Classification of PFOA is Not Supported by Animal Evidence**

SAB should recommend that EPA undertake a more in-depth weight of evidence analysis for carcinogenicity given the lack of concordance between human epidemiological data and the animal carcinogenicity studies for PFOA and questions about relevance of the primarily benign neoplasms identified in the animal studies. EPA concluded that PFOA is *Likely to Be Carcinogenic to Humans* based on evidence of kidney and testicular cancer in humans and testicular Leydig cell tumors (LCTs), pancreatic acinar cell tumors (PACTs), and hepatocellular adenomas in rats. This conclusion is noteworthy both because 1) the three available rat carcinogenicity studies have identified tumor types of questionable relevance to humans and that, by and large, do not progress to carcinomas despite lifetime exposures; and 2) evidence for the tumors with presumptive evidence in humans is not replicated in the animal toxicology studies (LCT are rarely observed in the category of human testicular cancer as well as an absence of kidney tumors in rats related to PFOA treatment).

The three lifetime exposure carcinogenicity studies for PFOA (Biegel et al., 2001; Butenhoff, Kennedy, et al., 2012; NTP, 2020) between them identified three types of neoplasms, LCT, PACT, and hepatocellular, primarily in males and almost exclusively benign adenomas. In the first two studies there was not a statistically significant increase in malignant carcinomas in any of these tissues (liver, testes, pancreas) in rats fed diets with 30 ppm (Butenhoff, Kennedy, et al., 2012) or 300 ppm ammonium perfluorooctanoate (AFPO; the ammonium salt of PFOA) (Biegel et al., 2001; Butenhoff, Kennedy, et al., 2012). In fact, the only carcinoma of any type in these tissues reported by Biegel et al. (2001) was a pancreatic acinar cell tumor in a single animal; the statistically significant increase in liver, Leydig cell, and pancreatic acinar cell tumor incidence in this study was wholly attributable to an increase in benign adenomas. Butenhoff et al. (2012) did report liver carcinomas in 5 of 50 male rats fed 300 ppm AFPO, but this was not statistically different than the 3 of 49 animals in the control group with liver carcinomas; liver tumor incidence, benign or malignant, was not increased in any dose for males or females in Butenhoff et al. (2012). Butenhoff et al. (2012) also reported that a pathology review of pancreatic tissues conducted after the original study report using updated diagnostic criteria did identify a slight increase in acinar cell hyperplasia in the 300 ppm dose group, but not adenoma or carcinoma.

The more recent study (NTP, 2020) included two lifetime carcinogenicity studies. In Study 1, time-mated female Hsd:Sprague Dawley® SD® rats were fed diets with 0, 150, or 300 ppm during gestation and lactation, then F1 males were fed diets with 0, 150, or 300 ppm, resulting in perinatal/postweaning exposures of 0/0, 0/150, 0/300, 150/150, and 300/300. Postweaning females were provided diets with a higher dose level (0, 300, or 1000 ppm) because of the faster PFOA excretion rate in female rats compared to males. Due to unanticipated toxicity in male rats, Study 1 was discontinued at week 21 for males and a second study (Study 2) evaluating only males using only perinatal dose levels of 0 and 300 ppm and lower postweaning dose levels (0, 20, 40, and 80 ppm). Therefore, tumor results for females are from Study 1 and for males from Study 2.

There was a statistically significant increase in hepatocellular adenoma 0/40, 0/80, and 300/80 ppm, but not carcinoma in male rats. The rate of combined adenoma or carcinoma was also increased at these dose levels, but that was predominantly driven by adenomas. In female rats, there was not a statistically significant increase in hepatocellular adenoma, carcinoma, or combined adenoma or carcinoma, at any dose up to the high dose of 300/1000 ppm. A similar pattern occurred with PACT, wherein males were more sensitive and neither sex had an increased rate of adenocarcinoma. Male rats had an increased rate of pancreatic acinar cell adenoma at all dose levels, though not clearly demonstrating a dose-response relationship, but not pancreatic adenocarcinomas at any dose. Females had no statistically significant increase in adenomas, carcinomas, or combined adenomas or carcinomas at any dose. Unlike Biegel et al. (2001) and Butenhoff et al. (2012), NTP (2020) did not report an increase in Leydig cell adenomas at any dose. Additionally, it is worth noting, these three bioassays used different rat stocks for the evaluation. Sprague Dawley rats are outbred in that they are characterized by heterozygosity. Therefore, it is inappropriate to consider outbred rat “stocks” to be genetically distinct rat “strains.” The rat stock used by the NTP was Hsd:Sprague Dawley® SD®; and the rat stocks used by Butenhoff et al. and Biegel et al. were Crl:COBS CD(SD)BR and Crl:CD BR (CD), respectively. 3M has consulted with two expert laboratory veterinarians at the Charles River Laboratories (which has the largest animal breeding programs in the world) for the technical definition between a rat stock vs. strain. They validated our concern that it is scientifically inappropriate to consider different rat “stocks” as equivalent to different rat “strains”. Therefore, these independent bioassays were conducted spanning across approximately 40 years and they collectively demonstrated consistent neoplastic findings in Sprague Dawley rats with chronic dietary exposure to PFOA. The newly released study by the NTP did *not* report any additional neoplastic findings in the rats with chronic dietary exposure to PFOA compared to the previous studies. In addition, there was no early age of onset associated with PFOA exposures, as the NTP study confirmed that additional *in utero* exposure to PFOA did *not* potentiate the neoplastic response.

Of note, EPA reported increased rates of the three tumor types in the PFOA MCLG document, focusing almost exclusively on positive results, and without providing clear delineation of results for benign adenomas vs. malignant carcinomas. However, this approach misses the opportunity to fully evaluate consistency across studies, where the negative results are equally as important as the positive results, and the level of evidence for progression to a carcinoma. The simple presentation in the table below demonstrates a lack of consistency or progression that requires additional discussion to fully evaluate the weight of evidence.

**Statistically significant increased rates of adenomas and carcinomas reported in animal carcinogenicity studies**

	Leydig Cell		Pancreatic Acinar Cell		Hepatocellular	
	Adenoma	Carcinoma	Adenoma	Carcinoma	Adenoma	Carcinoma
<b>Biegel et al (2001)</b>	Yes	No	Yes	No	Yes	No
<b>Butenhoff et al. (2012)</b>	Yes	No	No	No	No	No
<b>NTP (2020)</b>	No	No	Yes	No	Yes	No

Adenomas are benign growths arising from glandular epithelial tissue. Although adenomas are not cancerous, they may over time become malignant tumors and as such are considered precancerous and potentially adverse. However, taken in the context of studies in which adenomas did not progress to carcinomas in rats fed very high doses of PFOA over a lifetime, it is apparent that PFOA demonstrated little if any carcinogenic potential. Public health agencies do consider benign tumors in evaluating carcinogenic potential, but sufficient evidence of carcinogenicity requires inducement of malignant tumors as well (EPA, 2005; NTP, 2015). For example, NTP (2015) states that “Sufficient evidence of carcinogenicity from studies in experimental animals” requires “[a]n increased incidence of malignant and/or a combination of malignant and benign tumors.” NTP (2015) states specifically that “[t]he spectrum of neoplastic response, from pre-neoplastic lesions and benign tumors to malignant neoplasms of a specific tumor type is relevant for the evaluation of whether increases in benign tumors are likely to progress to malignancy.” U.S. EPA (2005) states: “Observation of only benign neoplasia may or may not have significance for evaluation under these cancer guidelines. Benign tumors that are not observed to progress to malignancy are assessed on a case-by-case basis.” The PFOA Draft MCLG Document provided no context or nuance regarding the observation that statistically significant increases in tumors, when they occurred, were because of benign tumors, nor does it address the fact that these data do not meet the stated definition of “*Sufficient evidence of carcinogenicity*” described by NTP.

The PFOA Draft MCLG Document provides minimal discussion of potential mechanisms of carcinogenicity as it relates to the human relevance of the three tumor types identified in two of the three animal carcinogenicity studies. Evidence indicates that the hepatocellular tumors, LCT, and PACT are linked to a common mode of action involving PPAR $\alpha$  agonism, a mode of action with limited relevance to humans (Biegel et al., 2001; Corton et al., 2018). The only comment on this provided in the Draft MCLG Approach is that IARC “concluded that there is moderate evidence for many potential mechanisms for PFOA-induced toxicity (including PPAR $\alpha$ ).” In contrast, the (EPA, 2016) assessment highlighted a PPAR $\alpha$ -mediated mode of action as likely for rat liver tumors and discussed evidence for a PPAR $\alpha$ -mediated mode of action for both LCTs and PACTs. EPA concluded: “There are some data that provide support for the hypothesis that the PPAR $\alpha$  agonism MOA is wholly or partially linked to each of the observed tumor types. The data support a PPAR $\alpha$  MOA for the liver tumors and thus are indicative of lack of relevance to humans. PPAR $\alpha$  activation also could play a role in the other tumor types observed, but more data to support intermediate steps in the proposed MOAs are needed.” NTP (2020) also highlights the uncertain relevance of this MOA for humans, stating that the increased rate of hepatocellular neoplasms “could be related at least in part to the PPAR $\alpha$  activity, which reviews of studies suggest that the human liver is not as sensitive to PPAR $\alpha$  activity as rodents.”

The PFOA Draft MCLG Document also does not address the critical point that LCTs are not biologically relevant for humans (Steinbach T.J. et al., 2015). There are important differences between rats and humans in hormonal response and physiology that demonstrate the lack of relevance of rat LCT to humans, described in detail by Steinbach et al. (2015).



The issues associated with the significance and relevance of the animal toxicology findings require more detailed evaluation in the PFOA Draft MCLG Document in order to provide a transparent weight of evidence evaluation. As stated in EPA (2005) cancer assessment guidelines:

“In borderline cases, the narrative explains the case for choosing one descriptor and discusses the arguments for considering but not choosing another. For example, between “suggestive” and “likely” or between “suggestive” and “inadequate,” the explanation clearly communicates the information needed to consider appropriately the agent’s carcinogenic potential in subsequent decisions.”

A narrative statement clearly communicating the conclusion about cancer classification, according to the guidelines, is a critical aspect of any cancer assessment. The importance is further accentuated because the only new substantive studies available since the 2016 assessment are one human epidemiologic study (Shearer et al., 2021; discussed above) and one animal toxicology study (NTP 2020) that is essentially consistent with the two previous studies (Biegel et al, 2001; Butenhoff et al, 2012) showing statistically significant increases in benign adenomas of 2 of the 3 types (LCT, PACT, hepatocellular, for which human relevance has been debated) identified in one or both of the previous studies, but not increases in carcinomas despite lifetime exposures. SAB should recommend that EPA reevaluate its cancer classification and more clearly articulate its conclusions, identifying the evidentiary support, and consider whether a lower classification is better supported by the scientific literature.

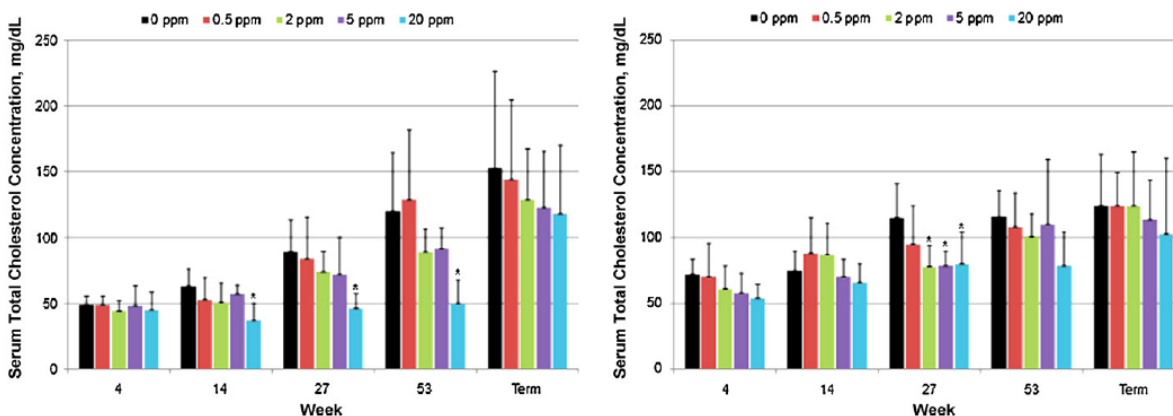
#### **E. Non-Cancer Effects: Cardiovascular Disease and Animal Studies**

Animal toxicology studies do not support a relationship between PFAS exposure and elevated serum lipids. In the Draft MCLG Document for PFOS, EPA concluded that evidence from human studies is consistent with a positive association between PFOS exposure and both total and LDL cholesterol. EPA’s conclusions regarding PFOA were similar, although noting less consistency in the response. EPA additionally concluded there was a positive relationship for PFOA and serum triglycerides. In all cases, EPA noted that the relationships were population-specific, with some serum lipid markers apparently affected in some sub-populations but not others.

EPA ultimately selected increased serum total cholesterol from the Dong et al., 2019 cross-sectional study as the only outcome/study for deriving PODs for both PFOS and PFOA. Dong et al., 2019, identified as a *medium* confidence study by EPA, analyzed U.S. National Health and Nutrition Examination Survey (NHANES) data to analyze temporal trends in PFAS biomonitoring concentrations and associations between cholesterol levels and PFAS exposure. Dong et al., 2019 reported small, positive associations between PFOS/PFOA and total cholesterol levels in their cross-sectional study.

In terms of the overall weight of evidence, the animal toxicology evidence cited in the Draft MCLG Documents is not in concordance with the human data. Rather, it demonstrates the opposite effect if anything. EPA documents that when serum lipids were affected at all in rat studies, they were generally decreased. For example, in the chronic dietary toxicity and

carcinogenicity studies with PFOS in Sprague Dawley rats, serum total cholesterol was decreased with PFOS exposure, especially in males (Butenhoff, Chang, et al., 2012; see figure below). This negative association with serum cholesterol is consistent with data from other studies with PFOS (Bijland et al., 2011) and in some studies with PFOA (NTP, 2020). Furthermore, a negative association between PFOS exposure and total cholesterol and other serum lipids has also been reported in non-human primates (Goldenthal et al., 1979; Seacat et al., 2002).



Mean serum total cholesterol was reduced in male rats (left panel) fed 20 ppm PFOS compared to controls (statistically significantly on Weeks 14, 27, and 53). There were statistically significant reductions in mean serum cholesterol occurred in female rats (right panel) on Week 27 in the 2, 5, and 20 ppm dose groups. Although not statistically significant, cholesterol appeared lower in 20 ppm dose group females on Week 53 and at terminal sacrifice. (\*statistically significant compared to the time-matched controls,  $p \leq 0.05$ ) (Butenhoff, Chang, et al., 2012)

Rather than explore the significance of not just inconsistent, but apparently opposite effects (increased total cholesterol in humans, decreased in rats) on the overall weight of evidence, EPA cites both as “a disruption in lipid metabolism” and dismisses the discrepancy as due to “known differences between the serum lipid composition in human and animals” and notes only that “biological significance of the decrease in various serum lipid levels observed in these animal models regardless of species, sex, or exposure paradigm is unclear.”

Instead of dismissing the animal data because of its apparent incongruity with the conclusions EPA draws from the epidemiologic data, the Draft MCLG Documents should fully assess both the animal and human data (as well as relevant mechanistic data that might explain such differences) with a detailed, transparent, and systematic weight of evidence review.

## II. MIXTURES FRAMEWORK

In EPA's Mixtures Framework<sup>14</sup>, the Agency presents methods for risk assessments based on the assumption of dose additivity. These methods include 1) a screening level approach involving summing of hazard indices (HI approach) for all PFAS in a mixture using reference doses (RfDs), regardless of similarity in toxicity endpoint; 2) refinement of the HI approach by grouping and then summing HIs based on toxicity to the same target organ (TOSHI approach); 3) a method using relative potency factors (RPF approach) that incorporates a factor representing the relative potency of individual compounds at inducing a given key effect required to induce the health outcome relative to the potency of an index, data rich chemical; and 4) with limited data, EPA introduces the potential use of a method for developing mixture-based POD using a dose addition mixture benchmark dose modelling (BMD approach).

To commence the mixtures analysis, Step 1 (p.36), Identification of RfDs, EPA proposes four sources of information for identification of a chronic oral RfDs: "off the shelf" RfDs (including MRLs) from federal sources, state and other sources, then where these "off the shelf" assessments are not available, EPA proposes that the "user" find other hazard effect and dose response data for development of reference values ("RfVs").<sup>15</sup> Finally, where previous, traditional sources are not available, it proposes New Approach Methodologies ("NAM").

In the charge questions posed to the SAB, the PFAS Panel was asked to comment on the appropriateness of the proposed approach for a component-based mixture evaluation of PFAS assuming dose additivity, the suitability of the illustrated BMD approach for development of a relative potency factor (RFP), and the fitness of other implied assumptions and factors presented in the Mixtures Framework. 3M recommends that the SAB respond to this charge by noting that frequently the draft Mixtures Framework proposed approaches are at odds with the EPA guidance on mixtures (1986 and 2000)<sup>16</sup>, and are otherwise inappropriate or inadequately described. SAB should further recommend that EPA revise the Mixtures Framework to rectify these concerns.

### A. Identification of Reference Toxicity Values

For the first two source categories for toxicity values, the draft Framework refers to "off the shelf" assessments to provide toxicity values, e.g., chronic oral RfDs. While the first source is traditional, EPA assessments or other Federal level assessments that have mostly undergone extensive peer review and public comment, under "PFAS 1" a second set of sources for toxicity

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<sup>14</sup> EPA, Combined PFAS framework Final 11.5.21\_Edited\_Formatted\_11.9.21 508.pdf

<sup>15</sup> Page 35 of the Framework states: "Many states and others (e.g., international entities) are addressing rapidly evolving PFAS issues under their respective purviews, including the development of toxicological assessment documents. Although there is overlap in the landscape of PFAS evaluated (or currently being evaluated) across federal, state, and international agencies, at the state/international level, there may be assessment values available for a broader array of PFAS in the context of this framework, these will be collectively referred to as "PFAS 1"[..]"

<sup>16</sup> EPA (Environmental Protection Agency). 1986. Guidelines for the Health Risk Assessment of Chemical Mixtures. EPA/630/R-98/002. EPA, Risk Assessment Forum, Washington, DC. <https://www.epa.gov/risk/guidelines-health-risk-assessment-chemical-mixtures>. EPA (Environmental Protection Agency). Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. EPA/630/R-00/002. EPA, Risk Assessment Forum, Washington, DC. August 2000.

values is identified. In a departure from traditional guidance, EPA calls for reliance on ‘state or other sources’ for assessments. These later sources are observed to provide widely varying toxicity guidance levels that suffer not only from inconsistency, which pose the problem of whether to select the highest, lowest or some median value, but also from high degree of variability in quality, peer review, use of judgmental uncertainty choices, and completeness of study considerations. In a component mixture assessment, not only could outcomes be inconsistent, but a poorly supported, atypically low, outlier reference value (s) could dominate the final health risk assessment outcome. EPA needs to reconsider these recommendations. The lack of data on any particular substance should not lead the risk assessor to provide what could be inappropriate substitutes and unfounded outcomes.

The next source of toxicity values is even more problematic. In the absence of “off the shelf” assessments, EPA guides the mixtures assessor to “study hazard effect and dose-response data” (PFAS -2) to derive reference values. While EPA directed attention to its methods and sought consultation with experts in the field, no adherence to any scientific process is described in detail or assured. Clearly, this could result in derivation of reference values that have not undergone formal peer review and public comment, bringing into question the quality of the toxicity value used in the component HI.

In both PFAS-1 and -2 risk assessment outcomes would be expected to vary enormously across various assessments depending on the choices of the particular assessor. The use of external or assessor-derived values allows for potential cherry-picking and the current discussion in the Framework does not provide guard rails to prohibit bias in the selection of studies to incorporate. This guidance is not reliable and should be reconsidered by the Agency.

For the selection of PODs that are used in the RFP approach, both the Mixtures Framework and the Draft MCLG Documents err on the side of extreme caution, by guiding users to the lowest possible POD without regard for the sufficiency of evidence that the endpoint chosen poses a relevant human health risk. Whether a health threat exists – the hazard assessment - regardless of the dose response characteristics is fundamental to an unbiased and transparent health risk assessment. Every POD derived or used should be accompanied by a hazard identification/weight of evidence for the effect end/disease point, which spells out the several lines of evidence including human, animal, and mechanistic, to assign greater weight to evidence where all lines converge, and lesser weight to weak or suggestive evidence where the lines of evidence are contradictory or missing. As recommended in the 2005 guidelines, quantitation generally is not performed where the evidence is too weak.

The process, as proposed, could lead to selection of a study for a particular component in a PFAS mixture that dominates the mixtures perceived risk, an issue raised in EPA’s Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures, August 2000 (“2000 Mixtures Guidance”):

“The other concern with a large number of chemicals in the mixture is that one poorly studied chemical may dominate the response estimate. An excessive response estimate could arise from improper statistical analysis or toxicological procedures employing highly sensitive animal species.” p.124

The 2000 Mixtures Guidance further provides the following cautionary remarks:

“The component-based procedures discussed earlier for dose-response assessment and risk characterization are intended only for simple mixtures of a dozen or so chemicals. The uncertainties and biases for even a small number of chemical components can be substantial. Component-based methods are particularly susceptible to misinterpretation because the listing of chemical components in a mixture is often misconstrued as implying a detailed understanding of the mixture toxicity and, by inference, the estimated mixture risk. The risk characterization must include a discussion of what is known as well as what is missing or poorly understood in order to convey a clear sense of quality and confidence in the risk assessment.” p.76

And,

“Whenever an assessment is based on component toxicity values, the risk characterization must discuss the quality of the individual chemical estimates that are used.” p.79

And,

“[] an evaluation of the data may lead the user to decide that only a qualitative analysis should be performed. This generally occurs in cases where data quality is poor, inadequate quantitative data are available, data on a similar mixture cannot be classified as “sufficiently similar” to the mixture of concern, exposures cannot be characterized with confidence, or method-specific assumptions about the toxicologic action of the mixture or of its components cannot be met.” p.xiv/xv

The Mixtures Framework does not acknowledge any of this guidance. At a minimum, this guidance provides scientific justifications that should be addressed. EPA does not recognize the recommended application to simple mixtures, implications of the lack of an MOA to imply confidence in common toxicity endpoints, requirement for full risk characterization, or consideration of using a qualitative analysis where data quality is poor. EPA resorted to methods for deriving toxicity values based on relative potency approaches without presenting sufficient and complete considerations of weight of evidence, which is not scientifically defensible. In doing so, EPA finds toxicity without providing evidence of a common mechanism of action for the chosen target health effect. Instead, for quantification of relative toxicity values, EPA proposes using PODs from disparate sources (as discussed above) and novel approaches that can bypass natural body defenses (see discussion of NAMs below). The SAB should request the EPA consider these points and the guidance noted above in its proposed procedures for use of “off the shelf” and assessor derived reference/toxicity values.

Where previous, traditional sources of toxicity values are not available, EPA next proposes a “PFAS-3,” the development of New Approach Methodologies (NAM) based reference values (“RfVs”). Use of these methods for direct application in deriving a toxicity value is superficial, premature, and lacks precedent. The NAM are outside the realm of standard

practice for chemicals that have not had MOA assessments. Indeed, NAM could potentially include: 1) *in vivo* study types not traditionally considered for risk assessment but rather used as supporting evidence for elucidating mechanisms of action and the relevance of findings in animal studies for humans (e.g., injection studies); 2) *in vitro* models, which bypass normal body defenses and certainly bypass MOA considerations such as understanding common MIEs and KEs; or 3) *in silico* approaches that rely on predictive modeling. This search for a potential common endpoint for PFAS compounds is improper given that these compounds demonstrably have clear differences in pathophysiological effects and common MIE and KEs have not been identified. The proposed application of NAM to reach regulatory decisions is entirely novel and EPA has no precedent for applying these methods in a Tiered approach.

The Mixtures Framework examples of chemical classes where dose additivity and/or relative potency have been used are inapplicable to the circumstances here and do not support EPA's novel action. In most or all of the examples, the chemicals in the class have a shared molecular initiating event ("MIE") (e.g., dioxins and induction of the aryl hydrocarbon receptor) and/or converge on a common KE (e.g., pyrethroids and altered neuronal excitability) in an adverse outcome pathway leading to a shared adverse outcome. EPA's Mixtures Framework states, "in the absence of detailed molecular mechanisms for most PFAS, it is considered a reasonable health-protective assumption that PFAS which can be demonstrated to share one or more KEs [key events] or adverse outcomes will act with toxicological similarity to produce dose-additive effects from co-exposure." Mixtures Framework at p.23. But without some evidence, EPA cannot simply assume a shared key event to justify using dose-additivity. Both the 1986 and 2000 Mixtures Guidance documents are founded on sound scientific principles that emphasize the role of mechanistic understanding when deciding to combine risks for component mixtures. Contrary to EPA's implied suggestion, these examples do not support the concept that evidence is only needed that individual chemicals in the class affect the same organ system or clear health outcome without evidence of a shared MIE and/or KE.

In section 3.4 of the Mixtures Framework document, EPA discusses evidence for shared MIEs and/or KEs for various outcomes, but the discussion lacks a systematic or critical evaluation of the scope and relevance of these data. For example, the Mixtures Framework discusses *in vitro* studies of activation of peroxisome proliferator activated receptor alpha (PPAR $\alpha$ ) as evidence of a shared MIE but does not provide discussion of the relevance of these data in the context of demonstrated species-specific differences in PPAR $\alpha$  and responses related PPAR $\alpha$  activation. This lack of context is compounded by the lack of meaningful discussion of this issue in the Draft MCLG Documents. Other potential MIE (activation of constitutive androstane receptor) are introduced briefly, and only in the context of studies of PFOA and PFOS. The Mixtures Framework mentions several outcomes as examples of shared evidence of toxicity, but it is difficult to discern from the discussion which outcomes EPA considers as KEs, which as markers/precursors, and which as apical endpoints. The discussion is cursory and does not include the presentation and full analysis of any one adverse outcome pathway (AOP). This is a critical aspect and should be a precondition for application of an assumption for shared MOA and use of an RPF approach.

The NAM approaches included are not practical, transparent, descriptive, or consistent with current EPA practice and guidance. Rather, they invite nearly any *in vitro* or *in silico* study to be incorporated without the benefit of peer and/or expert review (such as SAB) or public

comment. Indeed, the Mixtures Framework appears to focus far more on finding a way to arrive at the lowest POD and RfD rather than first fully understanding and presenting a full assessment of the available data. SAB should recommend that EPA revise the framework to fully explain its analysis in a revised document and then provide stakeholders an adequate opportunity to review and comment.

## **B. Assumption of Dose Additivity**

EPA's Mixtures Framework does not provide a detailed, transparent, or clear discussion of why the assumption of dose additivity that is the basis for the methods and approaches that follow are appropriate for PFAS. Rather, the rationale seems to be "PFAS are an emerging chemical class of concern" and "MOA data are limited or not available for many PFAS." Instead, the Framework needs to detail the data available that support OR contraindicate a similar MOA, and detail what critical information is necessary to fill the admittedly large data gaps; a roadmap for eventually determining whether an assumption of dose additivity is appropriate.

Notably, the quote from the 2000 Mixtures Guidance used in the Mixtures Framework and Charge to justify bypassing MOA comes from a table in EPA 2000 Section 2.6.1.2 *User Fact Sheet: Relative Potency Factors*, under the heading "Assumptions."<sup>17</sup> However, neither the Mixtures Framework nor the Charge include the full quote, which is:

"Based on dose addition which carries with it assumptions of same mode of action and similarly shaped dose-response curves across the components. The common mode-of-action assumption can be met using a surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)" p.29

Similarly stressing MOA, the 2000 Mixtures Guidance also notes,

"The minimum data needed for development of an RPF approach include: (1) a known or suspected common mode of action shared by the class of compounds; (2) a quantitative dose-response assessment for the index compound; and (3) pertinent scientific data that allow the components to be meaningfully compared to the index compound in terms of relative toxicity." p.109

And,

"Included in the definition of the class should be the understanding of the common mode of action leading to the observed toxicologic effects, the chemical similarity of the compounds, and the identification of the spectrum of toxicologic impacts shared by the class." p.110.

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<sup>17</sup> It should also be noted that the Framework or Charge do not mention the different wording is used in the table related to Hazard index (HI) (section 2.6.1.1. User Fact Sheet: Hazard Index): "Applies dose addition, which carries with it assumptions of same mode of action and similarly shaped dose-response curves across the components. The "common mode-of-action" assumption can be met by using a surrogate of same target organ."

EPA's Mixtures Framework does not clearly address the criterion of "similarly shaped dose response curves." Whereas the 2000 Mixtures guidance notes,

"A separate HI should be calculated for each toxic effect of concern (U.S. EPA, 1986, 1989a). The target organs to be addressed by the HIs should be decided for each particular mixture assessment. The assessor should compare the dose-response curves for the different toxic effects with the estimated exposure levels (and routes) to ensure that those effects most relevant to the environmental exposure are addressed. When certain toxic effects are known to occur, but at much higher exposure levels than those being assessed, then the HI for those effects may not need to be evaluated, but an explanatory note should be included in the discussion of assumptions and uncertainties for the mixture assessment." p,86

EPA has not complied with this portion of the 2000 Mixtures guidance.

Additionally, "surrogate of toxicological similarity, but for specific conditions (endpoint, route, duration)" has greater meaning than just showing that certain PFAS in drinking water can be grouped by health endpoint regardless of MOA. The 2000 Mixtures Guidance notes that a "common mode-of-action' assumption can be met by using a surrogate of same target organ" for the hazard index approach (Figure 2.6.1.1, EPA 2020). However, the bar for dose additivity is higher for the RPF approach (Figure 2.6.2.2, EPA 2020). The assumption for use of an RPF approach must be based on dose addition which carries with it assumptions of same mode of action and similarly shaped dose-response curves across the components. The "common mode-of-action" assumption can be met using a surrogate of toxicologic similarity, but for specific conditions (endpoint, route, duration). Although the 2000 Mixtures guidance is not specific about what would constitute a surrogate of toxicologic similarity to support the RPF method, by its contrast to the hazard index approach it is clearly more than just effects on the same target organ endpoint. At a minimum, the Mixtures Framework needs to fully explore the level of evidence needed and describe why PFAS do or do not meet adequate criteria to fulfill a reasonable assumption for a common MOA. At present, the EPA must consider that this MOA information is largely lacking and, further, that differences in MOA may be indicated by carefully considering the data that are available.

Although the Mixtures Framework states that MOA is 'optimal', EPA justifies not using MOA because "MOA data are limited or not available for many PFAS"<sup>18</sup> and then presents its approaches "in the interim." EPA's 2000 Mixtures Guidance explicitly states that a common MOA as well as similarly shaped dose-response curves are necessary for the assumption of dose additivity:

"4.4.2.5.3. Assess mode of action. It is necessary to describe the mode of action of the class of compounds underlying the health effects for which the RPF was developed. A common mode of action for the class is the basis for the assumption of dose additivity. However, in some cases the class may be linked by common effect with only suggestive or indirect information concerning the underlying mode of action. The description of the

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<sup>18</sup> The mention of chemical classes for which MOA has been used to support dose additivity does not advance the argument that MOA can be side-stepped for PFAS.



RPF must answer the question, “to what degree do the scientific data support the assumption of a common mode of action?” p.113

Furthermore, EPA’s 2000 Mixtures Guidance notes,

“For example, Feron et al. (1995) discuss studies where even at the same target organ (the nose), differences in mode of action led to other than dose additive response. Dose-additive models may be an adequate default procedure for chemicals affecting the same target organ but may not be the most biologically plausible approach if the compounds do not have the same mode of toxicologic action.” p.66

The Mixtures Framework does not appear to provide an answer to the basic question, “to what degree do the scientific data support the assumption of a common mode of action?” SAB’s recommendations to EPA should request that the Agency answer this and allow the public to review EPA’s assumptions.

### C. BMD Approaches

#### 1. Equation for mixture BMD

EPA uses an equation to calculate the mixture BMD (Equation 4-5 in EPA Document No. 822-21-003, p. 54), which it states is similar to the Berenbaum equation (as cited in the 2000 Mixtures Guidance). When 3M reviewed the Berenbaum equation in the 2000 Mixtures Guidance, Equation 4-5 is not the same, and EPA gives no explanation as to how it modified this Berenbaum equation and derived the equation it is using. (See comparison of equations below). The Berenbaum equation referenced in the EPA 2000 guidance on mixtures is on the top. Equation 4-5 that EPA is using in the current mixtures draft is shown on the bottom. At a minimum, the SAB should recommend the Mixtures Framework clarify the equation use and the changes made from prior guidance. Peer review of this work cannot be completed without further clarification and assessment from the Agency.

<p>where:</p> $I = d_1/D_1 + d_2/D_2$ <p><math>d_i</math> = dose of <math>i^{\text{th}}</math> chemical, and</p> <p><math>D_i</math> = dose of <math>i^{\text{th}}</math> chemical that produce the response of 0.05.</p>	$t_{add} = \left( \sum_{i=1}^n \frac{a_i}{BMD_i} \right)^{-1}$
<p>In the bottom equation, <math>t_{add}</math> is the mixture dose (I in the top equation) in mg/kg/day, <math>a_i</math> are the fixed proportions of the component PFAS in the mixture, and <math>BMD_i</math> is the <math>i^{\text{th}}</math> chemical BMD value.</p>	

## **2. Precedent for BMD approach**

In Section 1.5 of the EPA Mixtures Framework, EPA outlines various state, national, and international approaches to address PFAS mixtures. 3M notes there is nothing stated here about this BMD approach, so it would appear, based on information presented by EPA, that such an approach is unprecedented. Time limitations to comment on the EPA Mixtures Framework prevent further research to verify this point, but SAB should recommend that EPA provide a clearer understanding of other applications of the BMD approach, if any.

What is more, the 2000 Mixtures Guidance document mentions BMD analyses, but does not appear to use them in the way that EPA is attempting to use it in the Mixtures Framework. The 2000 Mixtures Guidance discusses use of the BMD approach in the context of applying the BMD to the hazard index, which is considered a separate approach in the Mixtures Framework. The BMD approach in the current mixtures draft document is different and it is not clear how it relates to prior EPA guidance. The SAB should provide comments on this and whether the current approach has been adequately explained given this departure from precedent.

## **3. EPA's examples of the mixture BMD approach**

EPA's examples of the mixture BMD approach are hypothetical and it is unclear how practical they could be in the real world. In these examples, EPA did not even use measured concentration data (although there is a section in Section 1.3 of the Mixtures Framework on the occurrence of PFAS mixtures in the environment, and EPA apparently monitors this). Tables 4-15 and 4-16 of the Mixtures Framework do not identify which PFAS are in the mixture; just PFAS 1, PFAS 2, PFAS 3, or PFAS 4. It is clear that the examples are arbitrary and thus, it is not known how this method would work in practice. The Agency should use a real-world mixture of PFAS to address at least some of the substantial uncertainty in its approach.

In Table 4-15 of the Mixtures Framework, EPA presents its first hypothetical water sample, and BMD values for 3 endpoints (liver weight, reduced pup body weight, and reduced thyroid hormone concentrations) that apparently came from animal studies. EPA does not state what studies these were, which PFAS compound these endpoints came from, and it does not present the BMD analyses (or cite to where they may have performed these). At a minimum, the current assessment is incomplete and lacks clarity.

Also in Table 4-15 of the Mixtures Framework, EPA states that the liver endpoint produced the lowest mixture BMD, so would be the most sensitive effect domain for this mixture; below the table, EPA illustrates its use of Berenbaum equation 4-5 for this liver endpoint. EPA used the liver BMDs for the denominator, but does not explain what was done with the other endpoints for this hypothetical scenario and how those endpoints were incorporated. The SAB should recommend that EPA clarify how it derived the BMD values in their examples and explain its use of the dose-additivity equation.

**Table 4-15. Mixture BMD Approach: Hypothetical Water Sample 1**

	Measured Water Concentration (ug/L)	Mixing Ratio (Proportion)	Thyroid BMD (mg/kg/d)	Liver BMD (mg/kg/d)	Developmental BMD (mg/kg/d)
PFAS 1	10	0.02	0.24	0.044	0.01
PFAS 2	10	0.02	0.24	0.013	0.0051
PFAS 3	50	0.11	2.1	720	2.1
PFAS 4	400	0.85	70	0.1	0.7
Mixture Total	470	1.0			
DA Mixture BMD Calculation			4.16	0.094*	0.132

\*The lowest mixture BMD is converted to a mixture-HBWC for comparison to the measured concentration (i.e., 470 ug/L).

Application of Equation 4-5 to the example water sample in Table 4-15 to derive the DA Mixture BMD. This example is for the liver domain as it was the lowest mixture BMD in this example.

$$t_{add} = \left( \sum_{i=1}^4 \frac{a_i}{BMD_i} \right)^{-1} = \left( \frac{0.02}{0.044} + \frac{0.02}{0.013} + \frac{0.11}{720} + \frac{0.85}{0.1} \right)^{-1} = 0.094 \text{ mg/kg/d}$$

**Table 4-16. Mixture BMD Approach: Hypothetical Water Sample 2**

	Measured Water Concentration (ug/L)	Mixing Ratio (Proportion)	Thyroid BMD (mg/kg/d)	Liver BMD (mg/kg/d)	Developmental BMD (mg/kg/d)
PFAS 1	5	0.07	0.24	0.044	0.01
PFAS 2	50	0.71	0.24	0.013	0.0051
PFAS 3	10	0.14	2.1	720	2.1
PFAS 4	5	0.07	70	0.1	0.7
Mixture Total	70	1.0			
DA Mixture BMD Calculation			0.299	0.017	0.0068*

\*The lowest mixture BMD is converted to a mixture-HBWC for comparison to the measured concentration (i.e., 70 ug/L).

Again, at a minimum, the Mixtures Framework is incomplete and lacks clarity. In this regard, it should not be relied on by EPA for any decision-making. EPA is not clear on how it came to this conclusion about the liver endpoint being most sensitive and having the lowest BMD.

In Table 4-16 of the Mixtures Framework, EPA presents its second hypothetical water sample. In this example, EPA concluded that developmental effects were the most sensitive endpoint and had the lowest BMD. As with the first example, there is no explanation of how EPA arrived at this conclusion.

The Agency seems to be in search of the lowest resulting value without regard to the primary scientific foundations for how likely the effect is to occur in humans from these modeling efforts, i.e., there is no weight of evidence (hazard index) consideration presented.

#### **D. Limitations of EPA's BMD approach.**

As with the Relative Potency Factor (RFP) approach (also discussed in the Mixtures Framework), the user/risk assessor needs to have effect data for at least one common endpoint for all the PFAS in the mixture. Thus, this approach cannot be applied if there is no common endpoint. In addition, for most mixtures, the available dose-response data for the different component chemicals will be based on different conditions, such as differences in exposure duration or test species. According to the 2000 Mixtures Guidance, in the context of applying the BMD to the hazard index, the hazard index can use these BMDs only if some sort of standardization is applied so that the 1/BMD scaling factors describe a common scenario (2000 Mixtures Guidance at p. 83). The SAB should comment on the limited utility of this approach and whether a standardization factor should be applied for the different experimental conditions used to derive the BMD values.

EPA also states that for some mixtures with less well-studied PFAS, there may be no available dose-response data for calculating a BMD. Thus, this BMD approach cannot be applied to all PFAS mixtures (it will be limited by the available data). If data are limited on the individual compounds, the endpoints modeled may not capture all possible endpoints..

In the 2000 Mixtures Guidance, EPA states that: "Pharmacokinetic differences among the class of compounds should be identified because differences in the pharmacokinetics across species could substantially change RPFs developed from nonhuman data." 2000 Mixtures Guidance at p. 114. Not only do PFAS differ in pharmacokinetics across compounds as a class (i.e., shorter-chain compounds are eliminated more quickly than longer-chain compounds), but PFAS differ in pharmacokinetics across species (i.e., rodents eliminate them more quickly than humans). The SAB should comment on how these differences in pharmacokinetics might affect the BMD and thus the outcome of this approach.

In the Mixtures Framework, EPA's description of the BMD approach is incomplete, lacks rationale scientific foundations and is fraught with uncertainties. That EPA further fails to provide even minimum practical considerations for application in the real world is troubling. Without adequate scientific analysis and peer review, this approach should not become policy or guidance, nor should it be applied in Agency decision-making. Accordingly, the SAB should recommend that EPA reevaluate its BMD analysis.

### III. CVD RISK ANALYSIS

#### A. Epidemiology of PFOA/PFOS and cardiovascular disease (CVD), including high cholesterol

EPA's CVD Risk Analysis is intended to estimate population-level reductions in cardiovascular disease ("CVD") risk, as well as reductions in total cholesterol levels, that may result from reductions in drinking water exposure to PFOA and PFOS. The premise of this document, however, ignores the fundamental issue that PFOA and PFOS are not known to cause CVD or to increase total cholesterol levels. In the absence of a causal effect, any reduction in drinking water exposure to PFOA and PFOS would be anticipated to have no impact on CVD risk and total cholesterol levels in the target population.

In the Draft MCLG Document for PFOA, EPA acknowledges that the available epidemiologic evidence "did not provide consistent evidence for an association between PFOA and blood pressure"; and was "inconsistent" regarding any association between PFOA and hypertension or other CVD-related outcomes (EPA Document No. 822D21001, p. 191). For total cholesterol, EPA concludes that "the association was consistently positive in pregnant women, positive but less consistently so in adults and children, and generally null in workers" (EPA Document No. 822D21001, p. 192).

In the Draft MCLG Document for PFOS, EPA uses similar language, noting that the available epidemiologic studies "provided evidence for a positive association between PFOS and blood pressure, although the results were not always consistent between [systolic blood pressure] and [diastolic blood pressure], and one study reported an inverse association. The limited evidence for an association between PFOS and increased risk of hypertension was inconsistent ... Evidence for other CVD-related outcomes across all study populations was more limited and inconsistent." Draft MCLG Document for PFOS at p. 179. Regarding total cholesterol, EPA states that "the available evidence supports a positive association between PFOS and [total cholesterol] in the general population, including children and pregnant women," and that "[a]lthough PFOS appeared not associated with elevated [total cholesterol and low-density lipoprotein] in workers, this conclusion is uncertain as the occupational studies included in this review are limited in both quantity and quality." *Id.* at p. 179).

3M notes that most epidemiological studies of PFOA or PFOS in relation to total cholesterol levels have been cross-sectional in design, preventing a causal interpretation of their results, especially in light of plausible reverse-causal effects. Even in prospective studies, shared underlying physiological mechanisms that affect circulating PFAS levels and lipid levels can also influence results, leading to spurious associations. Specifically, alternative explanations for observed positive associations between serum PFOA or PFOS and serum lipid levels include common underlying physiological mechanisms (Frisbee et al. 2010), such as shared gut receptors; an effect of total cholesterol, low-density lipoprotein (LDL), and non-high-density lipoprotein (HDL) cholesterol on decreased kidney function (Schaeffner et al. 2003, Morita et al. 2010). They are also subject to confounding by numerous demographic, behavioral, and environmental factors that affect lipid levels (Thelle 1990), such as body size, which can affect PFAS clearance at background exposure levels (Longnecker 2006), and high-fat or low fiber diets, which increase circulating lipid levels and also could affect clearance of PFAS via

gastrointestinal excretion and thus be associated with higher serum PFOA and/or PFOS levels (Buck et al. 2011; Dzierlenga et al. 2021). The ability of cholestyramine to facilitate both PFOA/PFOS and lipid clearance further suggests that a confounding factor impacting the enterohepatic circulation of both PFOA/PFOS and lipids could explain the observed association (Johnson 1984; Ducatman 2021). Binding of PFOA and PFOS to circulating  $\beta$ -lipoproteins and albumin in blood also could be responsible for a non-causal positive association (Olsen and Zobel 2007, Seo et al. 2018). The premise of the CVD Risk Assessment ignores these critical interpretive limitations. SAB should recommend that EPA account for these issues in a revised assessment. Other recent reviews, including one conducted through a project involving 30 countries, the European Environment Agency, and the European Commission (Fragki et al. 2021) and one with co-authors from EPA (Andersen et al. 2021), concur that “the extent to which the relationships between PFOS/PFOA exposure and these altered levels of blood lipids are causal remains uncertain” (Fragki et al. 2021) and that “[t]he extent to which the relationship is causal is an open question” (Andersen et al. 2021). These reviews detail many of the data limitations and plausible alternative explanations that preclude attributing the modest lipid effects observed in some studies to PFAS. SAB should recommend that EPA address these limitations and alternative explanations before basing any risk assessment on lipid effects.

Nearly all epidemiological studies of PFOA or PFOS with respect to total cholesterol levels are also hampered by a one-time measurement of lipid levels, which can vary, sometimes substantially, within individuals over short and long time scales (Hegsted and Nicolosi 1987, Smith et al. 1993, Tolfrey et al. 1999). Additional methodological concerns in these studies are whether analyses were restricted to fasting blood specimens or to individuals not taking lipid-lowering medications. Without such restrictions, associations with total cholesterol levels could be biased in an unpredictable manner due to outcome misclassification, given likely correlations between misclassification error and potential confounders such as socioeconomic status and health care access.

In 2020, the former C8 Science Panel members and collaborators noted that of the “numerous” cross-sectional studies of associations between PFOA and lipid markers, and that most found a “clear positive association between serum PFOA and total cholesterol (TC) or low-density [lipoprotein] (LDL) cholesterol and a minority with positive associations with high-density lipoprotein (HDL) and triglycerides.” The authors noted, however, that these findings were susceptible to bias; that is, the apparently consistent statistical association may not be causal:

The positive association could reflect confounding, if for example regulation of serum level of both PFOA and cholesterol was correlated. Inter-individual variation in enterohepatic cycling of both PFAS and bile acids, the latter affecting serum cholesterol levels, has been postulated as a mechanism for such a correlation between PFAS and cholesterol (EFSA Panel on Contaminants in the Food Chain et al. 2018). Some observations lend support to this view. Correlation between PFAS and cholesterol excretion has been shown in patients with high levels of PFOS, another long chain PFAS, who were given cholestyramine, a drug known to reduce cholesterol, and which led to a sharp decrease in PFOS (Genuis et al. 2014).

Regarding PFOA and CVD, the same authors concluded that there was “no evidence of an association with heart disease,” despite the apparent associations with cholesterol (Steenland et al. 2020). The authors reasoned that an association of PFOA with higher levels of HDL cholesterol and/or lower levels of C-reactive protein might mediate a protective effect against cardiovascular disease; therefore, they stated, “it is plausible that there is a positive association of PFOA with raised cholesterol, yet no impact on the risk of cardiovascular disease” (Steenland et al. 2020).

In its MCLG documents EPA notes that it did not find convincing evidence of an associating PFOA or PFOS with CVD in 2016 and, since, then it has assessed 35 and 30 new epidemiological studies, for PFOA and PFOS, respectively, that examined CVD endpoints. EPA’s 2021 assessment did not find affirmative evidence for associations with CVD endpoints.<sup>19</sup> Consequently, models that predict risk of CVD outcomes such as the ASCVD model, cannot be assumed to be applicable to PFAS because of the large numbers of studies including newer ones that do not affirm CVD associations with PFAS.

For the above reasons 3M recommends the SAB consider the evidence for the lack of a CVD response from exposure to PFAS and the legitimacy of the exposure reduction-benefit analysis proposed by EPA.

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<sup>19</sup> See conclusory paragraphs in the PFOA MCLG Draft Document on pages 172, 175, and 176, and the conclusory paragraphs in the PFOS MCLG Draft Document on pages 162 and 165.

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